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Determination of the Presence and Levels of Heavy Metals and Other Elements in Raw and Commercial Edible Bird Nests

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ABSTRACT

Introduction: Heavy metals and other contaminants in food have been a concern to food industries, consumers and governing authorities. The purpose of this study was to determine the levels of heavy metals and other elements in edible bird nests (EBNs). **Methods:** Raw and processed (commercial) EBNs were used in the study. Raw EBNs were collected directly from five house farms in Peninsular Malaysia - Kuala Sanglang (Kedah), Pantai Remis (Perak), Kluang (Johor), Kota Bharu (Kelantan) and Kajang (Selangor). Processed EBNs were purchased from five Chinese traditional medicinal shops located in Peninsular Malaysia. The levels of 32 elements were determined by inductively coupled plasma-mass spectrometry and findings of the study were compared to the maximum regulatory limits set by the Standards and Industrial Research Institute of Malaysia (SIRIM) for EBNs. **Results:** Of the seven elements with maximum regulatory limits (As, Cd, Pb, Hg, Sn, Cu, Fe), one raw EBN was detected with mercury level of 70.180 ppb which was above the SIRIM permissible limit of 50 ppb. All the EBNs had iron levels above the SIRIM permissible limit of 30 ppb. The levels of the other 25 elements with no maximum regulatory limits (Ca, Mg, Na, K, P, Co, Cr, Mn, Mo, Se, Zn, Ag, Ba, Be, Bi, B, Li, Ni, Sb, Sr, Ti, U, V, Al, Zr) were also determined. **Conclusion:** The data obtained for the 25 elements with no permissible limits can serve as baseline data for further studies to establish their maximum regulatory limits.

Key words: Edible bird nests, heavy metal contamination, inductively coupled plasma-mass spectrometry

INTRODUCTION

Edible bird nests (EBNs) are the only nests of birds known to be edible to humans, and are made from the regurgitated saliva of the swiftlets, *Aerodramus* species (Marcone, 2005). EBNs have been popularly consumed for centuries as an expensive delicacy and a tonic believed to have many beneficial health effects (Hobbs, 2004). The *Aerodramus* swiftlets which originally breed in mountain and seaside caves, are commonly found in the Southeast Asian region (Sia, 1982). However, due to high demand for their nests, these swiftlets are now domestically bred in man-made house farms or abandoned shop houses in suburban areas that have been structurally modified to mimic the internal environment of the caves (Sia, 1982). Malaysia is the second largest producer of EBNs in the world, producing up to 277 tonnes of EBNs, worth RM 1.5 billion in the year 2010 alone (Bernama Report, 2011). However, in August 2011, the Malaysian EBN industry suffered a setback when China detected high nitrite levels in some

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of the red EBNs from Malaysia. Some of the nests were found to contain between 200-350 ppm of nitrite, which was way above the permissible level of 34 ppm set by World Health Organisation for all food products. Following this incident, many Standard Operating Procedures and regulations have been implemented and the Malaysia Bird Nest Alliance was established to monitor the quality of the EBN industry in Malaysia. The most recent protocol regarding requirements for exported EBNs has been agreed and established between the Agriculture and Agro-Based Industry Department of Malaysia and the authorities of China in September 2012. The maximum limits for heavy metals such as arsenic, cadmium, lead and mercury, and microbial contents such as bacteria, yeast and mold in EBNs were set by the Standards and Industrial Research Institute of Malaysia (SIRIM) to monitor the standards and quality of EBNs in Malaysia (Department of Standards of Malaysia, 2010; 2011; 2012). These regulations have been implemented and enforced by the Food Safety and Quality Division of the Ministry of Health, Malaysia.

The quality of EBNs has been challenged on several occasions in the past. Common adulterants such as karaya gum, red seaweed and *Tremella* fungus have been added in the production of EBNs in order to increase the net weights or nutritional contents, at a very much-reduced cost price (Marcone, 2005). An isolated case of organic arsenic intoxication after the consumption of EBN soup was reported in Vietnam (Luong & Nguyen, 1999).

The objective of this study was to determine the levels of heavy metals and other elements in both raw and processed EBNs. Certain elements such as cobalt (Co), chromium (Cr), copper (Cu), molybdenum (Mo) and zinc (Zn) are essential micronutrients necessary for human health at very low concentrations, but are toxic at higher concentrations (Alloway, 1995). Studies of heavy metals in the ecosystems have revealed that many areas near urban complexes, metalliferous mines, factories or major road systems contain anomalously high concentrations of these elements. The *Aerodramus* swiftlets are known to be insectivores and feed on insects (Langham, 1980) which in turn, feed on smaller insects or plants. Plants have been known to take up heavy metals from the soil (Tangahu *et al.*, 2011). Traces of heavy metals in the ecosystem could possibly be transferred to the salivary product of the swiftlets, which will then be consumed by humans in the form of EBN soup. Other sources of heavy metal contamination in EBN could be from within the swiftlet house farms such as rusty iron bars, lead-based paints, or mercury tainted water supply. Chemicals and materials used during the processing or manufacturing processes could also be a source of heavy metals to the EBNs. Some heavy metals such as arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg) cannot be biodegraded and are known to have long-term cumulative effects in humans, causing various diseases and disorders even at relatively low concentrations (Pehlivan et al., 2009). Therefore, it is important to study the levels of these elements in EBNs as heavy metals have been known to cause hazard to human health though two routes – inhalation or ingestion (Martin & Griswold, 2009).

The inductively coupled plasma-mass spectrometry (ICP-MS) is the current method recommended by the Food and Drug Authority of United States (FDA) for the analysis of food for the following elements: As, Cd, Cr, Pb and Hg. In this study, ICP-MS was used to determine the levels of heavy metals and other elements in the EBN samples.

METHODS

Collection of raw and processed (commercial) EBNs

The unprocessed (raw and uncleaned) EBNs were purchased from house farms in five different localities in Malaysia: Kuala Sanglang (Perlis; 6° 16′ 0″ North, 100° 12′ 0″

East), Pantai Remis (Perak; 4° 27′ 0″ North, 100° 38′ 0″ East), Kluang (Johor; 02° 01′ 30″ North 103° 19′ 58″ East), Kajang (Selangor; 2° 59′ 0″ North, 101° 47′ 0″ East) and Kota Bharu (Kelantan; 6° 8′ 0″ North, 102° 15′ 0″ East). Processed (commercial) EBNs were purchased from five different Chinese traditional medicine shops (Companies A-E). From each locality/shop, three to six nests were purchased and the nests were sealed in a plastic bag and transported to the laboratory.

