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Quantification of heavy metals and pesticides residues in labeled Moroccan *Euphorbia resinifera* honey from Tadla-Azilal

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Abstract

For their colony needs, bees harvest honey, honeydew, pollen and water from the environment, which contain natural products and various chemical contaminants. Most of the latters can be found in products consumed by humans and, consequently, may constitute a risk to public health. Honey product could be considered a bio-indicator of environmental pollution to assess the presence of metals, present in soil, and pesticides, used in agricultural practices and in the treatment of varroa in the bee colonies. In this context, this research was conducted on 10 samples of *Euphorbia resinifera* honey collected among beekeepers located in the protected geographical indication "PGI" production of Tadla-Azilal region. The aim of this work is to assess the quality of the honey in question regarding some environmental pollutants such as heavy metals (Pb, Hg and Cd) and pesticides residues (insecticides, fungicides, varroacides and others). The quantification of cadmium was done by atomic absorption spectrophotometry with graphite furnace (GF-AAS), while that of cadmium was performed by hydride generation-atomic absorption spectrophotometry cold steam mode (CV-AAS). Quantification limits (LOQ) of these methods were 0.07mg.kg^{-1} for Pb and 0.015mg.kg^{-1} for Hg and Cd. After extraction by the QuEChERS method, detection, identification and quantification of the 202 pesticide residues were done by liquid chromatography-tandem mass spectrometry (LC-MS.MS), gas chromatography–electron-capture detection (GC-ECD) and gas chromatography flame photometric detector (GC-FPD). In all the cases, analytical quantification limit of was around 0.01mg.kg^{-1}. With the exception of one case were Pb achieved a value of 0.3 mg kg^{-1}, results of this investigation reveal that all the other samples had residue concentrations lower than the pre-established limits for both pesticides and heavy metals. Consequently, we can conclude that the studied honey samples of Euphorbia resinifera are of acceptable or even good quality.

1. Introduction

Of all honey produced in Morocco, *Euphorbia resinifera* honey, produced in Tadla-Azilal area, named "Zaggoum honey" in Moroccan Arab language or «Tikiwt honey» in Amazigh language is probably one of the most characteristic and preferred for consumption and traditional medicine. This monofloral honey is characterized by a low intensity floral odor of phenol and a unique and specific flavor of *Euphorbia* plant. It has a bitter and peppery taste in the throat and its color is dark golden. It is produced in *Euphorbia resinifera* endemic mountain zone. The union beekeepers cooperatives in the Tadla-Azilal region (U.C.A.T.AZ) scored *Euphorbia resinifera* honey in the labeling process and success to asset the sesame of protected geographical indications "PGI" in 2013 [1]. In addition, this label was published in the European Union Official Journal through the public consultation documents on geographical indications of the Kingdom of Morocco [2].
Given the importance of this honey, this work was designed to assess its safety quality by looking for the possible presence of some contaminants of public health concern such as heavy metals and pesticides residues.

Due to their global application, these chemicals constitute a potential risk to human health [3-4]. In addition, cadmium [Cd], lead [Pb], mercury [Hg] and pesticides are known to be toxic to humans if Maximum Residue Limits (MRLs) are exceeded [5-6]. Indeed, the composition of honey is influenced by biotic and abiotic factors around the bee colony, i.e., floral sources, climate conditions, soil, and beekeepers practices [7].
The bees are exposed to numerous pollutants during their foraging activities: (1) their hairy bodies may hold easily environmental contaminants, and (2) they may be contaminated via food resources when gathering pollen and nectar from flowers or through water [8-9-10-11]. In this regard, honey reflects the chemical constituents of the plants from which the bees collect their food, and the content of trace elements can indicate the botanical origin of a particular honey [12-13]. Furthermore, honey, between other beekeeping matrices, is commonly used such as a bio-indicator for assessing the contamination by environment chemicals [14-15-16-17-18-19]. Recent studies indicated bees and honey were useful for monitoring the environmental pollutants but more accurately to detect differences in spatial and temporal environmental contaminations [20-21-22-23].
The present work was conducted to determine a the safety quality of *Euphorbia resinifera* honey through the verification of measurable amounts of heavy metals with major risk (Pb, Cd and Hg) and of 202 pesticides residues (39 Organochlorines "OCPs", 16 Synthetic Pyrethroïds "PYR", 53 Organophosphorus "OPPs", 94 other molecules) (Table 4) in comparison with MRLs adopted for these pollutants.

1. Experimental

1.1.*Area study*

Tadla-Azilal area is characterized by its important floral diversity with a grat number of trees, shrubs and herbaceous plants. *Euphorbia resinifera* is one of the specific wild plants that grows spontaneously in this area. It is encoutred exclusively in El Ksiba and Demnate localities and occupies an area of over 8000 hectars.

1.2. *Honey samples*

Ten (10) samples of honey unifloral *Euphorbia resinifera* were supplied directly from the cooperative U.C.A.T.AZ or collected from some U.C.A.T.AZ beekeepers (Table 1 and Figure 1). After collection, honey samples (500g) were labeled, placed in a cooler kept at 4°C, and immediately transferred to the laboratory where stored at -20°C pending analysis.

Table 1: Description of the studied honey samples

Samples	Location	Harvested season and Year
E1	Ait Mhamed	Summer 2013
E2	Tabaroucht	Summer 2013
E3	Ait Hamza	Summer 2013
E4	Elksibah	Summer 2013
E5	Tilougguite	Summer 2013
E6	Bzou	Summer 2013
E7	Afourer	Summer 2013
E8	Azilal	Summer 2014
E9	Ait Abbass -Ait Massad	Summer 2014
E10	Ait Bououlli	Summer 2014

1.3.*Metals analysis*

Reagents used in this study were HNO3, HCl, Ammonium dihydrogen phosphate, tin chloride and hydrochloric acid and double deionized water (Milli-Q Millipore, 18.2 MΩ/cm resistivity). All these reagents were of analytical grade. Plastic and glassware were cleaned by soaking in HNO_3 (1/9, v/v) and by subsequent rinsing with double deionized water and drying prior to use. Each element was supplied by Perkin Elmer and calibrations were prepared with standard solutions of $1g.L^{-1.}$ Each element of supplied by Perkin Elmer. Stock solution was diluted in HNO_3 (0.2%). Honey samples (0,5g) were digested with 5mL HNO_3 (65% v/v) and 2mL H_2O_2 (30% v/v) with Ethos (Milestone application lab., July, 1997). A blank digest was carried out in the same way. The digestion temperature, in Ethos 1 (Milestone) was 180°C. The program takes place in two steps, first-marks a 10min for ramp time and a 15min for hold time and the second step for cooling in 10min. Digested samples were diluted to a final volume of 50ml with double deionized water. For Hg, after digestion the sample wet, the mercury is reduced to the vapor state by tin chloride ($SnCl_2$) and assayed by atomic absorption spectrometry (Builder cold vapor). Mercury in honey samples were quantified using the spectrophotometer atomic absorption spectra A 220 with a VGA hydride generator 77.

