Pollution 2022, 8(3): 820-829
DOI: 10.22059/POLL.2022.334317.1259

RESEARCH PAPER

Determination of Heavy Metals in Honey samples from different Regions of the northeast of Algeria: according to an Urban Gradient

Bouden Mohammed Chafik[1, 2*], Belabed Ibrahim Adnène[1, 2]

[1] Affiliations et coordonnées des auteurs 1 EcoSTAq - Ecology of Terrestrial and Aquatic Systems Laboratory, University of Badji MOKHTAR at Annaba, Algeria
[2] UrbEco Team – Urban Ecology Team at EcoSTAq Laboratory, the University of Badji MOKHTAR at Annaba, Algeria

Received: 23.11.2021, Revised: 25.04.2022, Accepted: 01.05.2022

Abstract
This study aims to assess the pollution of honey by heavy metals based on the efforts of an urbanization gradient in the city of Annaba (Northeast Algeria) where the dosage of five heavy metals (Fe, Cr, Ni, Cu, Cd) was carried out in four sites. The level of heavy metals was determined by atomic absorption spectrophotometer. The results obtained indicated that even when all the samples were contaminated they were of good quality since the concentrations did not exceed the international standards. According to the results of heavy metal concentrations in the honeys studied, the most abundant element is Fe with an average concentration of 6.956 ± 2.045 (mg/kg), Cr 0.765 ± 0.197 (mg/kg), Ni 0.6005 ± 0.159 (mg/kg), Cu 0.21025 ± 0.065 (mg/kg) and Cd 0.01425 ± 0.005 (mg/kg). The heavy metals studied are present in all samples but trace amounts. Moreover, the comparison of the honey from the four sites indicates to us that the healthiest honey is that of the urban site.

Keywords: heavy metals; honey; urban; atomic absorption spectrophotometer; Annaba

INTRODUCTION

Honey is a unique gift of nature producing by honeybees with medical, cosmetic, and nutritional properties, it is the oldest natural food that human has utilized (Mahmoudi, R et al., 2016). Honey is defined as a natural supersaturated sugar solution, which has been consumed as a high nutritive value food; the latter is composed of a complex mixture of carbohydrates (Saxena et al., 2010). Its composition is a variable owing to the differences in plant species, environmental conditions, climate, and beekeeper contribution (De Rodrıguez et al., 2004; Küçük et al., 2007; Adugna et al. 2020). Beside these honey contains different sugars, organic acids, proteins, enzymes, hormones, yeast, vitamins, minerals, and heavy metals (Kujawski & Namieśnik, 2008; Wang & Li, 2011; Adugna et al. 2020).

The honeybee is more exceptional than it can appear. In addition to producing honey, it helps to transmit life thanks to their action of pollination, their passage from flower to flower plays an essential role in the increase in the quantity and the quality of the harvests of several cultures. (Biri, M., 2011). Moreover, several ethological and morphological characteristics make the honeybee a reliable ecological detector: it is an easy-to-breed, almost ubiquitous organism with modest food requirements; its body is covered with hairs, it takes samples from almost

* Corresponding author Email: chafikbouden@hotmail.fr

all environmental sectors (soil, vegetation, water, and air) during these foraging activities, thus providing several indicators for each season. (Porrini et al. 2003). The honeybee then acts as a detector of environmental pollution in two ways. Its signals either via high mortality rates, even blanket apicides, the presence of toxic molecules, or via the residues in honey, pollen, larvae, and so forth (Celli, 1983; Porrini et al., 2002).

The heavy metals are released into the environment by natural and anthropic sources, and as they do not decay and are characterized by latent toxicity, they are continuously present in the environment entering into the biological cycles (Perugini et al., 2011). Their rate is an important indicator of possible environmental pollution as well as another potential indicator of the geographical origin of honey (Anklam., 1998). Minerals presence and content in honey reflects the area surrounding the apiary; the foraging activity of the bees extends for about 10 km², and when the bees collect nectar or pollen, these elements are transferred, contributing to the levels in the honey (Perna,A et al.,.2021). In fact, the air and the soil contain heavy metals, mainly from industry and road traffic, which can also contaminate the honeybee colony and its products. (Bogdanov., 2006). Therefore, apiaries located near polluted areas (because of intense traffic, industrial contaminants) can help in monitoring of the heavy metals from the various sources. (Tuzen et al., 2007)

Hence, this study aims to analyze the content of heavy metals in the collected honey samples according to an urban gradient (i.e. urban, peri-urban and rural samples) and to compare them to detect the existing differences or similarities between the different honey samples. The urbanity gradient can influence the content of honey even in heavy metals?

MATERIAL AND METHODS

Our study was conducted in the extreme northeast of the Algerian Tell on the city of Annaba (latitude 36 °, 30 ′ N and 37 °, 03 ′ N; longitude 7 °, 20 ′ E and 8 °, 40 ′ E; the capital of the city located at coordinates: 36 ° 54′N and 7 ° 46 ′ E) **(Figure 1)**. This region located 600 km from the capital of the country Algiers, open on the Mediterranean coast for 80 km, it extends over 1439 Km² or 0.06% of the national territory (187 Km2, for the Daïra of Annaba, and 49 km2 for the Municipality of Annaba) (Belabed., 2017).