Microwave digestion

Each EBN sample was digested using hot concentrated nitric acid and a microwave digester (Milestone START D Microwave Digester, Germany) before being subjected to ICP-MS analysis. A mixture of 0.5 g of EBN sample, 7 mL HNO_3 (65%) and 1 mL $H2O_2$ (30%) was mixed in a large vessel and placed into the carousel of the microwave digester. The SK-10 high-pressure rotor was used, and the microwave digester was programmed to run in three steps at zero pressure : Step 1 - 15 minutes at 200°C, 1200 W; Step 2 - 15 minutes at 200°C, 1200W; and Step 3 - 5 minutes at 26°C with ventilation time of 20 minutes. After the completion of the microwave digestion, the vessels were left to cool down at room temperature for 30 minutes. The vessel caps were then removed and the samples were poured into nitric acid-treated 50 mL tubes respectively. The digested samples were diluted and tested at 1: 5 and 1: 1000 dilutions.

Detection of levels of 32 heavy metals and trace elements using ICP-MS

Before use, on a daily basis, a quality check was performed on the ICP-MS machine (Perkin Elmer, Waltham, Massachusetts, USA) using the 10 ppb Smart Tune solution (Perkin Elmer) containing beryllium, magnesium, cobalt, rhodium, indium, barium, cerium and lead to ensure that the nebulizer argon flow is optimised and the machine is calibrated and has optimal resolution and sensitivity. The replicate standard deviation for 5 replicates of the 8 elements found in the Smart Tune solution should be less than 5%. After the quality check, the ICP-MS was then calibrated using a calibration blank consisting of 1% nitric acid in ultrapure water and calibration standards (based on the analytes to be determined) which were prepared freshly by diluting the stock multi-elements standard solutions in 1% nitric acid at concentrations ranging from 5-200 µg/L. Once calibrated, the ICP-MS was then used to analyse heavy metals and trace elements in the EBNs. The 32 elements measured using ICP-MS were silver (Ag), aluminium (Al), arsenic (As), boron (B), barium (Ba), beryllium (Be), bismuth (Bi), calcium (Ca), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), mercury (Hg), potassium (K), lithium (Li), magnesium (Mg), manganese (Mn), molybdenum (Mo), sodium (Na), nickel (Ni), phosphorus (P), lead (Pb), antimony (Sb), selenium (Se), tin (Sn), strontium (Sr), titanium (Ti), uranium (U), vanadium (V), zinc (Zn) and zirconium (Zr).

Statistical analysis

Statistical analysis was performed using the SPSS Software (PASW 18). Student *t*-test was used to compare the mean levels of elements found in raw and commercial EBNs.

Ethics approval

This study was approved by the IMU-Joint Committee on Research & Ethics.

RESULTS

Elements with permissible regulatory limits

For the 7 elements with maximum regulatory limits set by SIRIM (As, Cd, Pb, Hg, Cu, Fe) and European Commission (Sn), all of the EBN samples had levels below the limits, except for two EBN samples which had mercury and iron levels (Figs. 1d and 1g) higher than the set limits. Arsenic levels in all the EBN samples which ranged from 0.055-34.450 parts per billion (ppb), were lower than the maximum regulatory limit of 1 ppm (Fig. 1a). Higher levels of As were recorded in two raw samples from Kedah, KE1 (11.31 ppb) and KE5 (34.34 ppb) but these nests