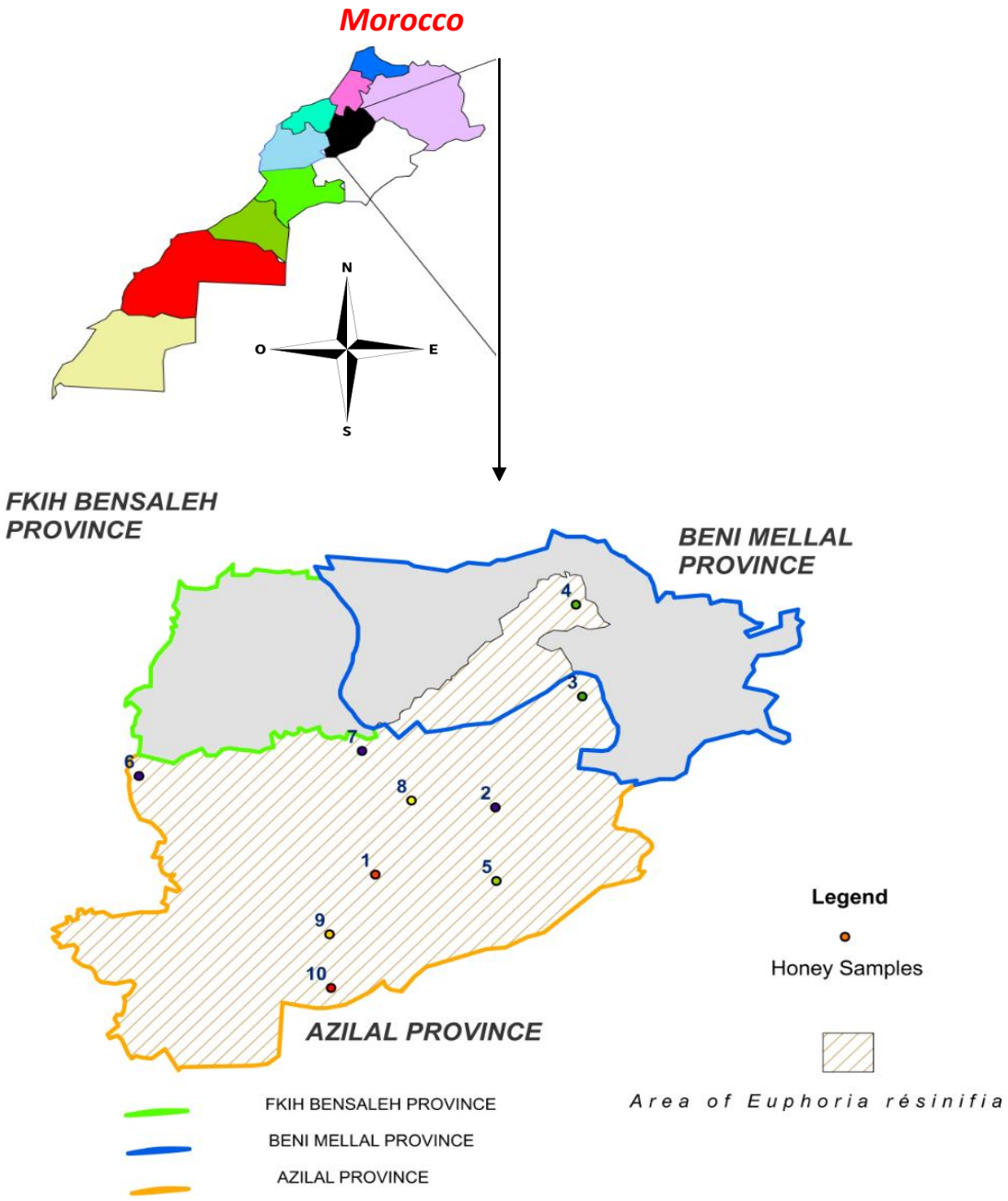

Figure 1: Sampling sites of *Euphorbia resinifera* honeys in Tadla-Azilal

The analysis of Cd and Pb were conducted according to the AOAC method [24]. The analysis was done by the graphite furnace atomic absorption spectroscopy (GF-AAS) using a Shimadzu AA 7000 GFA7000 atomic absorption spectrometer (Analyst 800 Perkin Elmer, USA) equipped with an auto-sampler for graphite furnace measurements. The atomic absorption signal was measured in signal absorption peak area mode against a calibration curve.

Temperatures for drying, mineralization, atomization and cleaning were 60, 120, 250, 700, 2000 and 2500°C, respectively. The spectral band width was 0.7nm. To improve sensitivity, the purge gas, argon, was interrupted during atomization. The identification of the different pollutants in honey samples was made by comparison with their respective authentic standards pattern. The instrumental settings optimizing programs and temperature of the spectrometer and heavy metals analysis are summarized in Table 2.

