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Determination of Heavy Metals and Potential Health Risk Assessment of Honey Harvested from the Tamale Metropolis of Ghana Using Atomic Absorption Spectrophotometer (AAS)

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ABSTRACT

Honey has been used as a bio monitor to determine environmental quality in the environments which are polluted by heavy metals, radioactivity and pesticides. The study sought to determine the levels of some heavy metals (Cd, Cr, Ni, Zn, Cu, Pb, As, and Mn) in honey and assess their potential health risk to consumers. Samples of honey were harvested from within 5km of the Tamale Metropolis of Ghana. A known weight (5g) of honey samples were digested using an oxi-acidic mixture of HNO_3/H_2O_2 and filtered into a 50ml volumetric flask. The contents of the minerals in the digests were analyzed using atomic absorption spectrophotometer (AAS). The analysis revealed that the concentrations of lead (79.815 ± 16.796 mg/Kg), nickel (15.785 ± 10.968 mg/Kg), copper (13.855 ± 10.213 mg/Kg) and manganese (8.215 ± 4.452 mg/Kg) in the samples exceeded the FAO/WHO maximum permissible limits. The elevated levels of these metals may be evidence of possible pollution agents (vehicular and industrial emissions, pollution of farm soils and pockets of water) in the study area. The Health Risk assessment (THQ< 1) showed no significant public health risk to consumers. However, we recommend regular monitoring of heavy metals from the natural environment of Tamale to mitigate possible future threats to public health.

Introduction

Honey is the sweetest product of nature, made by processing the nectar of flowers or plants blight. The chemical composition of honey consists of water, glucose, fructose, sucrose, dextrin, vitamins of group B, C, provitamin A, P, K, pantothenic acid, folic mineral salts of Ca, Na, P, Al, Fe, Si , Mg and some trace minerals (Ciobanu & Rădulescu, 2016).

Honey has a plethora of uses. Because of its sweetness, color and flavor, it is often used as a substitute for sugar. It has been found to be a very energetic food, possessing valuable nourishing, healing and prophylactic properties as a result of its chemical composition and predominant content of simple sugars (Pohl, 2009). Medicinally, it is used in treating coughs, sore throats, ulcers, ear aches, measles and eye diseases. Al-Waili *et al.* (2012) found honey to have potential therapeutic properties in infections, and wound healing hence used in cancer medicines.

Besides the nutrient and medical characteristics of honey, it is used as a bio monitor to determine environmental quality in the environments which are polluted by heavy metals, radioactivity and pesticides (Leblebici & Aksoy, 2008; Sanna *et al.*, 2000).

The main source of heavy metal concentration in honey has been traced to the soil; being transported to honey plants via the root system, passing to the nectar and eventually to the honey produced by the bees (Stankovska *et al.*, 2008). However, there is evidence that the presence of heavy metals in honey can emanate from anthropogenic factors (Bogdanov, 2006). When searching for nectar, honeydew, pollen and plant exudates within such a large territory, bees come into a contact with plants, air, water and soil. Where there is environmental pollution, they also become contaminated and carry the pollutants from surroundings into a hive themselves or in the raw materials collected. Some components of the environmental contaminants for production of honey and other beehive products are collected and brought to the hive from the distance up to 5 km (Ciemniak *et al.*, 2013), changing the honey composition and quality (Rodríguez García *et al.*, 2006; Üren *et al.*, 1998). This result in the accumulation of heavy metals, bacteria, pesticides and radioactive elements in the honey (Al-Waili *et al.*, 2012; Sitarz-Palczak *et al.*, 2015)

Heavy metals originate from two primary sources; natural sources and anthropogenic sources including metalliferous mining and industries, agrochemicals and mineral fertilizers, vehicle exhaust, sewage sludge and industrial wastes (Jiao *et al.*, 2015). According to Aljedani (2017) although the metals poison the bodies of honey bees, the honey bees are still able to travel great distances in the search for food and may not die directly from the poisoning of these metals. The metals, however, accumulate in their bodies helping them to play the role of detecting heavy metals in the environment. The evaluation of heavy metals content in honey has a twofold significance: the former one lies in the toxicity of these metals, with the consequent necessity to develop adequate analytical procedures for their monitoring; the latter one is suggested by the possibility of using bees and their products as bio-indicator of possible environmental their

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products as bio-indicator of possible environmental pollution (Mun˜oz & Palmero, 2005).