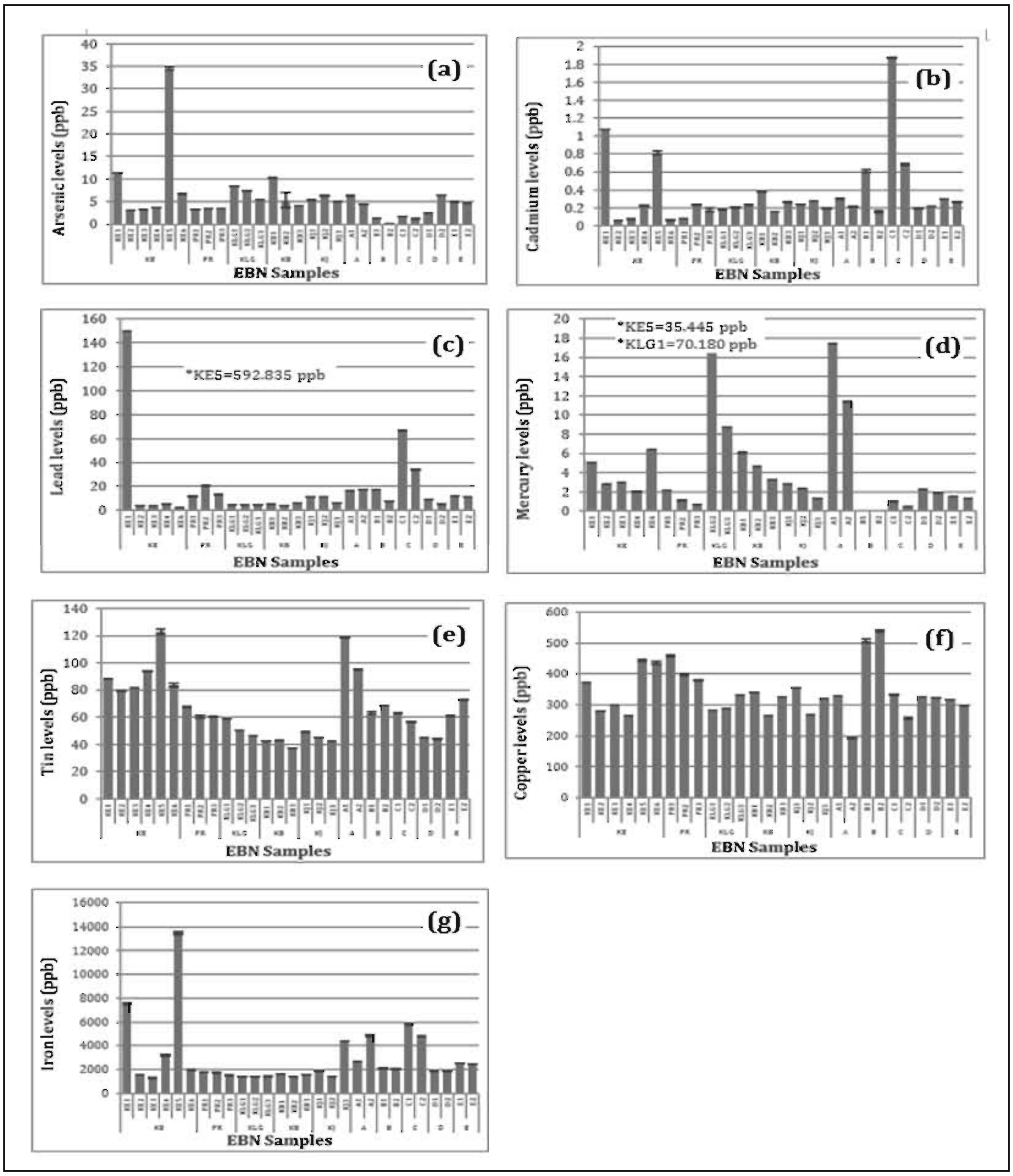

Figure 1. Levels of (a) arsenic, (b) cadmium (c) lead (d) mercury (e) tin (f) copper and (g) iron in the raw and processed EBN samples.
* indicates sample bars removed from graphical presentation due to high values.

had fallen to the floor of the house farms when collected. All the commercial EBNs had less than 10 ppb of As.

Cadmium levels in all the EBNs ranged from 0.060-1.870 ppb and were lower than the maximum regulatory limit of 1 ppm (Fig. 1b). The highest level of cadmium was detected in a commercial EBN sample, C1 at 1.870 ppb while two raw samples, KE1 and KE5 had higher Cd levels compared to the others, at 1.070 ppb and 0.815 ppb respectively.

Lead levels in all the EBNs ranged from 2.235 - 592.835 ppb and were lower than the maximum regulatory limit of 2 ppm (Fig. 1c). The lead level in sample KE5 has been removed from the graphical presentation due to its very high value (592.835 ppb). Raw sample KE1 also had a very high lead level at 149.770 ppb while commercial EBN samples from Company C were found to have higher levels of lead (67.005 ppb in C1 and 34.180 ppb in C2).

The mercury levels in all the EBN samples ranged from 0.060-70.180 ppb (Fig. 1d) and were lower than the 50 ppb SIRIM limit, except for a sample from Kluang (KLG1 with 70.180 ppb). KLG1 was a newly-formed nest with no hatchling, and was harvested directly from a house farm in Kluang. The other two nests with one and two hatchlings respectively (KLG2 and KLG3) which had been obtained from the same location had 17.490 ppb and 8.735 ppb of mercury respectively, levels which were comparatively higher than the levels in raw EBN samples obtained from other regions. The majority of the EBNs had mercury levels of less than 6 ppb. A sample from Kuala Sanglang (KE5) almost exceeded the set 50 ppb limit, with a level of 35.445 ppb of mercury. Interestingly, commercial EBN samples from Company A were found to have higher levels of mercury compared to those from other companies at an average of 14.413 ppb. However, mercury levels in commercial EBN from Company B were so low that only 0.060 ppb was detected in one sample, and levels were non-detectable in the other samples.

The levels of tin found in all the EBNs ranged between 37.145-122.940 ppb (Fig. 1e) which were lower than the regulatory maximum limit for tin set by the European Commission for canned food products at 200.000 ppb. However, higher levels of tin were detected in the raw samples KE4 and KE5 from Kedah, being 93.635 ppb and 122.94 ppb respectively, and in two commercial EBN samples from Company A (118.485 ppb in A1 and 95.225 ppb in A2).

Copper levels in all the EBNs ranged from 191.130 to 538.830 ppb, lower than 1000 ppb maximum limit (Fig. 1f). Interestingly, the highest levels of copper were found to be in the commercial EBNs from Company B, with both samples having 506.855 ppb and 538.830 ppb respectively. Three raw samples from Kedah (KE1, KE5 and KE6), and all the samples from Pantai Remis (PR1, PR2 and PR3) were found to have higher levels of copper compared with other raw nests.

A very interesting finding in this study was that all EBN samples contained iron levels that exceeded the maximum regulatory limit of 30 ppb (Fig 1g). The level of iron in all samples ranged from 1309.725 - 13496.320 ppb. The highest levels of iron among all the samples were in two samples from Kedah, KE5 (13496.320 ppb), followed by KE1 (7538.065 ppb). Among the commercial EBN samples, both EBNs from Company C and one sample from company A (A1) were detected with higher levels of iron at 5790.405, 4795.500 and 4823.090 ppb respectively.

Macro- and micro-minerals

Figures 2.1 and 2.2 show the range of values for a total of 11 micro- and macro-minerals. The range of values for calcium (Fig. 2.1a) and magnesium (Fig. 2.1b) were found to be between 116756.0-789562.0 ppb and 46526.0-153699.0 ppb respectively. The highest levels of calcium were detected in commercial samples from Company E, while the highest level of magnesium was detected from one raw sample from Kota Bharu (KB1). Overall, the raw nests obtained from Kedah and Perak had lower calcium levels than those obtained from Kluang, Kota Bharu and Kajang. The calcium levels in all the commercial nests were within the range of 300000-600000 ppb except for those of Companies B and E. The average level for magnesium was