Table 2: Instrument analytical parameters

Instrument parameters	Cd	Pb	Hg
	GF-AAS		CV-AAS
Lamp position	4	2	3
Lamp current(mA)	4	10	4
Slit width (nm)	0.7L	0.7L	0.5L
Slit height	Normal	Normal	Normal
Wavelength (nm)	228.8	283.3	253.7
Flame	-	-	Air only
Sample introduction	Sampler auto-mixing	Sampler auto-mixing	Manual
Measurement time (sec)	1.0	1.0	-
Replicate	2	2	-
Background correction	On	On	On
Precision	-	-	1.0
Default time (sec)	-	-	75

The recovery values obtained (98.6–102%), by a conventional standard calibration curve, were within the range of recommended AOAC values, with coefficient of variation (CV) ranged from 2.9 to 3.5% [24]. The quantification limits were set at 0.07mg.kg^{-1} for Pb and 0.015mg.kg^{-1} for Cd and Hg. All heavy metal concentrations were determined on a natural weight basis and expressed in milligram per kilogram (mg.kg^{-1}).

1.4 *Pesticides analysis*

The QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) extraction method, chemicals and reagents, determination methods of pesticides and their metabolites and validation parameters was conducted according to NF EN 15662, (2009) [25]. The procedure of Anastassiades M. *et al.* [26] was used for extraction and purification of pesticide residues from honey samples as described below. Each honey sample (5g) was weighed into a 50mL PTFE tube and dissolved in 10mL deionized water and homogenized and then 10mL of Acetonitrile was added by shaking for one minute. The internal standard is added GC (Bromophos) and LC-MSMS (Triphenyl Phosphate "TPP") at 0.1µg.g^{-1}. 6.5g, of saline buffer [MgSO4, NaCl, Trisodium citrate dehydrate, sodium hydrogenocitrate dehydrate (4g /1g/1g/0.5g)], were weighted and added into extraction tube (50mL) and shaken vigorously for one minute. The samples were centrifuged at 3000 rpm for 5 min. For purification, 8mL of aliquot was transferred into 15mL polyethylene tube and stored frozen at minimum 2 hours. Six (6) mL of the upper clear solution (extracts) was transferred into tube containing 150mg primary secondary amine (PSA) sorbent and 900mg anhydrous magnesium sulphate (SPE_d). The tubes were capped, then the extract with the sorbent/dessicant mixed vigorously for one minute and centrifuged at 3000 rpm for 5min. Dilution was carried out following 1/2 with water and homogenized on vortex. The final extract filtered through 0.20-0.45µm Nylon/ PTFE syringe filter (Whatman UK) and transferred to an injection vial and analyzed by LC/MS.MS. Otherwise, the multi-residues analysis by GC-ECD/FPD is performed on the acetonitrile extract under a nitrogen stream. The obtained residue was then dissolved with hexane/acetone (70/30) and 1mL of this mixture was purified on cartridge silica eluted with 5mL of hexane/acetone (70/30). Finally, 1µl of the sample was injected into GC-ECD and GC-FPD. The analytical conditions and confirmation of GC-ECD-FPD are shown in the Table 3.

The purified extract was injected on GC-ECD/GC-FPD and on 2MRM (Multiple Reaction Monitoring) using a screening program with two transitions. For LC-MS/MS, a separation of target analytes was performed using a gradient solvent system consisting of: (A) water/MeOH [95/5] at 5mM Ammonium formate and (B) MeOH at 5mM Ammonium formate. The flow was set at 0.250 mL min^{-1} and the column gradient program consisted of: 20% B ramped linearly to 90% B over 8min, then an hold time of 14min, before returning to 20% B for 15min kept for another 20 min.

The quantifications are performed with respect to the bead breaker range. If it exceeded the highest calibration point, the extract is diluted to integrate the upper bound of the curve. Without exception, quantification is carried out only to the extent that the regression coefficient of the calibration curve is greater 0.995. Otherwise, we repeated the process of quantification. The confirmation of GC results is done by retention time over the interval ± 0.2min.

The recovery values obtained by a conventional standard calibration curve were within the range of recommended values (70–120%), with RSD_s always less than 20% [27].

Table 3: Analytical conditions GC-ECD/FPD

	Analytical conditions		Confirmation conditions
	GC-FPD (OPPs)	**GC-ECD (OCPs and PYR)**	**GC-ECD (OCPs and PYR)**
Apparatus	Hewlett-Packard Model 6890		
Column	HP-5ms tested Ultra 2 Silicon, 25m length×0.32mm id and 0.25-µm film thickness.		HP-35ms, 30m length× 0.32mm id and 0.25µm film thickness.
Carrier gas	Nitrogen U, flow rate 2mL/min in constant flow Mode	Nitrogen U, flow rate 4mL/min in constant flow Mode, 15.83-psi pressure.	Nitrogen U, flow rate 2mL/min in constant flow Mode, 9.25 psi pressure
Injector	Oncolone, programmable temperature with Oven	240°C, Split/Splitless, Split vent : 98.6 mL/min -time: 0.75min	
Oven	60°C (2min)	60°C (2min)	
	25°C/min to 150°C (0min)	20°C/min to 150°C (0min)	
	10°C/min to200°C (5min)	10°C/min to200°C (5min)	
	25°C/min to 280°C (10min)	10°C/min to 260°C (10 min) 35°C/min to 280°C (15min)	
Detector	FPD, temperature 250°C, Gas Nitrogen U, Makeup: 60mL/min, air: 100mL/min, Hydrogen: 75mL/min	µECD, , temperature 310°C, Gas Nitrogen U, Makeup: 60mL/min,	
injected volume	1µL		

Table 4: Pesticides molecules and their metabolites assayed in this study as well as their quantification limits (LOQ)