The study sites were selected according to the different degrees of environmental pollution, especially due to urban and industrial activities. Knowing that honeybees forage within a radius of an average of 3 km around the hive (Bogdanov, S.2006), the bee's exhibition area is 27 km2 around the studied site. Thus, to establish the typology of an industrial site (rural, urban, etc.), it is necessary to consider the land use inside this area. To this end, 27 km2 areas of 4 sites were mapped by ArcGIS 10.8.

The first site is in the municipality of Sidi Amar inside the campus of the Badji MOKHTAR University of Annaba (Latitude N 36.815187, Longitude E 7.711402, altitude 38m). This site is an urban area characterized by a large urbanized area, an ornamental, and wild flora on campus's green spaces. In the foraging area located about 3km away, there are several plots of annual crops and market gardening. The heavy industries of the El Hadjar iron and steel complex and several other companies in the Mebouadja industrial zone represent a fixed source of pollution through gas and dust emissions. **(Figure 2)**

The second site is in the commune of El Bouni and more precisely in a meadow in the region of Chabbia, which is along the road N 44 (Latitude N 36.859173, Longitude E 7.71298, altitude 3m). Very remarkable road traffic and many other plots of grassland or wild vegetation as well as an agricultural activity centered mainly around livestock, in addition to some annual crops and market gardening, and the presence of another source of pollution such as a fixed industrial activity (industrial area of El Bouni) and a mobile source of pollution (road traffic) in the foraging area. **(Figure.3)**

Fig. 1. Sampling site map.

The third site is in the commune of Annaba on the heights of the plain of Oued Forcha (Latitude N 36.915934, Longitude E 7.728565, altitude 72m), and characterized by an urban area without industrial activity. Weed vegetation and a large formation of cork oak around the hives with the presence of some dwellings. This site has no fixed sources of pollution and road traffic is almost absolutely absent simply because the land is inaccessible by car. **(Figure.4)**

The fourth site is in the commune of Annaba, more precisely in the city center, inside the Christian cemetery (Latitude N 36.91028, Longitude E 7.756232, altitude 13m) and characterized by an urbanized surface, an important exotic flora than the other types of landscape (public garden), remarkable automobile traffic, and an industrial and agricultural activity absent in the foraging area. This site presents a mobile source of pollution generated by road traffic. **(Figure.5)**

The number of colonies in each research apiary varied between two and three because of the temperament of the bees, especially in urban sites as the species used during this study is the local species *Apis mellifèra intermissa* known for its aggressiveness. There were therefore 10 research colonies in total in the 4 apiaries.

The type of hive used is Langstroth type with 10 frames, the most widespread in Algeria for these characteristics. All the hives are exempt from any chemical treatment so as not to interfere with the results of analyses. The colonies of honeybees were kept from winter to summer in the same apiary.

Samples were collected in late summer (August 2019). Each sample (100 g) was kept in a plastic jar and stored in the dark at 4 ° C.

A honey sample (5 g) was placed in a crucible and reduced to ash in an oven at 600 ° C for 12