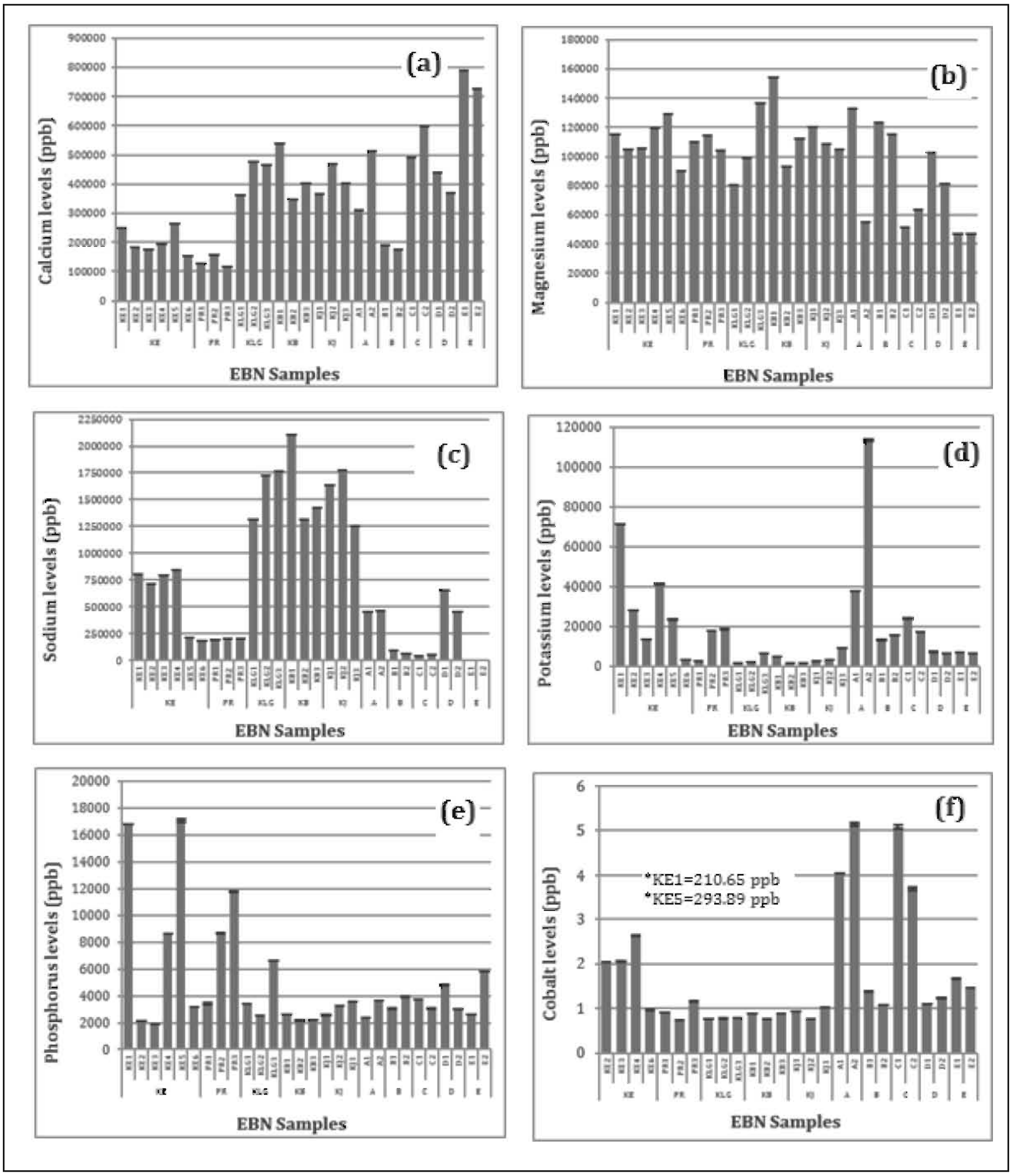

Figure 2.1. Levels of macro- and micro-minerals: (a) calcium, (b) magnesium, (c) sodium (d) potassium (e) phosphorus (f) cobalt in raw and processed EBN samples.

* Indicates sample bars removed from graphical presentation due to high values in raw and commercial EBN samples.

higher in raw EBNs (111208 ppb) compared to commercial EBNs (81800 ppb).

For sodium (Fig. 2.1c), the range of values detected were between 42393.0-2105375.0 ppb. Similar to calcium, high sodium levels were seen in raw EBN samples from Kluang, Kota Bharu and Kajang. The sodium levels in the raw EBNs obtained from Kedah (KE5 and 6) and Pantai Remis (PR1-3) were below