Pesticides	**LOQ ($mg.kg^{-1}$)**
Organochlorines by GC-ECD: *Alachlore, Aldrine, Binapacryl, Captane, Chinomethionate, Chlordane-Alpha,Chlordane-Gamma, Chlorobenzilate, Chlorothalonil, DOT, (PP-DDD, PP-DDE, OP-DOT, PP-DDT), OP-DDD, OP-DDE, Dichlofluanide, Dichlofopmethyl, Dicofol, Dieldrine, Endosulfan-alpha, Endosulfan-beta, Endosulfan-sulfate, Endrine, HCB, HCH-alpha, HCH-delta, HCH-gamma (Lindane), Heptachlore, lprodione, Methoxychlore, Procymidone, Pyridabene, Quintozene, Tetradition, Tolyfluanide, Triademefon, Trillate, Trifluraline, Vinchlozoline.*	0.01
Organophosphorus by GC-FPD: *Azinphos-éthyl, Azinphos-méthyl, Bromophos éthyl, Bromophos méthyl, Cadusaphos, Chlorfenvinphos, Chlorméphos, Chlorpyriphos-méthyl, Coumaphos, Demeton-S-méthyl, Diazinon, Dichlorvos, Diméthoate, Disulfoton, Edifenphos, Ethion, Ethoprophos, Ethrimphos, Fenamiphos, Fénitrothion, Fenthion, Fonophos, Formothion, Fosthiazate, Heptenophos, Isazophos, Isofenphos, Malathion, Méthamidophos, Méthidathion, Mevinphos, Monochrotophos, Omethoate, Paraoxonéthyl, Parathion-éthyl, Parathion-méthyl, Phenthoate, phorate, Phosalone, Phosalone, Phosmet, Phoxime, pyrimiphos étyl, Pyrimiphos métyl, Profenophos, Prothiophos, Pyrazophos, Pyridafenthion, Quinalphos, Tetrachlorvinphos, Tolclofos méthyl, Thiometon, Vamidothion.*	0.01
Synthetic Pyrethrinoides by GC-ECD: *Alphamethrine, Bifenthrine, Cyfluthrine, Cyhalothrine-Lamda, Cypermethrine, Cypermethrine béta, Cypermethrine zéta, Deltamethrine, Esfenvalerate, Fenpropathrine, Fenvalerate, Fluvalinate, Permethrine, Tetramethrine, Tralomethrine*	0.01
Screening multi-residues by LC-MSMS : *Abamectin, Acétamiprid, Aldicarbe, Azoxystrobine, Benalaxyl, Bendiocarbe, Bifenazate, Boscalid, Bromuconazole, Bupirimate, Buprofezine, Butoxide de piperonyl, Carbaryl, Carbendazine, Carbofuran, Carbosulfan, Chlorpyriphos-éthyl, Clofenterzine, Cycloxidim, Cyproconazole, Cyprodinil, Cypromazine, Desmedipham, Difenoconazole, Diffubenzuron, Dimethomorph, Diniconazole, Epoxiconazole, Ethiofencarbe, Famoxadone, Fenamidone, Fenarimole, Fenazaquine, Fenbuconazole, Fenhexamid, Fenpropymorph, Fluazifop butyl, Flucarbazone, Flufenoxuron, Fluopicolide, Flusilazole, Flutriafol, Hexaconazole, Imazalil, Imidacloprid, Indoxacarbe, Iprovalicarbe, Kresoxim méthyl, Linuron, Lufenuron, Metalaxyl-M, Méthiocarbe, Méthomyl, Methoxyfenozide, Metribuzine, Myclbutanil, Novaluron, Oxadixyl, Oxamyl, Oxycarboxine, Penconazole, Phenmedipham, Pirimicarbe, Prochloraz, Promecarb, Propamocarbe, Propaquizafop, Propargite, Propiconazole, Propoxur, Pymetrozine, Pyraclostrobine, Pyrimethanil, Pyriproxifen, Retenone, Spinosad, Spiroxamine, Spiromesifen, Tebuconazole, Tebufenozide, Tebufenpyrad, Tetraconazole, Thiabendazole, Thiacloprid, Thiametoxam, Thiophanate methyl, Triadimenol, Triazophos, Triflumizole, Triflumuron, Trifloxystrobine, Zoxamide.*	0.01

2. Results and discussion

Residual levels of heavy metals detected in *Euphorbia resinifera* honey samples are given in Table 5. Since MRLs for heavy metals are not established for honey in both Moroccan and European regulations, discussion of our results will be based on Byrne [28] and Bogdanov [29] recommendations [28,29] which limits of 0.1mg.kg^{-1} and 1mg.kg^{-1} for cadmium and lead respectively. In addition, no limit was suggested in the literature for mercury and, thus, the MRL for this compound in this study is the lowest European value set for foodstuff, which is 0.02mg.kg^{-1}. This limit was also the basis for setting of our analytical LOQ, which is equivalent to 0.015mg.kg^{-1}.

Table 5: Residual levels (mg.kg^{-1}) of metal contaminant from monofloral *Euphorbia resinifera* honey samples

Samples	Metal contaminants		
	Pb (mg.kg^{-1})	Cd (mg.kg^{-1})	Hg (mg.kg^{-1})
E1	< *LOQ* [a]	< *LOQ*	< *LOQ*
E2	< *LOQ*	< *LOQ*	< *LOQ*
E3	< *LOQ*	< *LOQ*	< *LOQ*
E4	< *LOQ*	< *LOQ*	< *LOQ*
E5	< *LOQ*	< *LOQ*	< *LOQ*
E6	< *LOQ*	< *LOQ*	< *LOQ*
E7	0.3±0.01	< LOQ	< LOQ
E8	< *LOQ*	< *LOQ*	< *LOQ*
E9	< *LOQ*	< *LOQ*	< *LOQ*
E10	< *LOQ*	< *LOQ*	< *LOQ*

[a] *LOQ for Pb : 0.07mg.kg^{-1} LOQ for Cd and Hg : 0.015mg.kg^{-1}*

Out of the ten analyzed honey samples, a single positive case of 0.3mg.kg^{-1} of Pb was recorded, but this value is less than the maximum limit of 1mg.kg^{-1} suggested by Bogdanov [29]. This contamination could be caused by the use of galvanized or welded metal hardware [32-33]. Similar studies conducted on *Euphorbia resinifera* [34] and on other Moroccan honeys [33-36] revealed the presence of very low residual concentrations of lead.

In other countries, lead detected in honey were from 0.037 to 0.95mg.kg^{-1} in Spain, 0.18 to 0.12mg.kg^{-1} in Turkey, 0.18-0.12mg.kg^{-1} in the Kingdom of Saudi Arabia and from 0.22 to 0.28mg.kg^{-1} in Algeria. The highest concentrations of lead (14, 15.5 and 19mg.kg^{-1}) were found in orange, sesame and clover Egyptian honeys (Table 6).

Concerning cadmium residues, the detected levels from *Euphorbia resinifera* honey are below the limit of quantification (LOQ) of 0.015mg.kg^{-1}, and are also similar to those found by Chakir *et al.*, (2011) [36] in Moroccan honeys. Relatively higher levels of cadmium were reported in honeys from different countries (Table 7).