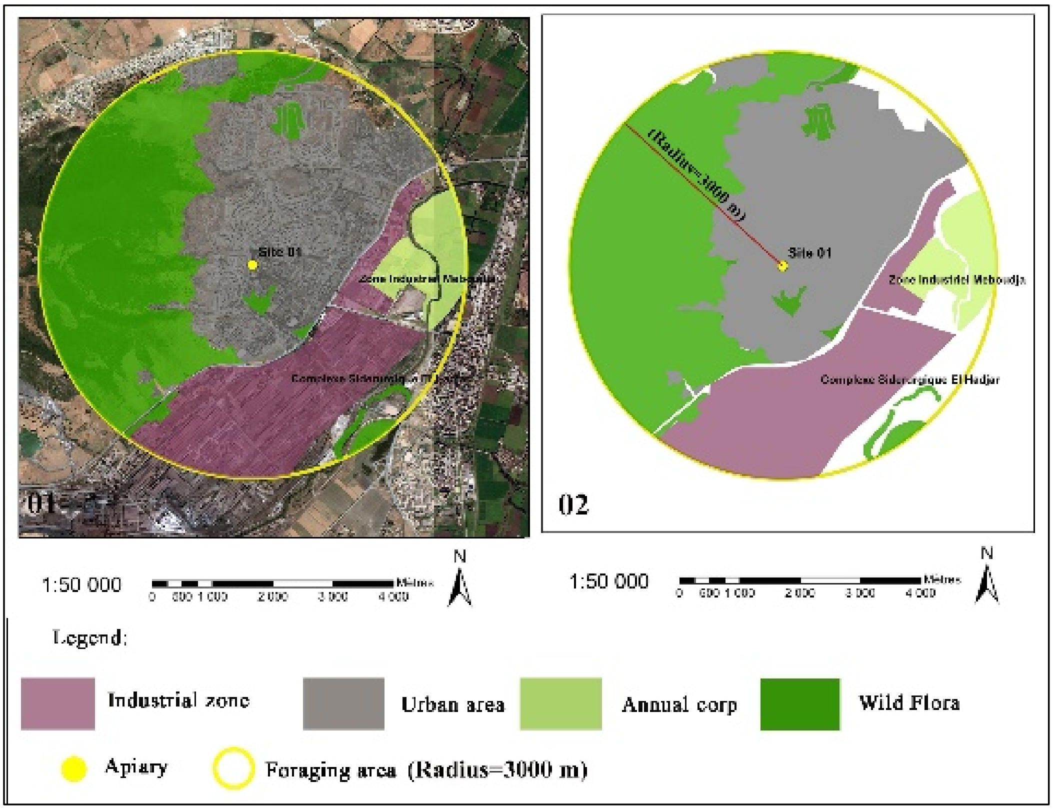

Fig. 2. The landscape surrounding sampling site (**Site 01**).

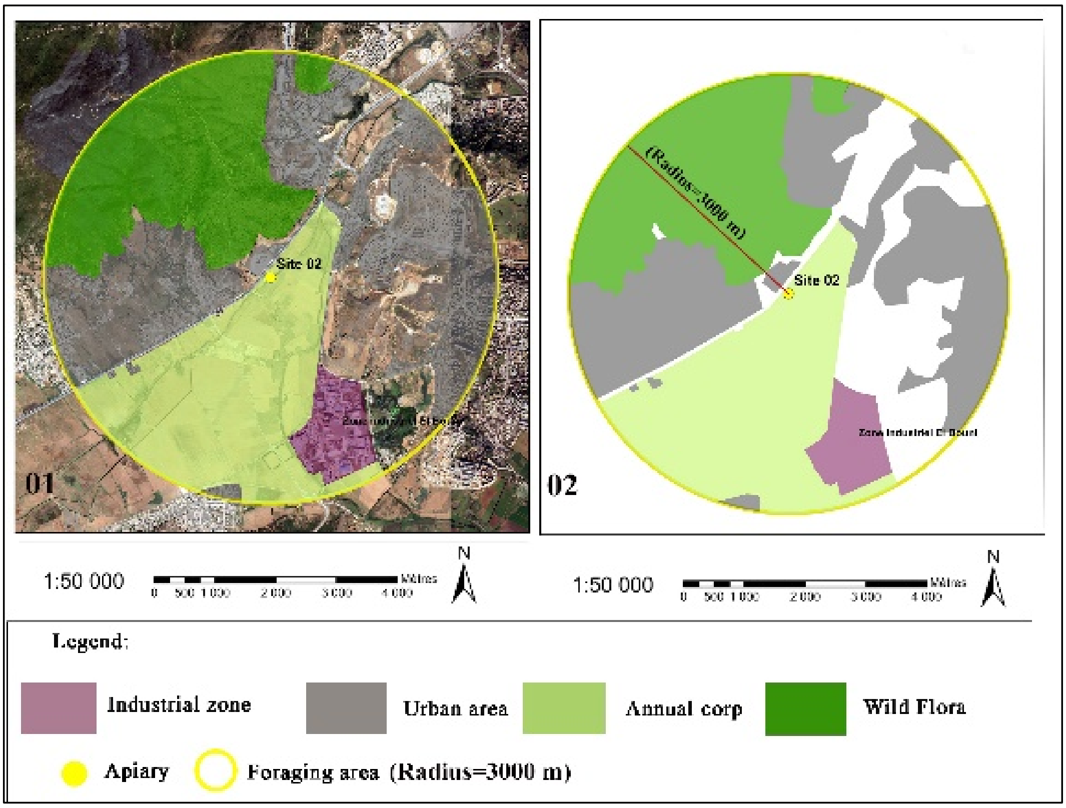

Fig. 3. The landscape surrounding sampling site (**Site 02**).

