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► To cite this version:

Guillaume Losfeld, Jean-Baptiste Saunier, Claude Grison. Minor and trace-elements in apiary products from a historical mining district (Les Malines, France). Food Chemistry, 2014, 146, pp.455-459. 10.1016/j.foodchem.2013.08.105 . hal-03172545

HAL Id: hal-03172545

<https://hal.science/hal-03172545>

Submitted on 17 Mar 2021

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Minor and trace-elements in apiary products from a historical mining district (*Les Malines*, France)

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A B S T R A C T

The trace-elements (TE) contents of honey, royal-jelly and beeswax from a historical Zn–Pb mining district have been investigated to assess potential contamination. In spite of high levels of heavy metal (As, Cd, Tl, Pb) in wastes left after mining stopped, apiary products appear to be relatively free of TE contamination. For honey, the following average levels ($\pm$ standard error) were observed: Zn $571 \pm 440 \mu\text{g kg}^{-1}$, Pb $26 \pm 20 \mu\text{g kg}^{-1}$, Tl $13 \pm 10 \mu\text{g kg}^{-1}$, Cd $7 \pm 6 \mu\text{g kg}^{-1}$ and As $3 \pm 4 \mu\text{g.kg}^{-1}$. These results bring additional data to the potential impact of brownfields left after mining on apiary products. They also bring new data to assess potential risks linked with honey consumption and discuss legal TE contents in honey and other food products from apiaries.

Keywords:

Honey

Apiary products

Trace elements

Historical mining

Guide values

1. Introduction

With regards to TE in honey and apiary products, various studies have been undertaken with different aims. Some focus on the use of TE to certify geographical origins (Hernandez, Fraga, Jimenez, Jimenez, & Arias, 2005; Terrab, Gonzalez, Diez, & Heredia, 2003; Terrab, Recamales, Gonzalez-Miret, & Heredia, 2005) or to assess quality (Devillers et al., 2002; Pisani, Protano, & Riccobono, 2008). The use of apiary products for TE biomonitoring has also been extensively investigated (Bilandzic et al., 2011; Lambert et al., 2012; Tuzen, Silici, Mendil, & Soylak, 2007) and reviewed (Bogdanov, 2006; Pohl, Sergiel, & Steck, 2009).

Contradictory conclusions were found with regards to the use of apiary products for TE environmental monitoring. Tuzen et al. (2007) suggested the use of honey, while according to Bogdanov, Haldimann, Luginbuhl, and Gallmann (2007), low TE concentrations and high natural variations should deter from using honey. In the case of Pb recent studies also showed that honey was the least affected matrix from beehives, but could nonetheless be used for environmental monitoring (Lambert, et al., 2012; Mihaly Cozmata, Bretan, Mihaly Cozmata, Nicula, & Peter, 2012). In spite of abundant data from the literature, reference levels for noxious TE in honey are difficult to find (Devillers et al., 2002), and additional data on the potential impacts of a heavily contaminated environ-

ment on honey could improve insights and provide additional material to assess the potential risks. The present investigation of the TE content of apiary products from a historical Zn–Pb mining district (*Les Malines*, southern France) aims to address these topics.

The study area, located in southern France (Fig. 1) displays drastic contrasts in its landscape. It includes a national park (*Parc National des Cévennes*, established 1970) and a Special Area of Conservation (SAC) of the *Natura 2000* network (European Union wide network of nature protection established under the 1992 Habitats Directive), where population density is low (<50 people per km²). Most of the land outside villages is covered by holm oak (*Quercus ilex*) forests and chestnut (*Castanea sativa*) coppices (Gondard, Romane, Grandjanny, Li, & Aronson, 2001). Thus we could expect apiary products from the area to show background TE levels.

Yet the region also has a mining history: *Les Malines*, where an estimated 1 Mt. ore was extracted from 1885 to 1991 remains the largest Zn–Pb mine ever exploited in France (Leguen, Orgeval, & Lancelot, 1991). Former mines were listed from national surveys (BRGM., 2012) and confronted with local knowledge. Locations where soil and apiary products were collected are indicated on Fig. 1.

Previous studies on the *les Malines* mining district reported high TE levels in soil and the occurrence of several metal hyperaccumulator plant species (Escarre et al., 2011; Grison et al., 2010). High TE levels in fish have been reported in a similar context elsewhere in the region (Monna et al., 2011), but to date no studies have been conducted on apiary products in the area. As positive relationships regarding Pb traceability were validated along the soil–melliferous

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Fig. 1. Location of historical mines and sampling sites (sample names in accord with Table 2 and 3).

flora–honeybees–honey chain (Mihaly Cozmuta et al., 2012), such a study was necessary to assess honey quality from a heavily contaminated area.