Finally, every study clearly shows that differences in concentrations of metals in honeys are related to the geographical location of apiaries taken it either to regional [72], national [35-15] or international scales [49].

In terms of pesticides, the simultaneous determination of the different classes (organochlorines, organophosphates, pyrethrins, pyrethroids and other molecules) using QuEChERS extraction method followed by chromatography analysis, showed that the residual concentrations in all honey samples are below the quantification limit (Table 8). Similarly, to our results, Bogdanov reported no measurable residues of insecticides in honey [13]. Additionally, results obtained by Naccari *et al.*, [73] in honey collected from different regions of Sicily showed that residue levels of most pesticides were all under the limit of detection. In our cases, the very low concentrations of pesticides detected in *Euphorbia resinifera* honey are consistent with the fact that the studied area (Tadla-Azilal PGI) is far from areas with intensive agricultural and industrial activities using pesticides and other polluting chemicals. Similar investigations conducted on different types of honey of India and Spain showed the presence of pyrethrins and other pesticides [62-65-66]. Another study on honey from Spain and Portugal revealed the presence of organochlorine residues, such as gamma-HCH, HCB and its isomers α-HCH and β-HCH, with concentrations ranging from 0.03 to 4.31mg kg^{-1}. In the United States (USA), variable residual levels of several pesticide molecules were also detected in honey [71].

In the end, it has to be concluded that pesticides residues detected in *Euphorbia resinifera* honey of the Tadla-Azilal are far below the Moroccan and European MRLs given in Table 8 [74].

Table 6: Levels of lead (Pb) reported in honey samples from different countries

Concentration of "Pb" in honey (mg.Kg^{-1})	Country	Author - Year
0.149[a]	*Germany*	[37]
0.14-1.2	*Nederland*	[38]
0.133[a]	*New Zeeland*	[39]
0.048[a]	*Poland*	[40]
0.34[a]	***Morocco (East)***	[35]
4.2-6.3	*Egypt*	[41]
0.86-1.88	*Egypt*	[42]
0.13-0.95	*Spain*	[43]
0.01-0.11	*Chili*	[32]
0.00717- 0.08578	*China*	[44]
0.06-0.34	*Iran*	[45]
0.15-8.22	*Israel*	[46]
1.017[a]	*Malysia*	[47]
0.008-0.1	*Turkey*	[10]
0.02-1.81	*# Country's*	[48]
0.0764[a]	*Italy*	[49]
0.842[a]	*Romania*	[50]
0.2-1.5	*Central Anatolia*	[51]
0.037[a]	*Spain*	[52]
0.02-15	*Turkey*	[51]
0.081[a]	***Morocco***	[34]
0.21-0.89	*Romania*	[53]
0.04-0.23	*Nigeria*	[54]
4.13-21.59	*Croatia*	[55]
0.118[a]	***Morocco***	[36]
0.98[a]	*Poland*	[56]
0.07-0.23	*KSA*	[57]
<0.9833-1.5348	*USA*	[58]
0.06-0.11	*Germany*	[57]
0.047[a]	*France*	[59]
0.22-0.28	*Algeria*	[60]
<0.07[b]	*Morocco (Middle Atlas)*	*Present study*

[a] *Mean value*
[b] *Over 10 samples, only one sample list a LOQ= 0.3mg.Kg^{-1}, the remaining nine have a LOQ <0.07mg.Kg^{-1}.*

Table 7: Contamination of honey with Cd and Hg from different countries

Concentration in honey (mg.Kg^{-1})			
Cd	***Hg***	***Country***	***Author - Year***
-	0.01-0.5[b]	Egypt	[41]
-	0.01-0.05[b]	Chili	[32]
-	6.10-3-0.018[b]	Turkey	[10]
-	0.09-0.24[b]	Turkey	[51]
1- 4[b]	0.13-0.9[b]	Croatia	[55]
0.08-0.25[b]	-	France	[70]
0.015[a]	-	Morocco	[34]
0.04-0.16[b]	-	KSA	[57]
0.15[a]	-	Malysia	[57]
0.08[a]	-	Australia	[57]
0.01[a]	-	Morocco	[36]
0.7042-1.5854	-	USA	[58]
0.018-0.019[b]	-	Algeria	[60]
0.33-0.53	2.37-3.51[b]	Iran	[45]
<0.015[d]	<0.015[d]	Morocco (Middle Atlas)	Present study

[a] *Mean value -* [b] *Concentrations with value between x- y -* [c] *Average concentrations -* [d] *Limit of quantification (LOQ)*

Table 8: Quantification (LOQ) and Maximum Residues Limits (MRLs) of different classes of pesticides from analyzed honey.

Families of pesticide residues tested	Nbr[e]	LOQ ($mg.kg^{-1}$)	Results [a] ($mg.kg^{-1}$)	MRLs[c][74] ($mg.kg^{-1}$)
Organochlorines (OCPs)	39	0.01	< *LOQ*	0.01[b]
Synthetic Pyrethroids (PYR)	16	0.01	< *LOQ*	0.01[b]
Organophosphorus (OPPs)	53	0.01	< *LOQ*	0.05[b]
Others molecules (OTH)	93	0.01	< *LOQ*	0.05[b]

[a] *Note that no result above the LOQ ($0.01 mg.kg^{-1}$) was detected*
[b] *The lowest EU MRL for foodstuffs according to Directive 2000/42 /EC*
[c] *MRL ($mg.kg^{-1}$) of honey in Moroccan law [74] : Acétamiprid : $0.05 mg.kg^{-1}$ - Boscalid : $0.05 mg.kg^{-1}$-Cycloxidim : $0.02 mg.kg^{-1}$-Cyproconazole : $0.05 mg.kg^{-1}$-Fluazifop-P- butyl : $0.02 mg.kg^{-1}$-Flufenoxuron : $0.05 mg.kg^{-1}$- Imazalil : $0.05 mg.kg^{-1}$-Metalaxyl-M : $0.05 mg.kg^{-1}$-Propaquizafop : $0.05 mg.kg^{-1}$-Tebufenpyrad : $0,05 mg.kg^{-1}$-Tetraconazole : $0.02 mg.kg^{-1}$-Thiametoxan : $0.01 mg.kg^{-1}$-Thiophanate methyl : $1.0 mg.kg^{-1}$*
[e] *Number of molecules and their metabolites tested*

Conclusion

According to the Moroccan and European food safety and food quality standards, results of this study indicate that monofloral *Euphorbia resinifera* honey of Tadla-Azilal region is of good quality with regards to pesticides and heavy metal residues. This also denote that the "PGI" production area of *Euphorbia resinifera* honey is not polluted by these chemicals and confirm the " mountain honey" label of this product which is well preferred by Moroccans for both consumption and medicinal uses.

