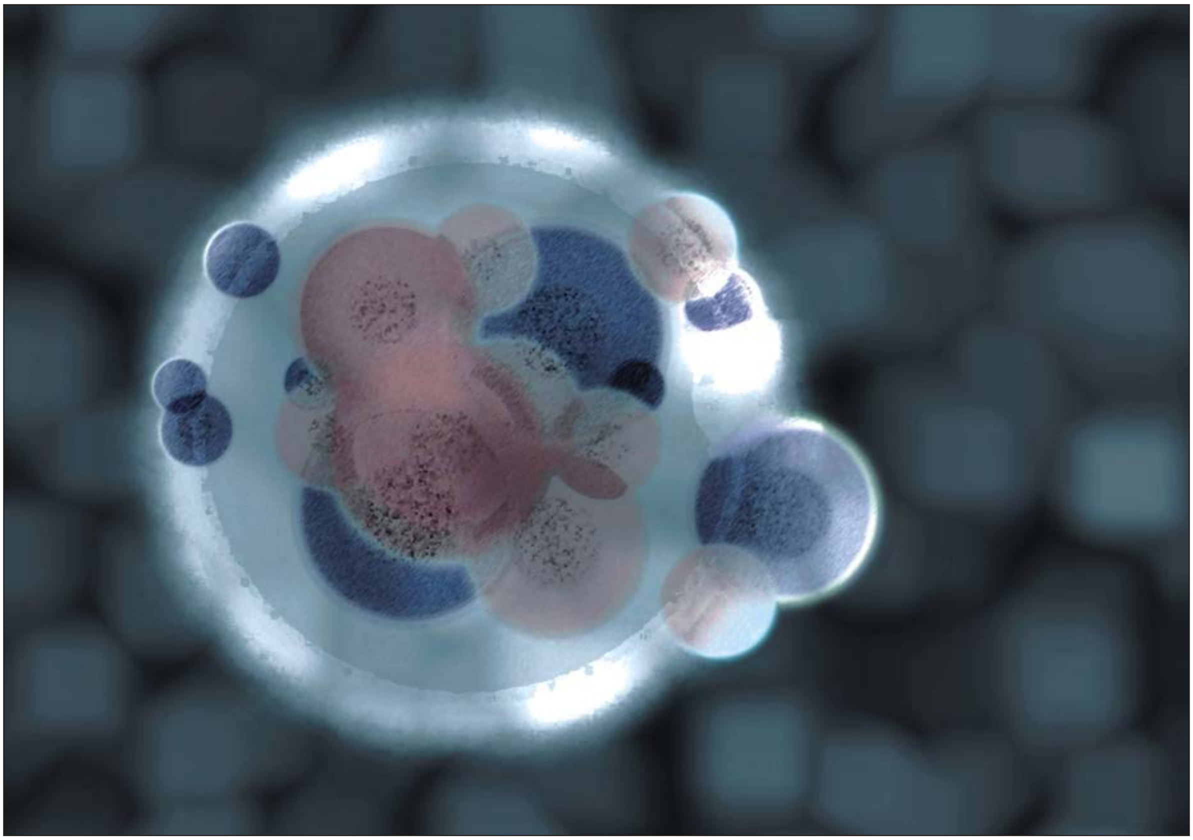

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Metals Concentrations in Eggs of Domestic Avian and Estimation of Health Risk from Eggs Consumption

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Abstract: This study is focused on evaluating the trace metals levels in eggs of four species of domestic birds that collected from commercial farm located in Kajang city, Malaysia. The concentrations of Al, Cr, Ni, Zn, As, Cd and Pb were measured using inductively coupled plasma mass spectrometry (ICP-MS). Additionally, this paper carries out probabilistic risk analysis methods to quantify As, Zn, Cd and Pb bioaccumulation in egg content to assess the range of exposures for the people who consume the contaminated eggs. The models applied include a probabilistic bioaccumulation model to account for this metals accumulation in egg and a human health exposure and risk model that accounts for hazard quotient and lifetime risk for humans consuming contaminated eggs. Generally, result showed that eggs in four species accumulated relatively dissimilar levels of metals. Zn is found in high levels while, Cd burden occurred in less levels in all birds eggs. Quail eggs were gathered elevated levels of Zn, Al and Pb. However, As exist in high concentration in chicken eggs. The associated risk was assessed using Hazard Quotient (HQ). Values of hazard quotient were ranged 0.5-0.03, 0.09-0.03, 0.1-0.06 and 0.2-0.03 for As, Zn, Cd and Pb, respectively. The risk quotient revealed that the intake of the heavy metals by eating eggs does not pose any apparent threat to the local people as none of the HQ of the heavy metals exceeds the limit of 1.