2. Methods and materials

2.1. General

All reagents used in this study were of high purity grades: commercial HNO_3 , HCl and H_2O_2 were obtained from the Sigma–Aldrich TraceSELECT® range. All solutions were prepared using double deionised water (Milli-Q Millipore 18.2 MΩ cm at 21 °C).

WEPAL IPE 110 and house standards were used as reference materials for apiary products analyses. BIPEA soil sample 402 (inter laboratory certification) was used as a reference material for soil analyses. For each digestion procedure, blank and standard digestions were carried out the same way and analysed for controls. ICP-MS analyses were performed with a Thermo scientific X Series II ICP-MS (Plateforme AETE – Hydrosociences, Montpellier – France) using In and Bi as internal standards.

2.2. Apiary products

Collection of apiary products was performed by beekeepers according to their usual harvest plans. Chestnut honey is collected from June to July. Four apiaries located in Saint-Laurent-le-Minier (*les Avinières*), Saint-Bresson, Aulas and Majencoules were sampled as stated in Table 3. Honey samples were obtained in generic glass jars used by beekeepers for honey retail, so as to assess the ele-

mental content of honey as sold to potential consumers. Each jar contained 500 g honey, which allowed for measurement replicates and quality control using the standard addition method. Royal jelly and beeswax were taken directly from the hives and stored in clean polyethylene jars. An open vessel wet-digestion protocol adapted from Tuzen et al. (2007), proved efficient to recover on average 93% of the TE from apiary products. Samples were warmed to 30 °C and homogenised before 3 g samples were taken for analysis. 12 ml of an oxi-acidic mixture (2:1 HNO_3 : H_2O_2) were added per gram sample and the resulting solution was heated at 100 °C until dry (6 h). Dry residues were solubilised in HNO_3 (2.5% v:v) for analysis.

2.3. Soil

Samples were taken from horizon A (Table 3). S1–S3 are not actually soil but mining waste and tailings; S4, S6 and S7 are a mixture of mineral soil and humus found around beehives (S4, S7) and pastoral areas in Saint-Laurent-le-Minier (S6); S5 and S8 are a mixture of mineral soil and waste expelled from beehives by bees. Soil samples were treated according to a house procedure: dried at 60 °C until the weight was stable, sieved to 2 mm and approximately 120 mg were treated with (i) 4 ml H_2O_2 (ii) 4 ml HNO_3

Table 1
French guide values for soils TE content ('sensitive use' mg kg⁻¹ dry weight).

Element	Zn	As	Cd	Tl	Pb
FIVs	9000	37	20	10	400

Table 2Soil elemental contents (mg kg⁻¹ dry weight).

Sample	Type	Location	Distance from mine ^a (m)	Cr	Mn	Cu	Zn	As	Cd	Tl	Pb
S1	Mine spoils	<i>Les Avinières</i>	0	13	248	11.2	86 706 [*]	4 247 [*]	408 [*]	317 [*]	66 388 [*]
S2	Mine tailings	<i>Les Avinières</i>	0	na	559	981	125 456 [*]	734 [*]	1 605 [*]	37.1 [*]	88 472 [*]
S3	Mine tailings	<i>Les Malines</i>	0	na	1310	929	56 999 [*]	103 [*]	386 [*]	6.45	3 686 [*]
S4	Soil around beehives	<i>Les Avinières</i>	250	37.2	1131	29	3 684	147 [*]	8.79	16.1 [*]	1 234 [*]
S5	Soil under beehives	<i>Les Avinières</i>	250	29.9	745	21.2	2 513	125 [*]	7.47	9.5	858 [*]
S6	Soil	<i>St-Laurent</i>	500	37.3	696	89.1	2 879	75 [*]	8.26	3.6	1 787 [*]
S7	Soil around beehives	<i>St-Bresson</i>	1500	19.4	3 767	30.8	50.8	159 [*]	<dl	4.8	379
S8	Soil under beehives	<i>St-Bresson</i>	1500	12.3	1 842	48.3	202	127 [*]	<dl	2.6	369

<dl = value below detection limit.