Pollution of honey with heavy metals poses risks to consumers especially when the metals reach their toxicity levels. Heavy metals can accumulate in human vital organs especially liver and kidney leading to progressive toxic effects through different mechanisms such as interference with essential metals, oxidative stress, and interaction with cellular macromolecules (Sobhanardakani & Kianpour, 2016). Majority of the metals possess carcinogenic and mutagenic proprieties. Once they are assimilated is very difficult to remove them from the human organism (Tabi, 2015). Heavy metal pollution affects the production and quality of crops, as well as the quality of the atmosphere and water bodies, thereby threatening the health and life of animals and human beings via the food chain (Ru *et al.*, 2013). Some of the heavy metals such as Cadmium and chromium due to their toxicity effects, accumulates in some vital organs especially in the kidney and liver causing human cancer (Sobhanardakani & Kianpour, 2016)

In recent years, the safety and quality of honey products have become an international issue (Wang *et al.*, 2011). Hence for the safe consumption of honey, the presence of heavy metals in honey and the associated health risks need to be evaluated (Ru *et al.*, 2013). Some countries like China, Italy, France, Croatia, Slovenia, Poland and Turkey have determined the levels of different heavy metals in honey and made comparison with the international standards (Aghamirlou *et al.*, 2015). Unfortunately, in Ghana, where Tamale is rapidly advancing in population, industries, farming and vehicles, there is a limited literature on heavy metals level in honey and the ultimate risk it poses to consumers. It is against this backdrop the research is conducted to determine the levels of heavy metals in the honey from Tamale and the risk related to its consumption.

Materials and Methods

Source of honey samples

A total of 20 different samples of wild harvested honey were bought from the honey harvesters in the Tamale Metropolis. The samples were obtained from August to November 2017. The samples were stored in cleaned labeled glass jars and sent to the Ecological Laboratory (ECOLAB) at the University of Ghana, for analysis.

Determination of Heavy Metals in Honey

5g of the honey was weighed using an analytical balance, transferred into a beaker, digested using oxi-acidic mixture of HNO_3/H_2O_2 and filtered into a 50ml volumetric flask. This mixture was heated up to 120℃ for 3hr and brought to a volume of 25ml with de-ionized water. The blank digestions were repeated using the same procedure (AOAC. 1985; Mbiri *et al.*, 2011). Blanks were prepared to check for background contamination by the reagents used. For quality assurance, honey samples were digested thrice along with blanks to minimize error. All reagents were of analytical reagent grade. Double distilled deionized water (Milli-Q Millipore 18.2 MΩ-cm resistivity) was used for all dilutions. The instrument was calibrated with a series of standard solutions supplied by Merck (Germany). Also, all metal content determinations were performed with five replications. The digested honey samples were analyzed for the heavy metals (Cd, As, Pb, Cu, Ni, Mn, Cr and Zn) using atomic absorption spectrophotometer (PinAccle 900T).

Statistical analysis

The statistical analysis was performed using the IBM SPSS 22 software (SPSS Inc, IBM Company, Chicago, III., USA).

The obtained results were first carried out using descriptive statistics. The mean, standard deviation, minimum and maximum levels of the heavy metals in the honey were compared to the FAO/WHO permissible limits. Additionally, to study the correlation of the heavy metal concentration in the honey samples, Pearson's correlation was employed. All statistical analysis was performed at $\boldsymbol{p < 0.05}$

Potential Health Risk Assessment of the honey

The health risk from consumption of honey by the local inhabitants of Tamale Metropolis was assessed based on the THQ. The THQ is a ratio of determined dose of a pollutant to a reference dose level. If the ratio is less than 1, the exposed population is unlikely to experience obvious adverse effects. The methodology for estimating THQ is described in details by the U.S. EPA (US EPA, 2000). The dose calculations were carried out by using the standard assumption from an integrated U.S. EPA risk analysis considering an average adult body weight of 70.0 kg, and 15.0 kg for children (Sobhanardakani & Kianpour, 2016). Furthermore, children are more sensitive to pollutants. There will be a certain amount of discrepancy in health risks between the age groups and the locality of the inhabitants. In this respect, the THQ was determined based on the methods by Chien *et al.* (2002) described by the following equation:

$$THQ = \frac{E_F\ E_D\ F_{IR}\ C}{R_{FD}\ W_{AB}\ T_A} \times 10^{-3}$$

Where E_F is exposure frequency (365 days/year); E_D is the exposure duration (70 years), equivalent to the average lifetime (Sobhanardakani & Kianpour, 2016); F_{IR} is the food ingestion rate (Kg/person/day); The ingestion rate of honey for adults and children is considered to be 0.001Kg per person per day (Akbari *et al.*, 2012); C is the metal concentration in food (mg/Kg); R_{FD} is the oral reference dose (mg/kg/day); W_{AB} is the average body weight (70. 0 kg for adults and 15.0 kg for children), and T_A is the averaging exposure time for non-carcinogens (365 days/year × number of exposure years, assuming 70 years in this study). R_{FD} indicates oral reference dose of metal (which was 0.001, 0.003, 0.02, 0.30, 0.04, 0.004, 0.14 and 0.0003 mg kg^{-1} day^{-1} for Cd, Cr, Ni, Zn, Cu, Pbs, Mn and As respectively).