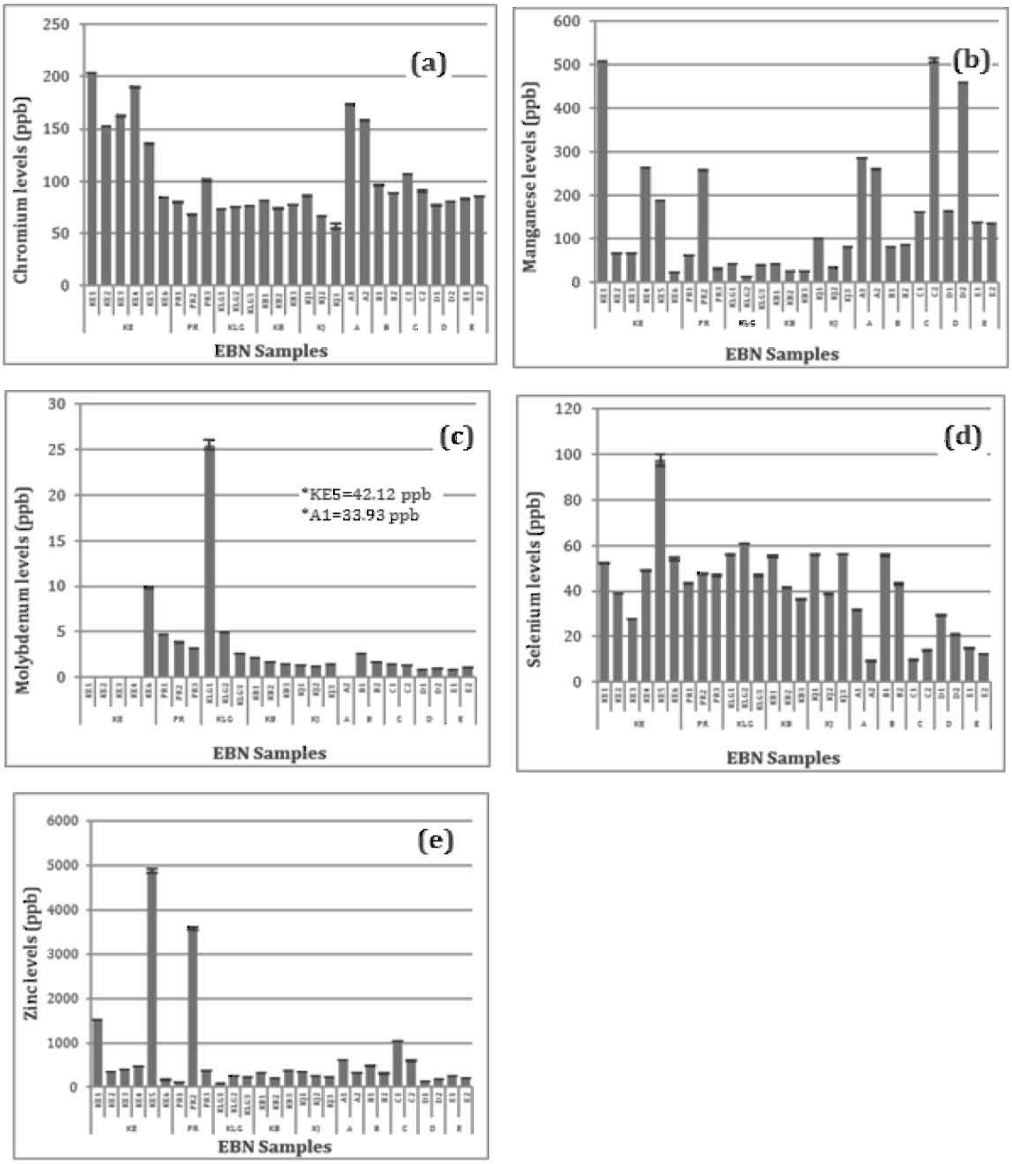

Figure 2.2. Levels of micro-minerals: (a) chromium (b) manganese (c) molybdenum, (d) selenium and (e) zinc in raw and processed EBN samples.
* Indicates sample bars removed from graphical presentation due to high values.

250000 ppb. However, low (< 500000 ppb) or undetectable levels (Company E) of sodium were detected in all the commercial EBN samples except for Company D (D1).

The levels of potassium (Fig. 2.1d) ranged between 1288.01-113285.54 ppb. The highest level of potassium was seen in a commercial sample from Company A, followed by two raw samples from Kuala Sanglang (KE1 and 4). The levels of potassium were lower than 20000 ppb for the majority of the raw and commercial nests.

The levels of phosphorus (Fig. 2.1e) in the EBN samples were between 1858.65 and 17091.13 ppb; the highest level was in sample KE5. Overall, the majority of the raw and commercial EBNs had less than 4000 ppb phosphorous level.

The range of values for cobalt (Fig. 2.1f) was very wide, between 0.74-293.89 ppb. The highest level of cobalt was 293.89 ppb (in KE5), while the lowest was from Perak (PR2) with only 0.74 ppb of cobalt. Twenty-six out of 28 EBN samples contained cobalt levels of less than 6 ppb. Only two samples from Kedah were detected with levels relatively much higher than the rest - samples KE1 (210.65 ppb) and KE5 (293.89 ppb).

The range for chromium (Fig. 2.2a) was 56.48-203.33 ppb, where relatively high chromium levels were detected in EBN samples from Kedah and commercial EBN samples from Company A. Manganese (Fig. 2.2b) was detected in EBN samples at a range of 11.57-508.47 ppb with three samples from Kedah (KE1, KE4, KE5), one sample from Perak (PR2), both samples from Company A, and one sample each from Companies C and D showing relatively higher levels.

Molybdenum (Fig. 2.2c) was found to be below detectable levels for samples obtained from Kedah (KE1-4) and one EBN sample from Company A. Higher values were detected in the raw sample KE5 (42.12 ppb) and commercial sample A1 (33.93 ppb). Both raw and commercial EBNs were found to have selenium levels between 1.985-97.5 ppb (Fig. 2.2d). The raw sample KE5 was found to have the highest level of selenium. The average level of selenium in raw EBNs (47.45 ppb) was found to be higher than the commercial EBNs (24.07 ppb).

Zinc (Fig. 2.2e) was detected in EBN samples at a range of 92.085 - 4869.795 ppb, with the highest levels in EBN samples from Kedah (KE5, 4869.795 ppb) and Perak (PR2, 3578.01 ppb). The other samples showed zinc levels of less than 2000 ppb.

There was no specific trend for the levels of macro-minerals in both raw and commercial EBNs, as each individual sample showed different mineral profiles.

Other 14 elements

The levels of the other 14 elements (Ag, Ba, Be, Bi, B, Li, Ni, Sb, Sr, Ti, U, V, Al, Zr) in the EBNs are presented in Figs. 3.1a-f and 3.2a-h. Very low amounts (< 1 ppb) of beryllium (Fig. 3.1c), lithium (Fig. 3.1f), antimony (Fig. 3.32b), uranium (Fig. 3.2e) and vanadium (Fig. 3.2f) were detected in all EBN samples. The range of values for silver (Fig. 3.1a), bismuth (Fig. 3.1d), boron (Fig. 3.1e), strontium (Fig. 3.2c) and titanium (Fig. 3.2d) were found to be between 0-50 ppb in all EBN samples.

Barium levels were found to be distinctively higher in all the commercial EBNs compared to raw EBNs (Fig. 3.1b), with average levels of 159.54 ppb and 22.26 ppb respectively. For beryllium levels, an EBN sample from Kedah (KE5) had the highest amount among all the EBN samples (Fig. 3.1c).

Levels of bismuth in all EBNs were lower than 15 ppb, except for sample A1 from company A (35.67 ppb) (Fig. 3.1d). Boron levels (Fig. 3.1e) were found to be less than 5 ppb in all EBN samples with the highest levels found in EBN samples from Kedah (KE5, 242.58 ppb) and Perak (PR2, 178.215 ppb). Lithium was found to be higher in all the commercial samples than the raw EBNs, except for two samples from Kedah (KE1, KE5) and one sample from KB (KB1) (Fig. 3.1f).