^a Distance from the closest mine, which is *les Avinières* for samples collected in *Saint-Laurent-le-Minier* and *Font-Bouillens* for samples collected in *Saint-Bresson*.^{*} Values above FIVs; na = not available.**Table 3**Apiary products elemental contents (mg kg⁻¹ or µg kg⁻¹ fresh weight)

Sample	Location	Distance from mine ^a (m)	Season	Mg	P	Ca	Mn	Zn	As	Cd	Tl	Pb
H1	<i>les Avinières</i>	250	04.2011	80.5	40.7	187	5.81	0.806	1	9	6	3
H2	<i>St-Bresson</i>	1500	05.2011	122	78.4	204	9.17	0.556	1	4	1	35
H3	<i>les Avinières</i>	250	05.2011	125	115	219	6.77	1.4	3	22	37	101
H4	<i>les Avinières</i>	250	06.2011	44.8	65.6	76.7	3.22	0.905	1	3	6	11
H5	<i>les Avinières</i>	250	07.2011	163	66.6	277	10.5	0.613	1	1	12	9
H6	<i>St-Bresson</i>	1500	06.2011	101	80.1	200	7.86	0.429	8	6	13	5
H7	<i>St-Bresson</i>	1500	07.2011	66	82.3	168	10.6	0.429	2	3	19	25
H8	<i>Aulas</i>	4000 ^b	2011	32.8	59.4	36.4	1.38	<dl	<dl	1	12	6
H9	<i>Majencoule</i>	7500 ^b	2011	145	62.8	206	12.8	<dl	1	1	3	14
RJ	<i>les Avinières</i>	250	05.2011	33.8	61.7	123	0.304	0.906	5	7	<dl	168
W	<i>les Avinières</i>	250	05.2011	167	78.8	305	16.1	1.520	12	6	13	<dl
Unit				mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	µg kg ⁻¹	µg kg ⁻¹	µg kg ⁻¹	µg kg ⁻¹

<dl = value below detection limit.

^a Distance from the closest mine, which is *les Avinières* for samples collected in *Saint-Laurent-le-Minier* and *Font-Bouillens* for samples collected in *Saint-Bresson*.^a Distance from the mine located in *Bez-et-Esparron*.^b Distance from the *les Jumeaux* mine in *Sumène*.

(iii) 4 ml HNO₃:HCl (1:3). In each step, the resulting solution was heated at 100 °C until dry. Dry residues obtained after step (iv) were solubilised in HNO₃ (2.5% v:v) for analysis.

3. Results and discussion

3.1. Soil TE content

In a risk-based approach, French authorities issued guideline values for contaminated land management as stated in [Table 1](#). Fixed Impact Values (FIVs), based on toxicity studies, consider the chronic risks to public health related to the actual land use: 'sensitive use' values were selected here due to the occurrence of human activities (housing, beekeeping). These values were defined to be used in a specific scoring framework, but may also provide information on the level of concern for a specific site ([Carlton, 2007](#)).

Measurements of the TE levels in soils are shown in [Table 2](#). S1 and S2 taken from the *les Avinières* mine spoils and tailing ponds show the highest TE content, with values for Zn, As, Cd, Tl and Pb largely above the FIVs. Soil from the *Les Malines* tailing ponds (S3) was very similar, except for its lower Tl content. Our measurements confirm that mining waste from the area should be considered highly contaminated. The other soils were all below the FIVs for Zn and Cd meaning that the Zn or Cd contents of samples S4 through to S8 should not be considered as potential threats to public health. Values for As, on the other hand, all exceed FIVs with an average 3.4-fold FIV for As. In this case, the potential toxicity and high concentrations of As should be taken seriously. Tl and Pb are more contrasted with a few values above the FIVs. However, there is a clear trend of a decrease in Tl and Pb concentrations

([Table 2](#)) as the distance from the mines increased. Such a phenomenon is very likely to be related to dust transport by winds, a phenomenon previously reported for other historical mines ([Sondergaard, Asmund, Johansen, & Elberling, 2010](#)).

One case of Pb early stage poisoning was reported in the area. It highlights the crucial need for further investigation in contaminant linkages. Water and wind erosion may result in widespread TE contamination with potential impacts on human health, activities and livelihoods ([Conesa & Schulin, 2010](#)). Precautionary measures such as restrictions on the use of local wells for water supplies, and on the trade of locally produced vegetables are currently enforced. To our knowledge, the possibility of safe beekeeping in such a context was never considered.