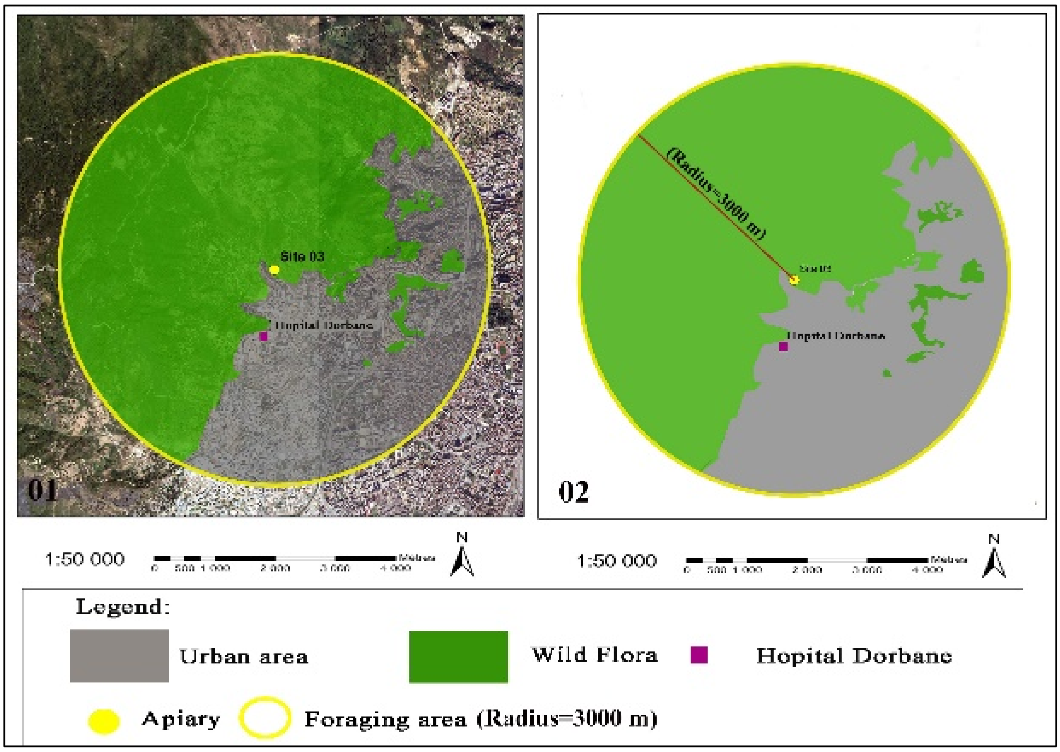

Fig. 4. The landscape surrounding sampling site (**Site 03**).

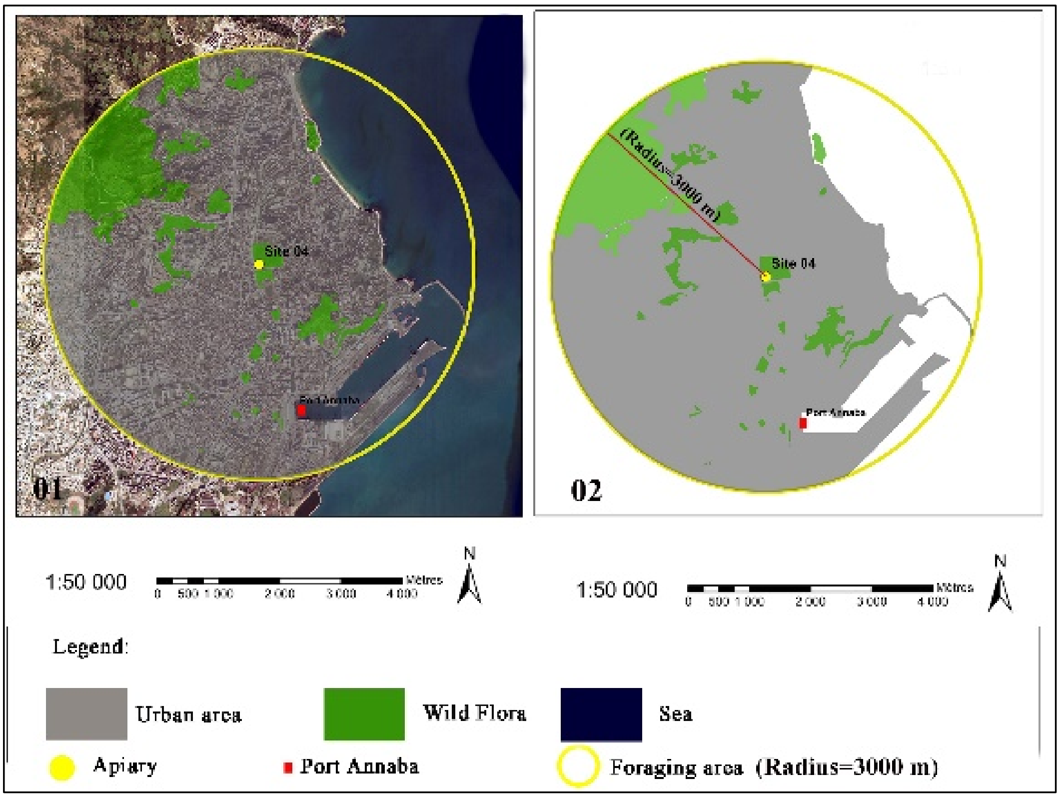

Fig. 5. The landscape surrounding sampling site (**Site 04**).

hours until the ash set; characterized by a color ranging from white to gray. The ash was treated with 2 ml of 65% HNO_3 solution. Peroxide (30% pure H_2O_2) destroys the possibly organic part left after combustion. Samples were placed in 25 ml vials and topped up with distilled water.