Aluminium levels were generally relatively higher in the commercial EBNs compared to the raw EBNs, with an average of 11616.921 ppb (range: 434.680 ppb – 39055.980 ppb) in commercial EBNs, and 912.971ppb (range: 230.875 ppb – 6437.310 ppb) in raw EBNs (Fig. 3.2g).

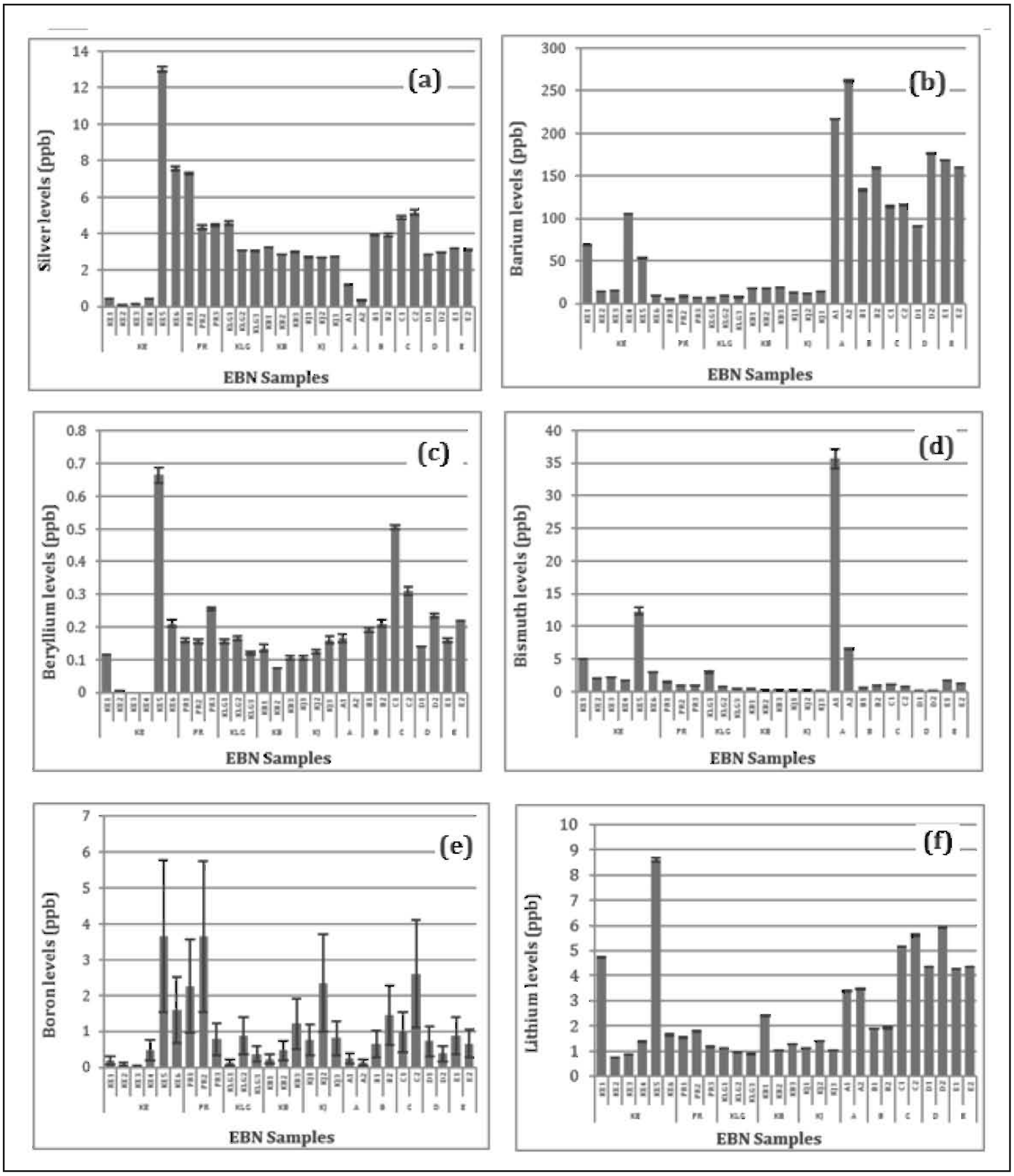

Figure 3.1. Levels of 6 elements tested in this study: (a) silver, (b) barium, (c) beryllium, (d) bismuth, (e) boron and (f) lithium in raw and processed EBN samples.

Nickel was detected in all the EBN samples, with mean levels of 56.14 ± 70.16 ppb (Fig. 3.2a) while antimony levels in all EBNs were found to be below 10 ppb, with relatively higher levels seen in two samples from Kedah (KE4 and 5) and both