To maintain this quality, it is essential that public authorities, in cooperation with beekeepers organization, establish and ensure the implementation of the guide to "good beekeeping practices" which describes standardization and rationalization of beekeeping techniques, manufacturing and storage process for the "PGI" *Euphorbia resinifera* honey.

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Conflict of interest - The authors declare that there no conflicts of interests.

References

1. Approval order of Moroccan Ministry of Agriculture and Sea Fishing no.1721-12 of 18 April 2012, Recognizing the PGI "*Euphorbia* Honey of Tadla-Azilal" and homologation of its specifications. *Moroccan Off. Bulletin.* 6074 (2012), 2524.
2. European Union. Geographical indication from the Kingdom of Morocco C232/C. *European Comm. Act*. (2013) 13-16.
3. Pico Y., Font G., Molto J.C., Manes J. In Nollet L.M.L. (ed.) *Food Analysis*. p. 717. HPLC. Dekker, New York. Chapter 17. (1996)
4. Buekers J., Steen Redecker E., Smolders E. Science of the Total Environment, Honey as an indicator of heavy metal contamination. *Sci. Total. Environ.* 407 (2009) 3431-3438.
5. Bratu I., Georgescu C. Chemical contamination of bee honey: Identifying sensor of the environment pollution. *J. Cent. Eur. Agric.* 6. (2005) 467-470.
6. Tuzen M., Soylak M. Trace heavy metal levels in microwave digested honey samples from Middle Anatolia, Turkey. *J. Food Drug Anal*. 1 (2005) 343-347.
7. White J.W., « Composition of honey », ed. crane honey: a comprehensive survey. *Heinemann, London*. (1979) 157-158.
8. Przybylowski P., Wilenzynka A. Honey as an environmental marker. *Food Chem.* 74 (2001) 289-291.
9. Rashed M.N., Soltan M.E. Major and trace elements in different types of Egyptian mono-floral and non-floral bee honeys. *Rev. J. Food Comp. Anal*. 17 (2004)725-735.
10. Tuzen M., Silici S., Mendil D., Soylak M. Trace element levels in honeys from different regions of turkey, *Rev. Food Chem.*103 (2007) 325-330.
11. Balayiannis G., Balayiannis P. Bee Honey as an Environmental Bio-indicator of Pesticides' Occurrence in Six Agricultural Areas of Greece, *Arch. Environ. Contam. Toxicol.* 55(3) (2008) 462. http://dx.doi.org/10.1007/s00244-007-9126-x.

12. Celli G., Maccagnani B. Honeybees as bio-indicators of environmental pollution, *B. Insectol.* 56 (1). (2003)137-139.
13. Bogdanov S. Contaminants of bee products. *Apidologie.* 37(1) (2006) 1-18.
14. Yarsan E., Karacal F., Ibrahim I.G., Dikmen B., Koksal A., Das Y.K. Contents of some metals in honeys from different regions in Turkey., *Bull Environ Contam. Toxicol.* 79 2007) 255-258.
15. Frias I., Rubio C., Gonzáles-Iglesias T., Gutiérrez A.J., Gonzáles-Weller D., Hardisson A. Metals in fresh honeys from Tenerife Island, Spain, *Bull Environ Contam. Toxicol.* 80 (2008) 30-33.
16. Kalbande D.M., Dhadse S.N., Chaudhari P.R., Wate S.R. Biomonitoring of heavy metals by pollen in urban environment. *Environ. Monit. Assess*. 138 (2008) 233-238.
17. Achudume A.C., Nwafor B.N. The ecological assessment of metal in local brands of honey in Southwest Nigeria. *Afr. J. Agric. Res.* 5 (2010) 2608-2610.
18. Van der Steen J.J.M., de Kraker J., Grotenhuis T. Spatial and temporal variation of metal concentrations in adult honeybees (*Apis mellifera* L.) *Environ Monit. Assess*. 184(7) (2012) 4119-26. http://dx.doi.org/10.1007/s10661-011-2248-7.
19. Morgano M.A., Teixeira Martins M.C., Rabonato L.C., Milani R.F., Yotsuyanagi K., Rodriguez-Amaya B. Inorganic contaminants in bee pollen from Southeastern Brazil., *J. Agric. Food Chem.*. 58 (2010) 6876-6883.
20. Sevlimli H., Bayulgen N., Varinioglu H. Determination of trace elements in honey by INAA in Turkey. *J Radioanal Nucl. Chem.* 165 (1992) 319–325.
21. Feller-Demalsy M.J., Vincent B., Beaulieu F. Mineral content and geographical origin of Canadian honeys. *Apidologie.* 20 (1989) 77–91.
22. Jones K.C. *Water Air and Soil Pollut.* 33 (1987) 179-189.
23. Codreanu I., Sfetcu I., Codreanu M., Goran G. Correlation between the lead residues in honeybees and the apiary location. *Lucrări Stiintifice Medicină Veterinară*. XLII (2009) 301-305.
24. AOAC, Association of Analytical Communities. Metals and other elements at trace levels in foods. Official Method Analytical AOAC. *AOAC Bull.* 1 (1990) 237.
25. Norme NF EN 15662, Aliments d'origine végétale - Méthode polyvalente de détermination des résidus des pesticides par CG-SM et SL/SM/SM avec extraction/partition avec de l'acétonitrile et nettoyage par SPE dispersés - Méthode QuEChERS [In French], (2009).
26. Anastassiades M., Lehotay S. J., Stajnbaher D., Schenck F. J. Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and "Dispersive Solid-Phase Extraction" for the Determination of Pesticide Residues in Produce, *J. AOAC Int.*. 86 (2) (2003) 412-431.
27. European Commission. (2010). Guidance document on pesticide residue analytical methods. [SANCO/825/00 rev. 8.1]. [Online] Available in http://ec.europa.eu/food/plant/pesticides/guidance_documents/docs/guide_doc_825-00_rev8_en.pdf. [Accessed November 10, 2016].
28. Byrne D., EC Commission Decision (draft) Amending Annex II to Council directive 92/118/EEC b. (2000).
29. Bogdanov S. Contaminants of bee products. *Apidologie*. 37 (2006) 1-18. http://dx.doi.org/10.1051/apido:2005043
30. European Union. Regulation (EC) No 470/2009 of the European Parliament and of the Council of 6 May 2009. *Off J European Union* 16.6.2009 L 152/11. (2009).
31. Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. *Off J European Union* L 15/220.1.2010. (2010).
32. Fredes C., Montenegro G. Heavy metals and other trace elements contents in Chilean honey, *Rev.Ciencia e Investigacion Agraria.* 33 (2006) 50-58
33. Sodré G.S., Marchini L.C., Orgeda L., Domingues A., Zucchi, Virgilio Franco Nascimento Filho, Ivani Pozar Otsuk, Augusta Carolina de Camargo Carmello Moreti, Determination of chemical elements in africanized *Apis mellifera* (Hymenoptera: Apidae) honey samples from the State of Piauí, Brazil, *Quím. Nova*. 30 (4) (2007) 920-924. http://dx.doi.org/10.1590/S0100-40422007000400030.
34. Naman M., Faid M., El Adlouni C. Microbiological and Physico-Chemical Properties of Moroccan Honey, *Int. J. Agri. Biol.* 7(5) (2005) 473-476.