The calibration lines are made from three standard solutions (1, 2 and 3 mg/L) which are prepared from a stock solution of known concentration (100 mg/L), using distilled water. The concentrations of the five heavy metals (Fe, Cr, Ni, Cu, Cd) in the solutions obtained were determined by Flame Atomic Absorption Spectrophotometer (SAA) at the Laboratory of Industrial Analysis and Materials Engineering (LAIGM) University of May 8th, 1945 Guelma.

Three replicates were performed for each sample,

Descriptive statistics for each parameter were calculated using Microsoft Excel 2019 software and the results are processed by parametric tests. The concentrations of each element were compared between sites using the t-test (with a significance level = 0.05), using the STATISTICA 12.5 software, Stat soft.

RESULTS AND DISCUSSION

Five heavy metals were quantified for each honey (Fe, Cr, Ni, Cu, and Cd). Table 01 show the mean and standard deviation of the heavy metals contents in honey samples.

The results study (Table.01) indicate that the concentrations of heavy metals assayed show differences for each element depending on the location of the sites and their characteristics (flora, presence or absence of fixed and mobile sources of pollution, as well as the degree of urbanization).

As a result, the presence of heavy metals in the beehive products depends on several conditions and the different places visited by the honeybees during their flight to collect the various products from outside the hive (Water, Nectar, Pollen and, Propolis).

Based on our findings, Fe concentrations ranged from 4.22 to 8.96 mg/kg, and the analysis by the t-test gives us very highly significant differences between the four sites ($t = 6.801$; $p = 0.006$; ***). With a maximum of 8.96 mg/kg for site 01 (Sidi Amar) and a minimum of 4.22 mg/kg for site 04 (Christian cemetery).

Regarding Fe content, our results are not too far from those found by (Yaiche Achour et Khali., 2014), which are 1.95 mg/kg to 6.37 mg/kg. The minimum and maximum Fe contents observed are 1.95 mg/kg for eucalyptus honey from the Httatba region and 6.37 mg/kg for jujube honey from the Laghouat region. (Yaiche Achour et Khali., 2014). Nevertheless, our results are superior to those found by (Pehlivan and Gül., 2015) in Turkey, which was from 1.71 to 3.61 mg/kg, and well as those found in Serbia by (Spirić et al., 2019) who described values from 0.77 to 3.94 mg/kg. As for (Nega et al., 2020) in Ethiopia, they too found values below ours, namely 0.59 to 5.39 mg/kg.

The concentration of this element in our samples was maybe related to environmental

Table 1: the mean and standard deviation of the heavy metals contents (mg/Kg) in honey samples.

Heavy metals	Site 01 N=2	Site 02 N=3	Site 03 N=3	Site 04 N=2
Fe	8,96 ±0,1228	6,697±0,1201	7,947 ±0,652	4,220 ±0,0378
Cr	0,974 ±0,0329	0,812 ±0,0305	0,775 ±0,0204	0,499 ±0,0217
Ni	0,567 ±0,0052	0,690 ±0,0334	0,753 ±0,06	0,392 ±0,0216
Cu	0,277 ±0,0114	0,192 ±0,0037	0,244 ±0,0073	0,128 ±0.030
Cd	0,020 ±0,0022	0,017 ±0,0013	0,011 ±0,0015	0,009 ±0,0017

Note: the mean ± standard deviation, N=Number of beehive pollution due to anthropogenic activities near study sites such as the steel complex of El Hadjar. The dust dispersion is remarkable with the highest Fe values in Site 01 (Sidi Amar), which happens to be the closest to the steel complex of El Hadjar on the other hand by comparing it to that of Site 04 (Cemetery Christian). Despite the purely urban ecosystem, the Fe rate in this latter is two times lower than that of the Site (01). Our values are much lower than those found by (Draiaia., 2016). The values found by the latter vary between 60.62 and 201.07 mg/kg with an average of 103.51 ± 6.64 mg/kg. According to (Draiaia., 2016) the among honey studied in the region: Bargouga, Al Matar, Hjar Diss, and El Bouni in the wilaya of Annaba, the high content of Fe explained given the pollution developed by the El Hadjar steel complex located near the area where the hives placed.

The highest concentration of Cr was found at site 01 (Sidi Amar) with a value of 0.974 mg/kg compared to site 04 (Christian cemetery) with 0.499 mg/kg. Analysis by the t-test gives us very highly significant differences between the four sites (t = 7.755; p = 0.004; ***). These levels are higher compared to the different results described by authors such as (Yaiche Achour et Khali., 2014) in Algeria (0.023 mg/kg), (Pehlivan and Gül., 2015) in Turkey (0.001 to 0.036 mg/kg), (Nega et al., 2020) in Ethiopia (0.22 to 0.46 mg/kg) as well as (Aghamirlou et al., 2015) in Iran (1.2203 to 0.17237 mg/kg). These differences could be due to industrial companies present in the honeybee foraging area which can affect honey through their pollutants, which can contain high levels of toxic elements such as heavy metals (Cr). Our results are lower than those of (Draiaia., 2016), who found high levels of Cr with an average of the order of 1.34 ± 0.90 mg/kg among studied samples of honey collected in the region of Annaba.