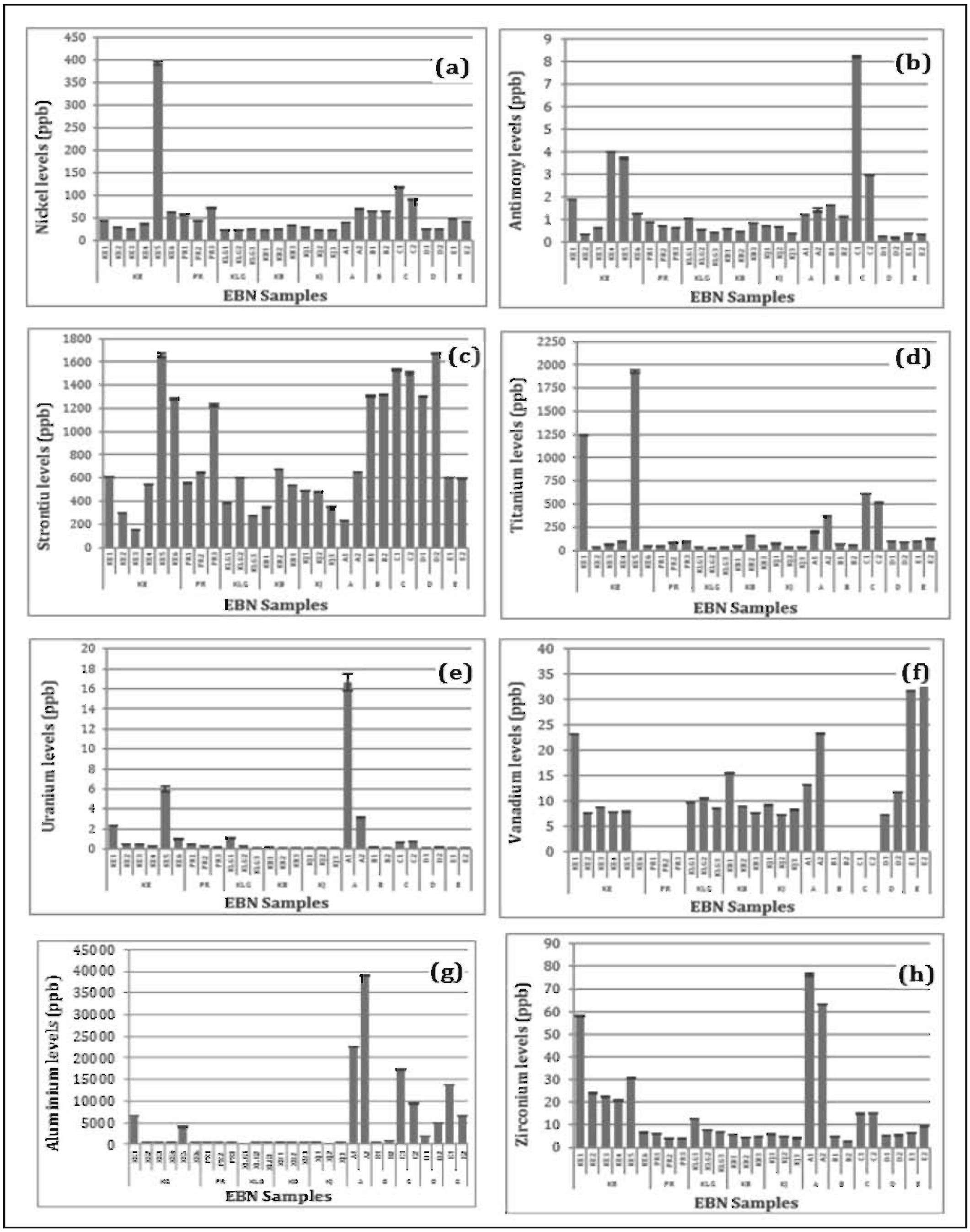

Figure 3.2. Levels of 8 other elements tested in this study: (a) nickel, (b) antimony, (c) strontium, (d) titanium, (e) uranium, (f) vanadium, (g) aluminium and (h) zirconium in raw and processed EBN samples.

EBNs from Company C (Fig. 3.2b). The majority of EBN samples were found to have > 200 ppb of strontium, the highest level being 1671.435 ppb, detected in EBN from Company D2, followed by 1657.095 ppb, detected in an EBN from Kedah (KE5) (Fig. 3.2c). Less than 500 ppb of titanium were detected in all EBN samples, except for two samples from Kedah (KE1 and 5) at 1239.7 ppb and 1927.5 ppb respectively (Fig. 3.2d).

Vanadium was also not detected in 8 EBN samples from Perak, Company B and Company C, and one sample from Kedah (Fig. 3.2f). The mean level of zirconium detected in all the EBN samples was 15.64 ± 19.25 ppb (Fig. 3.2h).

In summary, calcium, magnesium and sodium levels ranged between 4,000 to 1,026,000 ppb while beryllium, cadmium, antimony and uranium levels were less than 2.5 ppb. The average levels of silver, arsenic, mercury, molybdenum, phosphorus, zinc, boron, cobalt, lead, selenium, titanium, magnesium and sodium were higher in the raw EBN samples, compared to the commercial EBN samples, while all the other elements were found to be higher in the commercial EBN samples compared to the raw EBN samples.

DISCUSSION

For the seven elements with maximum regulatory limits (As, Cd, Pb, Hg, Sn, Cu, Fe), all EBN samples had levels that did not exceed the limits set by SIRIM, except for mercury and iron. Only one EBN sample from Kluang (KLG1) was found to have a mercury level of 70.18 ppb, which was higher than the set limit of 50 ppb. This EBN sample was a newly-formed nest with no prior hatching. The other two nests from Kluang (KLG2 and KLG3) were also found to contain higher levels of mercury compared to other raw EBNs. Since all the nests from Kluang showed relatively higher levels of mercury, this contamination by mercury could possibly be explained by the influence of the location. The swiftlet house-farm in Kluang where these nests were harvested from was located in an industrial area, with many factories located nearby. However, the types of industries and whether there was emission of volatile mercury vapour from these factories were not studied.

It has been reported that industrial emission of mercury contributed to the total mercury in the atmosphere with solid waste disposal through incineration processes as the dominant source in North America (~ 40%), Central and South America (~34%), western Europe (~28%) and Africa (~30%), while coal combustion was the dominant source in Asia (~42%) and eastern Europe and Russia (~40%). Mining and smelting of zinc and lead represented the major industrial source of Hg in Oceania (~35%) (Pirrone, Keeler & Nriagu, 1996). Mercury has been reported to be strongly retained by soil components, especially within the nearest distance from the industrial emission site (Inácio, Pereira & Pinto, 1998).

Mercury in the environment has been shown to accumulate in both marine and terrestrial food chains, where the methylation of inorganic mercury to organic mercury has been proven (Linquist & Rodhe, 1985; Gnamuš, Byrne & Horvat, 2000). The nymphs of the burrowing mayfly *Hexagenia rigida* (Ephemeroptera) (Saouter *et al.*, 1993) have been found to accumulate mercury and they are known to be one of the largest prey items of the *Aerodramus* sp. swiftlets. Ingestion of mercury-contaminated food has been reported as the source of mercury in feathers of birds. Raw EBNs were found to have higher mercury levels in this study and traces of mercury-contaminated feathers in the EBNs could be the most likely cause for the high mercury levels found in the raw EBN samples from Kluang.