35. Belouali H., Bouaka M., Hakkou A. Determination of Some Major and Minor Element in the East of Morocco Honeys Through Inductively Coupled Plasma Optical Emission Spectrometry. *J. Apiacta*. 43. (2008) 17-24.
36. Chakir A., Romane A., Barbagianni N., Bartoli D., Ferrazzi P. Major and trace elements in different types of moroccan honeys, *Rev. Australian J. of Basic and App. Scien*ce. 5 (2011) 223-231.
37. Otto K., Jekat F. Experimental investigations on the contamination of a food with residues of lead, zinc, cadmium. *Ernahrungs Umschau*. 24 (1977) 107-109.
38. Kerkvliet J.D. Lead and cadmium contents of honey. *Maandschrift vorr Bijenteelt*. 85 (1983) 251-253.
39. Rowarth Jacqueline S. Lead Concentration in Some New Zealand Honeys, *J. Apic. Res.* 29 (3) (1990) 177-180. http://dx.doi.org/10.1080/00218839.1990.11101216.
40. Przybyłowski P., Wilczyńska A. Honey as an environmental marker. *Food Chem.* 74 (2001) 289-291.
41. Rashed M.N., Soltan M.E. Major and trace elements in different types of Egyptian mono-floral and non-floral bee honeys. *J. Food Comp. Anal.* 17 (2004) 725-735.
42. Taha E., Ali M. Determination of heavy metals content in cotton honey in Kafr Elshiekh Province, Egypt. *J Plant Protec Pathol.* 3. (2012) 1211–9.
43. Rut F-T., Perez-Bernal J.L., Bello-Lopez M.A., Callejon-Mochon M., Jimenez-Sanchez J.C., Guiraum-Perez A. Mineral content and botanical origin of Spanish honeys. *Talanta*. 65 (2005) 686–691.
44. Ru Q-M., Feng Q., He J-Z. Risk assessment of heavy metals in honey consumed in Zhejiang province, southeastern China. *Food Chem Toxicol*. 53 (2013) 256–62.
45. Samimi A., Maymand O.E., Mehrtabatabaei M. Determination of cadmium and arsenic pollution by bee honey based on the study on Ja'far abad area from Saveh city from Iran. *Water Geosci*. (2001) 199–2. [Online] Available from:http://www.wseas.us/e-library/conferences/2010/Cambridge/WHGE/WHGE-32.pdf [Accessed November 09, 2016].
46. Dag A., Afik O., Yeselson Y., Schaffer A., Shafir S. Physical, chemical and palynological characterization of avocado (Persea Americana Mill.) honey in Israel. *Int. J. Food Sci. Technol*. 41 (2006) 387–94.
47. Moniruzzaman M., Chowdhury M.A.Z., Rahman M.A., Sulaiman S.A., Gan S.H. Determination of mineral, trace element, and pesticide levels in honey samples originating from different regions of Malaysia compared to Manuka honey. *Bio. Med. Res. Int*. (2014) 1–10.
48. Bibi, S., Zahoor Husain, S., Naseem Malik, R., Pollen analysis and heavy metals detection in honey samples from seven selected countries. *Pakistan J. Bot.* 40 (2008) 507-516.
49. Pisani A., Protano G., Riccobono F. Minor and trace elements in different honey types produced in Siena County (Italy). *Food Chem.* 72 (2008) 89-95.
50. Tudoreanu L., Crivineanu V., Goran G.V., Codreanu, M.D. Models (nominal logistic regression and anova) for lead accumulation in honey. *Bulletin USAMV-CN*. 65 (2008).
51. Leblebici Z., Aksoy A., Determination of heavy metals in honey samples from Central Anatolia using plasma optical emission spectrophotometry (ICP-OES). *Pol. J. Environ. Stud*. 17 (2008) 549-555.
52. Frias I., Rubio C., Gonzáles-Iglesias T., Gutiérrez A.J., Gonzáles-Weller D., Hardisson A. Metals in fresh honeys from Tenerife island, Spain. *Bull. Environ. Contam. Toxicol.* 80 (2008) 30-33.
53. Codreanu I., Sfetcu I., Codreanu M., Goran G. Investigations concerning the lead residues in apiary products (honey wax). *Lucrări Stiintifice Medicină Veterinară.* XLII (2009) 306-309.
54. Achudume A. C., Nwafor B. N. The ecological assessment of metals in local brands of honey in Southwest Nigeria, *Afr. J. Agric. Res.* 5 (18) (2010) 2608-2610.
55. Bilandžić N., Đokić M., Sedak M., Kolanović B.S., Varenina I., Končurat A., Rudan N. Determination of trace elements in Croatian floral honey originating from different regions. *Food Chem*. 128 (2011) 1160-1164.
56. Roman A., Madras-Majewska B., Popiela-Pleban E. Comparative study of selected toxic elements in propolis and honey. *J. Apic. Res.* 55 (2011) 97-106.
57. Alqarni A.S., Ayman A., Oways A., Awad Mahmoud A., Mohammed A., Hannan A. Mineral Content and Physical Properties of Local and Imported Honeys in Saudi Arabia. *J. Saudi Chem Society*. (2012).
58. Birge W., Price D. Analysis of metals and polychlorinated biphenyl (PCB) residues in honeybees, honey and pollen samples collected from the Paducah gaseous diffusion plant and other areas. Draft report submitted to Jon Maybriar, Division of Waste Management Kentucky Department for Environmental Protection. [Online] Available from: http://www.uky.edu/Research/Superfund/images/pdf/22%20PCB%20Metals%20in%20bees%20honey%20 pollen%20030901%20Report.pdf [Accessed 2016 October].