The Ni contents found during our study vary from 0.392 to 0.753 mg/kg. Analysis by the t-test gives us very highly significant differences between the four sites (t = 7.552; p = 0.004; ***). Ni values vary from country to country, as Turkey's rates are from 0.02 to 0.87 mg/kg (Pehlivan and Gül., 2015) and, in Iran's northwest, the results are between 1.09449 to 0.06504 mg /kg (Aghamirlou et al., 2015). The honey analyses showed different average Ni values by region in Ethiopia (Nega et al., 2020) found Ni levels of 0.14 mg/kg in Debre Markos, 0.12 mg/kg in Bicheno, and 0.04 mg/kg in Dejen. In the main, the concentrations of Ni in honey are between 0.3 and 1.3 mg/kg. These concentrations can be accidental or mostly natural (Bogdanov., 2004; Yaiche Achour et Khali., 2014). The presence of Ni in honey shows that this type of pollutant is now omnipresent and that it is difficult to find uncontaminated areas. (Porrini et al., 2002)

Cu contents for all the varieties of honey dosed are in the range of 0.128 mg/kg to 0.277 mg/kg. With a maximum of 0.277 mg/kg for site 01 (Sidi Amar) and a minimum of 0.128 mg /kg for site 04 (Christian cemetery). Analysis by the t-test gives us very highly significant differences between the four sites (t = 6.464; p = 0.007; ***). Interestingly, we notice a decrease in the content of this metal as we move away from the industrial area of El Hadjar. Our results agree with the results obtained by (Nega et al., 2020) 0.268 to 0.279 mg/kg from Ethiopia and fall within the ranges found by other researchers. In monofloral honey, from Turkey (Pehlivan and Gül., 2015) found values ranged from 0.01 to 0.36 mg/kg. In Serbia, (Spirić et al., 2019) found values ranging from 0.09 to 0.92 mg /kg. (Aghamirlou et al., 2015) in northwestern Iran, values are from 0.02765 to 2.87274 mg/kg. However, our results represent a low Cu content compared to the values cited by (Yaiche Achour et Khali., 2014), 3.22 mg/kg eucalyptus honey from the region Httatba and 2.72 mg/kg for orange honey. Cu can be presented in the honey due to the Cu fungicides use into agriculture. The main man-made releases of Cu are from coal-fired power stations, metal production, waste incinerators, sewage treatment processes, and from the application of agricultural chemicals. Smaller amounts are also released naturally from the earth's crust (Oroian et al., 2016).Conversely, in Romania, the results obtained by (Bartha et al., 2020) are too high compared to all the previously mentioned results 33.00 to 2.00 mg/kg.

Cd values are low at trace levels in all samples, ranging from 0.009 to 0.020 mg/kg. Analysis by the t-test gives us highly significant differences between the four sites (t = 5.562; p = 0.011;

***). Values obtained in our study are comparable to those found in a survey of Algerian honey by (Yaiche Achour et Khali., 2014) found 0.018 to 0.019 mg/kg. In northwestern Iran, values ranging from 0.12588 to 0.00136 mg/kg by (Aghamirlou et al., 2015) and in Moroccan mono-honey in several localities values lower than 0.015 (mg/kg) (Moujanni et al., 2017). On the other hand, Cd levels detected in Turkish honey are much lower than ours ranging from 0.00023 to <0.0001mg/kg (Pehlivan and Gül., 2015).

While others have found much higher concentrations like the Romanian honey contained 3.81 to 0.05 mg/kg (Bartha et al., 2020). The presence of Cd at very high levels in honey is proof that there is a real pollution problem because Cd is not naturally present in the ecosystem at such high concentrations. Cd from metallurgical industries and incinerators is transported from the soil to plants and can then contaminate nectar and honeydew. Only a small part of the cadmium could reach the honey by air, mainly near the incinerators. (Bogdanov., 2006). In our case, the Cd levels obtained do not represent a danger. There are no specific maximum residual limits for Cd for honey, but a value of 0.1 mg/kg has been proposed by the European Union (Bogdanov., 2006). Our results remain below the limit set by the European Union.