Key words: Heavy metals, eggs, domestic avian, health risk, egg consumption

INTRODUCTION

Global environmental pollution through heavy metal lead to an increased interest in metals contamination of food-stuffs and amongst them eggs which symbolize an important part of human's diet especially children due the avian eggs is an important source of nutrients, containing all of the proteins, lipids, vitamins and minerals (Sparks, 2006). Food safety is a major public concern worldwide in recent decades due the growing demand for food safety has stimulated research regarding the risks associated with consumption of foods contaminated with pesticides, heavy metals or toxins (DMello, 2003). Additionally, Eggs used as evidence for environmental pollution since they can accumulate the heavy metals from diet and surrounding environment (Burger *et al*., 2009). Besides, avian eggs have been shown to be vulnerable to external application of toxicants and represent local exposure of the adults have laid them (Burger, 1993). Some of the heavy metals that are known as potentially toxic include aluminum, arsenic, cadmium and lead and other essential metals such as, zinc, nickel and chromium those play a definitive role in the intrinsic mechanisms regulating vital biological processes, toxic elements can be harmful even at low concentrations when ingested over a long period of time (Nolan, 1983; Young, 2005). Therefore, exposure to metals through consumption eggs may pose health risk especially for high eggs consumption populations. According to USEPA (1997) high levels of inorganic arsenic in food or water can be fatal. A high level is 60 parts of arsenic per million parts of food or water (60 ppm). Arsenic damages many tissues including nerves (peripheral polyneuropathy, axonal degeneration), stomach, intestines and skin. As well as, long term exposure to lower levels of cadmium in air, food, or water leads to a buildup of cadmium in the kidneys. Further, cadmium is cancer-causing and potentially mutation-causing, with severe sub lethal and lethal effects at low environmental concentrations (Eisler, 1985). Mills (1983) claimed that excess zinc can cause some feeding disorders and diseases and impairs the immune system. Several researchers, including Al-Saleh (1994) and Fraser *et al*. (2006) have reported that lead exposure causes neurotoxicity, which is characterized by histological, ultrastructural and neurochemical changes in the central nervous system, as well as behavioral shortfall.

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However, In Malaysia urban residents consume chicken and eggs more frequently than their rural counterparts, more men than women consumed chicken and eggs more frequently (Norimah *et al*., 2008). There is a lack of studies regarding metal levels in eggs of domestic birds in this area of Malaysia and whether these levels represent a human health risk.

Consequently, the objectives of this study were to determine concentration and distribution of Aluminum (Al), Chromium (Cr), Nickel (Ni), Zinc (Zn), Arsenic (As), Cadmium (Cd) and lead (Pb) in egg content of chicken, quail, guinea fowl and pigeon to assess differences among metals content for domestic avian species and to estimate the health risk to local consummators from ingestion of metal contaminated eggs.

MATERIALS AND METHODS

Reagents: The reagents with super quality, analytical grade Nitric acid (70%) and hydrogen peroxide (30%) were acquired from Merck (Darmstadt, Germany) along with the stock standard solutions of Al, Cr, Ni, Zn, As, Cd and Pb in concentrations of 1,000 mg L^{-1}. All the plastic and glassware were cleaned by soaking in dilute HNO_3 (10%) and were rinsed with de-ionized water prior to use.

Apparatus: A Perkin Elmer model Elan 9000 inductively coupled plasma-mass spectrometry (ICP-MS, USA) was used in current study. After calibrating the apparatus with standard solutions derived from commercial materials, it was optimized according to the manufacturing standards the cones and tubes were carefully cleaned to get rid of any possible residues.