3.2. TE content of honey

Ca, Mg and P with average levels (±standard deviation) at 175 ± 74.1, 97.7 ± 44.9 and 72.4 ± 20.6 mg.kg⁻¹ are the main cations present in honey ([Table 3](#)). Mn and Zn are next, with average levels of 7.57 ± 3.69, and 0.571 ± 0.440 mg.kg⁻¹, respectively, which were consistent with references herein. These elements are known for their physiological role and their presence in honey is of lesser concern.

A higher Zn content of statistical significance (Wilcoxon test, $z = -2.460$, $P = 0.0139$) could be measured in honey samples H1 and H3–5 collected in the vicinity of mine dumps (<250 m) compared to H2, H6–9 collected further away (>1500 m). However all honey from the region (H8 and H9 in particular) showed rather low Zn contents compared to the literature ([Table 4](#)). Floral composition was previously emphasised as a possible factor ([Bogdanov et al., 2007](#)) and the use of galvanised containers was reported as

Table 4
Maximum TE levels previously reported in honey (mg.kg⁻¹ fresh weight)

Element	Max	Source	Element	Max	Source
Mg	373	Pohl et al. (2009)	Cu	34.6	Pohl et al. (2009)
Al	132	Pohl et al. (2009)	Zn	113	Pohl et al. (2009)
P	398	Devillers et al. (2002)	As	1.93	Terrab et al. (2005)
Ca	900	Pohl et al. (2009)	Se	0.09	Terrab et al. (2005)
Cr	4.48	Gonzalez-Paramas et al. (2000)	Cd	5.78	Pohl et al. (2009)
Mn	82	(Stankovska, Stafilov, & Sajn, 2008)	Ba	2.63	Pisani et al. (2008)
Co	2.46	Pohl et al. (2009)	Hg	1.35	Pohl et al. (2009)
Ni	13	Pohl et al. (2009)	Pb	8.22	Pohl et al. (2009)

a major source of Zn (Gonzalez-Paramas et al., 2000): such containers were not used in this case. Thus historical mining appears to have little influence on the Zn content of honey, an observation consistent with those by Bogdanov et al. (2007) or Lambert, et al. (2012).

The presence of other TE could be related to historical mining, namely Pb (mean 23 – min 3 – max 101 µg.kg⁻¹), Tl (mean 12 – min 1 – max 37 µg.kg⁻¹), Cd (mean 6 – min 1 – max 22 µg.kg⁻¹) and As (mean 2 – min 0 – max 8 µg.kg⁻¹). H8 and H9, which reflect background TE levels in honey from the region, actually show the lowest As and Cd levels, but an exception occurs for Tl, with a high Tl level in H8. However, H3 harvested in the vicinity of the *les Avinières* mine dumps, shows the highest Pb (101 µg.kg⁻¹), Tl (37 µg.kg⁻¹) and Cd (22 µg.kg⁻¹) levels, which correlates with our data in soil. However statistical analysis cannot relate TE levels to the presence or absence of historical mines. No significant differences in Pb, Tl, Cd and As levels could be highlighted using distance to mine as an explanatory variable (Wilcoxon test $P > 0.05$). Besides, Pb, Cd and As levels observed here are all below the highest values previously observed in honey (Table 4), and within the concentration ranges previously described.

Our measurements appear to confirm the views expressed by Bogdanov et al. (2007). Apiary products TE content is not directly related to the mineral content of the foraging area, and cannot provide quantitative information on environmental TE levels. As a result, honey production in a historical mining area also appears to be safe.

3.3. TE content of royal jelly and beeswax

Measurements on other apiary products, including royal jelly (RJ) and bees wax (W) were also performed (Table 3). The most abundant elements in royal jelly are those necessary for larvae development: Ca, P, Mg, Zn and Mn, which is consistent with previous studies (Stocker, Schramel, Kettrup, & Bengsch, 2005). Leita, Muhlbachova, Cesco, Barbattini, & Mondini (1996) highlighted higher Cd, Pb and Zn content in royal jelly compared to the corresponding honey, which is not consistent with homeostasis observations by Stocker et al. (2005). In our case, the TE levels were comparable (H3, RJ).

Regarding elements that could be related to historical mining (Zn, Pb, Cd, Tl), concentrations in royal jelly are of the same order of magnitude as those previously reported outside mining districts (Bogdanov, 2006), and once again no impacts of mining could be inferred from statistical analysis of our measurements. The same trend was observed for beeswax, which had a low Pb, Cd and Tl content. The low mineral content of beeswax should be noticed and detract from using it for TE monitoring.