CONCLUSION

According to our results the most abundant element, on average, is Fe, with an average concentration of 6.956 ± 2.045 (mg/kg), followed by Cr 0.765 ± 0.197(mg / kg), Ni 0.6005 ± 0.159 (mg/kg), Cu 0.21025 ± 0.065(mg/kg) and Cd 0.01425 ± 0.005(mg/kg). From these results, we can establish a fairly clear picture of the presence of real pollution in the study region caused by the industrial presence in the city of Annaba. Firstly, we must highlight the rate of Fe present mainly by atmospheric and liquid discharges from the steel complex of El Hadjar in the study area. Secondly, the Cr level is the result of industrial discharges from the steel complex. Finally, the presence of Cd represents a real danger for various living beings (flora, fauna) as well as for public health. The concentrations obtained do not represent the true rate since some authors state that the bee filters heavy metals from the environment (Devillers and Pham-Delegue., 2002; Lequet., 2010) because it has a detoxification system that allows it to eliminate some of the pollutants encountered during its journey to feed. Therefore, an in-depth study on other products of the hive and the honeybee would allow us to have a very detailed picture of the state and the level of pollution in the region. According to (Kadem., 2005), it is confirmed that the El-Hadjar complex and more precisely the electric steelworks 1 and 2 are responsible for atmospheric emissions deposited in the soil and vegetation. The total quantity of this dust greatly exceeds the levels authorized by the WHO for all the constituent elements of this dust (Fe, Cu, Ni, Mn, Cr, Zn, Pb, etc.). Other studies focus on the diagnosis of the pollution of water resources in the sub-watersheds of the south-east of the Edough, samples were taken from Oued Forcha, Oued Sidi Harb, Oued Bouhdid and Oued Boudjemaa show high levels in heavy metals (Cr, Fe, Pb, Ni, Zn, Mn) coming mainly from liquid discharges from a few companies (galvanizing, metal processing, refinery, etc.) which are discharged either in the open air at the level of the wadis or at near Lake Fetzara (Amirat., 2012).

Thereby, the Annaba region is a region with a high risk of heavy metal pollution, and despite this, the honey samples analyzed were of good quality and much less polluted than the standards imposed by the European Union and the WHO. To conclude, the least polluted honey during our study happens to be the honey from Site 04 (Christian cemetery), this indicates that even if urban ecosystems tend to be more polluted than other ecosystems, Annaba's ecosystem is not too affected by pollution in general.

GRANT SUPPORT DETAILS

The present research did not receive any financial support.

CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

REFERENCES

Adugna, E., Hymete, A., Birhanu, G. and Ashenef, A. (2020). Determination of some heavy metals in honey from different regions of Ethiopia. Cogent Food & Agriculture (2020), 6: 1764182.

Aghamirlou, H.M., Khadem, M., Rahmani, A., Sadeghian, M., Mahvi, A. H., Akbarzadeh, A. and Nazmara, S. (2015). Heavy metals determination in honey samples using inductively coupled plasma-optical emission spectrometry. Journal of Environmental Health Science & Engineering DOI 10.1186/s40201-015-0189-8.

Amirat, S. (2012). Diagnosis of the pollution of water resources and EDR Health in the sub-watersheds of the South-East of Edough. Thesis for obtaining the Magister degree in Hydrogeology, Badji Mokhtar-Annaba University. 117p.

Anklam, E. (1998). A review of the analytical methods to determine the geographical and botanical origin of honey. Food Chemistry, 63 (4): 549-562.

Bartha, S., Taut, I., Goji, G., Vlad, I.A. and Dinulică, F. (2020). Heavy Metal Content in PolyfloralHoney and Potential Health Risk. A Case Study of Copșa Mică, Romania. Int. J. Approx. Res. Public Health 2020, 17, 1507. DOI: 10.3390/ijerph17051507

Belabed, A.I., Lebnaoui, S., Bouden, M.C., Brahmi, C.E. and Belabed-Zediri, H. (2017). The Use of a Bird Species as a Bioindicator: The case of Eurasian Jay (Garrulus glandarius) in the Edough Region (Northeastern of Algeria). Journal of Advanced Zoology, 38 (2) : 140-153.

Biri, M. (2011). Tout savoir sur les abeilles et l'apiculture. De Vecchi. 298pp.

Bogdanov, S. (2004) Beekeeping products, 23A Honey, Reviewed by the "beekeeping products" expert group 37 P.

Bogdanov, S. (2006) Contaminants of bee products. Apidology, 37 (1) 1-18.

Celli, G. (1983). - L'ape come insetto test della salute di un territorio.- In: *Atti XIII Congresso Nazionale Italiano Entomologia*, Sestriere – Torino, Italy (ARZONE, A., CONTI, M., CURRADO, I., MARLETTO, F., PAGIANO, G., UGOLINI, A.,VIDANO, C., Eds) 637-644.

De Rodrıguez, G.O., De Ferrer, BS., Ferrer, A. and Rodrıguez, B. (2004). Characterization of honey produced in Venezuela. Food Chemistry, 84 (4), 499–502.

Devillers, j. and Pham-Delegue, mh (2002). Honey bees: estimating the environmental impact of chemicals. Taylor & Francis Editions, London and New-York, 332 p.

Draiaia, R. (2016). Physico-chemical characterization and botanical name of Algerian honeys (Case of langstroth hives). Doctoral dissertation. Ph.D. thesis in Biochemistry. University Badji Mokhtar Annaba.319p.