Results and discussion

The aim of the study was to determine the levels of heavy metals in freshly harvested wild honey and conduct a potential human health risk assessment due to honey consumption in the Tamale metropolis of Ghana.

Table 1. Concentrations of heavy metals in honey from Tamale metropolis ($mgkg^{-1}$)

Heavy metals	Mean ± SD	Range
As	0.665 ± 0.108	0.389 – 0.822
Cr	2.655 ± 4.773	1.800 – 14.800
Cu	13.855 ± 10.213	6.500 – 39.000
Mn	8.215 ± 4.452	0.900 – 15.100
Ni	15.785 ± 10.968	1.200 – 44.100
Pb	79.815 ± 16.796	44.000 – 111.100
Zn	0.615 ± 1.996	4.200 – 8.100
Cd	0.625 ± 1.667	0.400 – 7.300

Source: Authors computation

The table 1 indicates the mean concentration, standard deviation and the range (minimum and maximum) of heavy metals in the honey samples from the Tamale Metropolis. The most abundant heavy metal detected was lead (Pb) with an average of $\boldsymbol{79.815mg/Kg}$ (ranged from $\boldsymbol{44.000 - 111.100mg/Kg}$). The minimum and average level of the lead exceeded the standard level of $\boldsymbol{300\mu g/Kg}$ recommended by FAO/WHO (Aghomirlou *et al.*, 2015).

Naturally, lead is found in the earth's crust and has been associated with illness in children and adult especially cardiovascular related diseases. Acute lead poisoning in humans causes severe damage in the kidneys, liver, brain, reproductive system and central nervous system, and even causes death (Dhahir & Hemed, 2015). Pb residues in food are mostly linked to human activities such as farming, industrial and vehicular emissions and storage places. In this study, lead concentration in the honey samples was probably due to the frequent farming activities and heavy vehicular emissions from the study area.

The least concentrations of manganese (Mn) and Nickel (Ni) were $\mathbf{0.900}mg/Kg$ and $\mathbf{1.200}mg/Kg$ while the highest concentrations $\mathbf{15.100}mg/Kg$ and $\mathbf{44.100}mg/Kg$ respectively. The mean concentrations of Mn ($\mathbf{8.215}mg/kg$) and Ni ($\mathbf{15.785}mg/Kg$) in the honey samples were also above the FAO/WHO (maximum permissible limits of $\mathbf{5.5}mg/Kg$ and $\mathbf{5}mg/Kg$ respectively (Aghamirlou *et al.,* 2015; Ogunkunle *et al.,* 2014). The average value of manganese obtained from the research was in agreement with other reported values of Mn for honey from the Czech Republic ($\mathbf{1.16-8.89}mg/Kg$) and the United Arab Emirates ($< \mathbf{0.01-10.31}mg/Kg$) (Oroian *et al.,* 2016), Chile ($\mathbf{0.01\,3.14}\mu g/Kg$) (Fredes & Montenegro, 2006), Venezuela ($\mathbf{0.4-1.67}\,\mu g/Kg$) and Morocco ($\mathbf{0.080-9.76}\mu g/Kg$) (Belouali *et al.,* 2008). However, the findings exceeded the level of manganese in honey from the Turkey ($\mathbf{0.32-4.56}mg/Kg$) (Oroian *et al.,* 2016). Similarly, the Nickel content in the honey samples was higher than that of Iran ($\mathbf{56.15} \pm \mathbf{54.32}\mu g/Kg$) but lower than those reported in Turkey ($\mathbf{480}\mu g/Kg$) (Sobhanardakani & Kianpour, 2016). Manganese is required by the body for enzyme functioning, nutrient absorption, wound healing bone development. The element manganese deficiency results in poor bone growth, joints pains and related fertility problems. Some other benefits of manganese includes; preventing anemia, alopecia, alleviating premenstrual syndrome, epileptic seizures and antioxidant protection. Mn may also be contained in foods such as, whole grains, beans, nuts, okra and cocoa. Mn is one of the most abundant metals in the soil and normally occurs as oxides and hydroxides. Its effects in the body mostly occur in the respiratory tract and the brains. Some symptoms of manganese poisoning includes; forgetfulness and hallucination. Manganese causes Parkinson disease and impotency in men when exposed for a longer time. The elevated levels of Mn in the honey samples from the study area may be due its presence in the dust through the air, industrial activities, burning fossil fuels and the surface waters.

Nickel helps in the production of the hormone prolactin and the aldosterone, stabilizes DNA and RNA. The presence of nickel in the honey sampled reported in the study area was due to contamination through the air, water and the soil particles (Aghamirlou *et al.,* 2015).