59. Lambert O., Piroux M., Puyo S., Thorin C., Larhantec M., Delbac F., Pouliquen H. Bees, honey and pollen as sentinels for lead environmental contamination. *Environ. Pollu.* 170 (2012) 254-259.
60. Achour H.Y., Khali M. Composition physicochimique des miels algériens. Détermination des éléments traces et des éléments potentiellement toxiques. [In French]. *Afrique Sci*. 10 (2) (2014) 127-136.
61. Blasco C., Vazquez-Roig P., Onghena M., Masia A., Picó, Y. Analysis of insecticides in honey by liquid chromatography–ion trap-mass spectrometry: Comparison of different extraction procedures. *J. Chromatogr. A.* 1218 (2011) 4892-4901.
62. Buldini P. L., Cavalli S., Mevoli A., Lal Sharma, J. Ion chromatographic and voltammetric determination of heavy and transition metals in honey. *Food Chem.* 73 (2001) 487-495. http://dx.doi.org/10.1016/S0308-8146 (01)00132-7
63. Zhang J., Gao H., Peng B., Li S., Zhou, Z. Comparison of the performance of conventional, temperature-controlled, and ultrasound-assisted ionic liquid dispersive liquid–liquid micro extraction combined with high-performance liquid chromatography in analyzing pyrethroid pesticides in honey samples. *J. Chromatogr.A.* 1218 (2011) 6621-6629. http://dx.doi.org/10.1016/j.chroma.2011.07.102
64. Zhu S. H., Wu, H. L., Li, B. R., Xia, A. L., Han, Q. H., Zhang, Y., Yu, R. Q. Determination of pesticides in honey using excitation–emission matrix fluorescence coupled with second-order calibration and second-order standard addition methods *Anal Chim. Acta.* 619 (2008) 165-172. http://dx.doi.org/10.1016/j.aca.2008.05.005
65. Mukherjee I. Determination of Pesticide Residues in Honey Samples. *Bull. Environ. Contam. Toxicol*. 83 (2009) 818-821. http://dx.doi.org/10.1007/s00128-009-9772-y .
66. Herrera A., Perez-Arquillue C., Conchello P., Bayarri S., Lazaro R., Yagu C., Arino A. Determination of pesticides and PCBs in honey by solid-phase extraction cleanup followed by gas chromatography with electron-capture and nitrogen–phosphorus detection. *Anal Bioanal. Chem*. 381 (2005) 695-701. http://dx.doi.org/10.1007/s00216-004-2924-63 .
67. Blasco C., Fernandez M., Pico Y., Font G. Comparison of solid-phase microextraction and stir bar sorptive extraction for determining six organophosphorus insecticides in honey by liquid chromatography-mass spectrometry. *J. Chromatogr. A.*1030 (2004) 77-85. http://dx.doi.org/10.1016/j.chroma.2003.11.037
68. Blasco C., Fernandez M., Pena A., Lino C., Silveira M. I., Font G., Pico, Y. Assessment of pesticide residues in honey samples from Portugal and Spain. *J Agric. Food Chem.* 51(2003) 8132-8138. http://dx.doi.org/10.1021/jf034870m.
69. Ioannidou M.D., Zachariadis G.A., Anthemidis A.N., Stratis J.A. Direct determination of toxic trace metals in honey and sugars using inductively coupled plasma atomic emission spectrometry, *Talanta*. 65(1) (2005) 92-97.
70. Devillers J., Dore J., Marenco M., Poirier-Duchene F., Galand N., Viel C. Chemometrical analysis of 18 metallic and nonmetallic elements found in honeys sold in France. *J. Agric. Food Chem.* 50 (2002) 5998–6007.
71. Reed Johnson M., Marion Ellis D., Christopher A., Mullin A., Frazier M. Pesticides and honey bee toxicity – USA. *Apidologie*. 41 3 (2010) 312-331.
72. Masiá A., Suarez-Varela M.M., Llopis-Gonzalez A., Picó Y. Pesticides and veterinary drug residues determination in food by liquid chromatography-mass spectrometry: A review. *Anal. Chim. Acta.* (2016). http://dx.doi.org/10.1016/j.aca.2016.07.023
73. Naccari C., Macaluso A., Giuseppe G., Naccari F., Ferrantelli F. Risk Assessment of Heavy Metals and Pesticides in Honey from Sicily (Italy). *J. Food Res*. 3 (2) (2014) 107-117
74. Approval order of Moroccan Ministry of Agriculture, Sea Fishing and Public Health n°156-14 of 17 January 2014. Maximum residue limits permitted for agricultural primary products and foodstuffs. [In French]. *Moroccan Off. Bulletin* n°6322bis. (2015) 238.
75. Bilandzic N., Dokic M., Sedak M., Varenina I., Kolanovic B.S., Koncurat A., Simic B., Rudan N. Content of five trace elements in different honey types from Koprivnica-Kri Evci County. *Slov. Vet. Res.* 49 (2012) 167–173.

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