The different levels of mercury detected in all the EBNs in this study could possibly be explained by the regional differences where these EBNs are harvested. The house-farms of other locations might be located further away from industrial plants, or in areas with less mercury contamination in the environment, causing a variable level of mercury deposition in the feathers of the swiftlets. Apart from swiftlet feathers, the indoor environments of individual house-nests where breeding and formation of nests takes place could have been the source of mercury contamination in EBNs such as interior latex paints (Beuterian *et al.*, 1991). It is not known whether the interior of the house-farms from which the EBN samples were collected were painted with these latex paints. There are no regulations set by SIRIM Malaysia regarding painting of the indoor walls of the swiftlet house-farms (Department of Standards Malaysia, 2012).

In this study, mercury was also detected in all but one commercial EBN samples. As commercial samples have undergone a process of cleaning and the removal of feathers and dirt, a more likely cause of mercury contamination would be the incorporation of traces of mercury into the components of the EBN itself, rather than bird feathers, which could not be removed by processing.

Low levels of mercury exposure would result in impaired development of motor and language skills during neonatal life and early childhood, but larger exposure can produce severe cognitive effects, including paresthesia, blindness, deafness, and with more severe exposure, fetal death and abortion (Goyer & Golub, 2003). The mercury levels detected in the EBN samples in this study do raise some concerns due to the fact that many pregnant women eat EBNs in large amounts as a tonic throughout their pregnancy, as EBN is believed to enhance nourishment to their bodies and to the foetus. The provisional tolerable weekly intake (PTWI) for total dietary exposure mercury, set at the 72th meeting of the Joint FAO/WHO Expert Committee on Food Additives in 2010, was 4 μg per kilogramme body weight per week (μg/kg bw/wk) (Joint FAO/WHO Expert Committee on Food Additives, 2010) equivalent to 4 ppb/kg bw/week. Consumption of four pieces of the EBNs of KLG2 (mercury level of 70.18 ppb) within a week would exceed the PTWI. This should serve as a red-flag to consumers as some may consume more than four pieces of EBNs per week.

All the EBN samples studied were found to have iron levels that exceeded the maximum regulatory limit set by SIRIM (30 ppb) (Department of Standards Malaysia, 2011). This may be due to the natural endogenous presence of iron in EBNs, affected by the dietary habits of the swiftlet and its regurgitation into the salivary products to form nests; or exogenous contamination of EBNs with iron, such as iron bars or rusty iron equipment found in the swiftlet breeding sites, leading to the incorporation of iron into the nests. The average level of iron detected in the raw EBN samples in this study (2810.819 + 3079 ppb) was found to be lower than the levels detected in a previous study conducted on the EBN in Peninsular Malaysia, which reported 1.2 + 0.1 mg/100 kg (equivalent to 12 000 + 1000 ppb) of iron in raw EBNs (Norhayati, Azman & Wan Nazaimoon, 2010). Only one of the raw EBN samples in this study corresponded with the average value - KE5, with iron levels of 13496 ppb. The average iron level detected in the commercial EBNs in this study (3092.893 + 146.2 ppb) was also found to be much lower compared to the levels detected in a previous study, which reported an average of 2.0 + 0.475 mg/100g (equivalent to 20000 + 4750 ppb) of iron in EBNs from Peninsular Malaysia (Norhayati *et al.*, 2010). The differences in the levels of iron could be due to seasonal variations and locations from which the nests were harvested. Different house-farms would have different internal environments, causing different levels of exposures of the swiftlets and nests to iron contamination. Iron is known to be an essential trace element in human health, required for red blood cell production, iron-dependent enzymes, cellular immune function and thermoregulation. In fact, the deliberate fortification of food with iron and the encouragement of dietary iron supplementation have been done to address issues of iron-deficiency in developing countries (Hurrell, 1997). The level of iron in the body is well-regulated by physiologic mechanisms. Thus, iron toxicity and overload in humans are rare, and have only been reported in patients with inherited and acquired iron metabolism disorders, or intravenous injection of large doses of iron (Herbert, 1987). However, these iron levels pose a very low possibility of toxicity when compared to the tolerable upper limits.

The levels of the 5 macro-minerals (Ca, Mg, Na, K, P) in this study were lower compared to findings from two previous studies (Marcone, 2005; Norhayati *et al.*, 2010) for both raw and commercial EBNs, except for the level of sodium in raw EBNs, whereby all the three studies detected values within a similar range. This could be due to the difference in sample size, regional and seasonal variations affecting the diet of the swiftlets, and different elemental analysis methods used. Both of the previous studies used Atomic Absorption Spectrometry (AAS) for the elemental analysis of EBNs, while ICP-MS was used in this study. The sensitivity, detection limit and precision of the ICP-MS and AAS may vary, and the sample collection and digestion methods may differ. The differences in levels of minerals in commercial EBNs can be explained by the differing amounts and ratios of minerals in the adulterants possibly incorporated during the processing of the EBNs such as karaya gum, red seaweed and *Tremella* fungus, rendering each adulterated EBN to have a differing mineral profile (Marcone, 2005).

From the elemental analysis results obtained in this study, it was found that the average levels of Ca, Mg, Na and P were higher in the raw EBNs compared to the commercial EBNs, possibly due to the natural presence of these minerals in EBNs that have been reduced after going through the cleaning processes prior to commercialisation. On the contrary, the levels of potassium were found to be higher in the commercial EBNs compared to the raw EBNs, most likely due to the introduction of potassium during the manufacturing process, or from the adulterants. However, the findings indicate that EBNs are not rich in macro-minerals such as calcium, sodium, magnesium, potassium and phosphorus. Therefore, the data obtained from this study could perhaps serve as a baseline for future studies on the individual or specific elements in the raw and commercial EBNs.

CONCLUSION

The levels of 32 elements were determined in raw and commercial farmed edible nests. Of the seven elements with maximum regulatory limits (As, Cd, Pb, Hg, Sn, Cu, Fe), mercury level above the permissible limit was detected in one raw EBN sample. All the EBN samples contained iron levels that exceeded the maximum regulatory limit set by SIRIM. For the other 25 elements (Ca, Mg, Na, K, P, Co, Cr, Mn, Mo, Se, Zn, Ag, Ba, Be, Bi, B, Li, Ni, Sb, Sr, Ti, U, V, Al, Zr) with no maximum regulatory limits, data obtained can serve as baseline data for further studies.

Conflict of interest

The authors declared that there is no conflict of interest with respect to research, authorship and publication of this manuscript.

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