Kadem, D.E.D. (2005). Evaluation and behavior of heavy metals (Cd-Cr-Cu-Ni-Pb-Zn and Mn) in soils used for agriculture and natural vegetation subjected-to atmospheric pollution. (El-Hadjar - Annaba - East Algerian). Influence of vegetation on the dynamics of these metals) Doctoral dissertation. Ph.D. thesis in ecology, Mentouri University of Constantine. 76p.

Küçük, M., Kolaylı, S., Karaoğlu, Ş., Ulusoy, E., Baltacı, C. and Candan, F. (2007). Biological activities and chemical composition of three honeys of different types from Anatolia. Food Chemistry, 100 (2), 526–534.

Kujawski, MW. and Namieśnik, J. (2008). Challenges in preparing honey samples for chromatographic determination of contaminants and trace residues. TrAC Trends in Analytical Chemistry, 27 (9), 785–793.

Lequet, L. (2010). Du nectar a un miel de qualité : contrôle analytique du miel et conseils pratiques a l'intention de l'apiculteur amateur. Thèse pour obtenir le grade de Docteur vétérinaire à l'université Claude-Bernard – Lyon ,195p.

Mahmoudi. R., Ghojoghi, A. and Ghajarbeygi, P. (2016). Honey Safety Hazards and Public Health. Journal of Chemical Health Risks 6(4) (2016) 249–267.

Moujanni, A., Terrab, A., Eddoha,R., Nasser,B., Benbachir,M., Tannaoui,M., Zouaoui,A. and Essamadi, AK. (2017). Quantification of heavy metals and pesticides residues in labeled Moroccan Euphorbia resinifera honey from Tadla-Azilal JMES, 2017, 8 (5), 1826-1836.

Nega, A., Mulugeta, E. and Abebaw, A. (2020). Physicochemical Analysis and Determination of the Levels of Some Heavy Metals in Honey Samples Collected from Three District Area of East Gojjam Zone of Amhara Region, Ethiopia. J AgriSci Food Res; 11: 279. DOI: 10.35248 / 2593-9173.20.11.279.

Oroian, M., Prisacaru, A., Hretcanu, E.C., Stroe S.G., Leahu, A .and Buculei, A. (2016). Heavy Metals Profile in Honey as a Potential Indicator of Botanical and Geographical Origin, International Journal of Food Properties, 19: 8, 1825-1836, DOI: 10.1080 /10942912.2015.1107578.

Pehlivan, T. and Gül, A. (2015). Determination of Heavy Metals Contents of Some Monofloral Honey Produced in Turkey Journal of Applied Pharmaceutical Science Vol. 5 (08), pp.042-045. DOI: 10.7324/JAPS.2015.50807.

Perna, A. M., Grassi, G., Gambacorta, E .and Simonetti, A. (2021). Minerals content in Basilicata region (southern Italy) honeys from areas with different anthropic impact. International Journal of Food Science & Technology 2021**,** *56* (9), 4465-4472. DOI.org/10.1111/ijfs.15112.

Perugini, M., Manera, M., Grotta, L., Cesarina Abete, M., Tarasco, R. and Amorena, M. 2011. Heavy Metal (Hg, Cr, Cd, and Pb) Contamination in urban areas and wildlife reserves: honeybees as bioindicator. Biol Trace Elem Res, 140, 170–176.DOI 10.1007/s12011-010-8688-z.

Porrini ,C., Ghini S., Girotti S., Sabatini A. G., Gattavecchia E. and Celli G. (2002). - Use of honey bees as bioindicators of environmental pollution in Italy. - In: *Honey Bees: Estimating the Environmental Impact of Chemicals*, (DEVILLERS J., PHAM-DELÈGUE M., Eds), Taylor & Francis, London, and New York, 186-247.

Porrini, C., Sabatini, A. G., Girotti, S., Ghini, S., Medrzycki, P., Grillenzoni, F., Bortolotti, L., Gattavecchia, E. and Celli, G. (2003). HONEY Bees and bee products as monitors of the environmental contamination. APIACTA 38 (2003) 63-70.

Saxena, S., Gautam, S. and Sharma, A. (2010). Physical, biochemical, and antioxidant properties of some Indian honeys. Food Chem. 118: 391–7.

Spirić, D., Ciric, J., Teodorovic, V., Nikolic, D., Nikolic, A., Radicevic, T. and Jankovic, S. (2019). Trace elements and heavy metals in multi-floral honey from Serbia IOP Conf. Series: Earth and Environmental Science 333 (2019) 012104.

Tuzen, M., Silici, S., Mendil, D. and Soylak, M. (2007). Trace element levels in honeys from different regions of Turkey. Food Chemistry, 103, 325–330.

Wang, J., and Li, QX. (2011). Chemical composition, characterization, and differentiation of honey botanical and geographical origins. In S. Taylor (Ed.), Advances in food and nutrition research (Vol. 62, pp. 89–137).

Yaiche Achour, H. and Khali, M. (2014). Physicochemical composition of Algerian honeys. Determination of trace elements and potentially toxic elements. Africa Science, 10 (2): 127 - 136.