3.4. Potential health hazards resulting from honey consumption

3.4.1. Defining acceptable TE levels in honey

The issue of potential health hazards related to the TE contamination of honey is usually left aside, possibly because of a lack of

Table 5
Maximum and minimum TE levels from regulation EC 1881/2006.

	Cd	Pb
Max	1 mg.kg ⁻¹	1.5 mg.kg ⁻¹
Product	Bivalve molluscs	Bivalve molluscs
Min	50 µg.kg ⁻¹	20 50 µg.kg ⁻¹
Product	Meat	Milk

legislation to define acceptable TE levels in honey and other apiary products (Devillers et al., 2002). It is stated by the *Codex Alimentarius*, that 'honey shall be free from heavy metals in amounts, which may represent a hazard to human health' (Codex Alimentarius., 2001). Yet these hazardous levels are not defined, and in the European Union for example, regulation EC 1881/2006, which set the maximum levels for certain contaminants in foodstuffs does not address apiary products. Nonetheless, levels measured here for Pb and Cd in honey are all below the maximum levels stated by regulation EC 1881/2006 (Table 5).

3.4.2. Bioavailability and consumption trends

Although TE bioavailability from honey is high (Pohl, Stecka, Greda, & Jamroz, 2012) consumption trends are determinant to assess the risks. For our sample with the highest TE content (H3 with 0.101 µg.g⁻¹ Pb and 0.022 µg.g⁻¹ Cd), a 20 g daily honey consumption for an average 65 kg person turns into a weekly intake of 0.218 µg Pb per kg body-weight and a monthly intake of 0.203 µg Cd per kg body-weight. These intakes only represent about 0.9% of the Provisional Tolerable Weekly Intake (PTWI) for Pb and 0.8% of the Provisional Tolerable Monthly Intake (PTMI) for Cd (fixed by the World Health Organisation, namely 25 µg Pb per kg body-weight per week and 25 µg Cd per kg body-weight per month) (Food, 2010). Thus honey appears to be a minor component in TE intakes, a result previously emphasised in other studies (Pohl et al., 2012).

Nonetheless measurements in honey from *les Malines* showed Tl levels sometimes superior to that of Pb or Cd. Tl is more toxic than Pb/Cd and commonly appears in Zn sulphide ores. Thus, it is found at high concentrations in Zn-mine tailings (Peter & Viraraghavan, 2005). Its presence in honey was not previously investigated. Additional data on the occurrence of this element in apiary products and other food products from the mining district could help assess the Tl content related to the presence of mine dumps and potential Tl impacts on health.

4. Conclusion

According to our results, in an environment heavily affected by historical mining, where soils are notoriously contaminated with TE, apiary products remain of good quality with regards to two aspects: (i) the levels of TE measured were consistent with those usually found in uncontaminated areas, as shown from literature datasets (ii) according to WHO guidelines, the consumption of honey appears to be safe: a simple calculation for Pb and Cd taking into

account the usually low honey consumption and its low mineral content, clearly show that it only introduces a very small fraction of these toxic elements into the diet. Consumption of other apiary products such as royal-jelly is very likely to be even lower, representing a very minor part of dietary minerals intakes. This could explain why reference levels for noxious TE in honey have so far not been set.

It also appears from our study, that apiary products should not be used for TE biomonitoring. In the case of historical mining, no clear contaminant linkage appear from mining waste to apiary products. The possible remediation options for historical mines are often subject to much discussion, although potential public exposure and impacts have not always been thoroughly investigated. In the case of *Les Malines*, our measurements showed that beekeeping could be safely performed and should not be restricted. It could maintain livelihoods as chestnut honey from the region is highly regarded for its unique taste. However, the case of other less studied TE, such as Tl, deserves further investigation.

Acknowledgements

The authors gratefully acknowledge the CNRS, the ANR ECOTECH (11ECOT01101), the FEDER and ADEME for financial support. Particular thanks go to the Ecole Polytechnique – Paris Tech for a PhD studentship. We would also like to thank Remi Freydier (Plateforme AETE – Hydrosiences, Montpellier – France) for ICP-MS analyses and Raphaële Leclerc (CEFE UMR-5175 platform of chemical analyses – LabEx CeMEB – Mediterranean Center For Environment and Biodiversity) for guidance in sample treatment. Finally, we would like to thank Mr. Daniel Favas, a beekeeper from Saint-Laurent-le-Minier, France and member of the local honeybees health preservation group (GDSA, Gard) for his active help in supplying samples.

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