The study also reported the pollution of Cu in the honey samples. It was worth noting that the average accumulation of Cu ($\mathbf{13.355}mg/Kg$) was above the maximum acceptable standards (10 mg/Kg) according to the Polish Standards PN-88/A-77626 (Roman & Popiela, 2011). The levels of Cu in the studied samples were higher than those in Jazan ($\mathbf{0.109}\,mg/g$), Al-Baha ($\mathbf{0.159}\,mg/g$), Asir ($\mathbf{0.268}mg/g$) and Makkah ($\mathbf{0.253}mg/g$) (Aljedani, 2017).

The rest of the heavy metals detected in the honey from the Tamale Metropolis ie Cd, Cr, Zn, Cu and As were below the WHO/FAO permissible standards. The decreasing order of concentration of the heavy metals in the honey from the study area is: Pb$> \boldsymbol{Ni} > \boldsymbol{Cu} > \boldsymbol{Mn} > \boldsymbol{Cr} > \boldsymbol{As} > \boldsymbol{Cd} > \boldsymbol{Zn}$.

Table 2. Pearson's correlation for the heavy metals in honey

	As	Cr	Cu	Mn	Ni	Pb	Zn	Cd
As	1	0.135	0.698	0.489*	0.594	0.235	0.617	0.712
Cr		1	0.438	-0.197	0.302	0.283	0.139	0.113
Cu			1	0.527*	0.568	0.322	0.231	0.353
Mn				1	0.295	0.670	0.093	-0.194
Ni					1	0.137	0.400	-0.398
Pb						1	0.030	-0.170
Zn							1	0.840
Cd								1

*Correlation is significant at the 0.05 level (2-tailed).

A Pearson's correlation was run to assess the relationship between the heavy metals concentration in the honey samples harvested within the Tamale metropolis. The Pearson correlation coefficients and their significance levels ($p < 0.05$) between all these rates are presented in Table 2. There was a moderate positive correlation between the Copper level and Manganese level in the sampled honey, $r(20) = 0.527$, $p < 0.05$, with Copper concentration level explaining about 28 % of the variation in manganese concentration level in the honey. Also, there was a moderate positive correlation between the Arsenic concentration and Manganese concentration in the sampled honey, $r(20) = 0.489$, $p < 0.05$, with Arsenic concentration level explaining about 24 % of the variation in manganese concentration level in the honey.

Table 3. The Health Risk Quotient (THQ) for individual heavy metal due to the honey consumption in the Tamale Metropolis.

Heavy metals	Adults THQ	Children THQ
As	$6.34 \times \mathbf{10^{-4}}$	$2.96 \times \mathbf{10^{-3}}$
Cr	$2.52 \times \mathbf{10^{-4}}$	$1.18 \times \mathbf{10^{-3}}$
Cu	$9.90 \times \mathbf{10^{-4}}$	$4.62\times \mathbf{10^{-3}}$
Mn	$4.69 \times \mathbf{10^{-3}}$	$2.19 \times \mathbf{10^{-2}}$
Ni	$2.26 \times \mathbf{10^{-4}}$	$1.05 \times \mathbf{10^{-3}}$
Pb	$1.63 \times \mathbf{10^{-4}}$	$7.60 \times \mathbf{10^{-4}}$
Zn	$5.86 \times \mathbf{10^{-7}}$	$2.73\times \mathbf{10^{-7}}$
Cd	$1.79 \times \mathbf{10^{-4}}$	$8.33 \times \mathbf{10^{-4}}$
TOTAL	$7.13\times \mathbf{10^{-3}}$	$3.33 \times \mathbf{10^{-2}}$

Source: Authors computation

Finally, all the computed health risk quotient (THQ) values of heavy metals were within the safe limits (THQ< 1) as shown on the Table 3. Also, the total health risk quotient values ranged from 1.79E-07 to 4.69E-03 for adults and from 2.73E- 07 to 2.19E-02 for children. The range for adults, children and their respective total were within the safe limit (THQ < 1) for this study. Therefore, people might have no potential significant health risk by consuming honey from the Tamale Metropolis of Ghana.

Conclusion

Findings from the studies showed that Lead (Pb), Nickel (Ni), Manganese (Mn) and Copper (Cu) reported high concentrations that were virtually above the maximum permissible limits prescribed by FAO/WHO. Their high pollution in the honey was probably due to the proximity of industries, vehicular and motors emissions, use of fertilizers and the frequent cultivation of crops such as corn. Other heavy metals residues such as As, Zn and Cd were very low and within the permissible limits proposed by WHO/FAO.

The health risk quotient value indicated that no possible health risk is associated with the consumption of honey from the Tamale Metropolis during the period of study.

Owing to some heavy metals contaminants in the honey and generally in the natural environment, regular monitoring should be observed to protect the public's health.

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