Sample collection and preparation: Eggs sample from chicken (*Gallus gallus*), quail (*Coturnix coturnix japonica*), guinea fowl (*Numida meleagris*) and pigeon (*Columba livia*) were collected from commercial farm located in a rural area in Kajang city(latitude 2°59′N, longitude 101°47′E), Selangor, Malaysia. About 36 eggs (for each species) were chosen randomly during the period (from May to October 2010), all eggs were weighed, chicken egg (40.79±4.01 g), guinea fowl egg (38.55±1.75 g), quail egg (11.38±0.87 g) and pigeon egg (12.16±1.35 g). To analyze the metals in egg content according to method previously described by Burger and Eichhorst (2005) with slight modification, the eggs were washed vigorously with de ionized water, then open carefully the eggshell separated from egg content, the egg content were homogenizes and put it in Petri dishes the samples were drying at 70°C for 24 h the egg content grain to powder by mortar, 0.5 g of the sample soaked in nitric acid 70% and hydrogen peroxide 30% overnight in room temperature, the digested completed in bloke thermostat (150°C) for 4 h until solution were clear. After cooling the solution was diluted to 50 mL with de ionized water, then were filtrated throw 0.45 µm acid resistant filter paper. The solutions were stored at 4°C for later metal analysis. Concentration of Aluminum (Al), Chromium (Cr), Nickel (Ni), Zinc (Zn) Arsenic(As), Cadmium (Cd) and lead(Pb) Were determined by inductively couple plasma-mass spectrometry (ICP-MS, model Perkin-Elmer Elan 9000 A). Each analysis was carried in duplicate, standard and blank samples were analyzed every 20 sample. All concentration were expressed in µg g^{-1} on dry weight basis. The accuracy of the applied analytical method was validated by accurate analysis of Certified Reference Materials (CRM) for lobster hepatopancreas (TORT-2, National Research Council Canada). All the runs were carried out in triplicate. The results obtained on the SRMs are showed in Table 1. The recoveries of all the metals were satisfactory.

Table 1: Analytical results for the Certified Reference Materials (CRM) and its certified values for each metal (µg g^{-1} dry weight)

Metals	Measured value	Certificate value	Recovery (%)
Cr	0.774±0.025	0.77±0.15	100.6±3.281
Ni	2.44±0.015	2.50±0.10	97.88±0.61
Zn	110.43±0.74	180.6	61.35±0.67
As	18.09±0.911	21.6±1.8	83.75±4.82
Cd	19.09±0.22	26.7±0.6	73.69±1.14
Pb	0.375±0.015	0.35±0.13	107.3±4.16

The CRM for Al is not available

Health risk estimation: To estimate the human health risk from consuming metal contaminated eggs, the methodology for estimation of target hazard offers an indication of the risk level due to pollutant exposure, this method was available in US EPA Region 111 Risk based concentration table (USEPA, 2000). It is described by following equation:

$$\text{Target hazard quotient (THQ)}=\frac{EF\times ED\times FIR\times C}{RFD\times WAH\times TA}\times 10^{-3}$$

where, EF is exposure frequency (from 365 days/year for people who eat egg 7 times a week to 52 days/year for people who eat egg one time a week. ED is the exposure duration (70 years), equivalent to the average lifetime; FIR is the food ingestion rate (egg: 37.2 g/person/day). C is the metal concentration in egg content (µg g^{-1}), RFD is the oral reference dose (USEPA, 1997; USEPA, 2000); WAB is the average body weight (64 kg, the reference weights for the age categories were derived from several local studies in Malaysia (Lim *et al*., 2000) and TA is the averaging exposure time for non carcinogens (365 day/year×ED). If HQ>1.00, indicating that there is a potential risk associated with that metal (Khan *et al*., 2008). The lifetime Cancer Risk (CR) for arsenic was obtained by using the chemical Slope Factor (SF) (USEPA, 1997) and the estimated metal exposure dose mg/kg/day (EED) in the following equation:

$$CR = EED \times SF, \text{ while the} EED = \frac{EF \times ED \times FIR \times C}{RFD \times WAH \times TA}$$

Statistical analysis: All calculations were performed using SPSS for Windows (vers. 18.0, SPSS Ltd., Woking, Surrey, UK). The descriptive statistics (mean values, standard deviation) for values of egg content were analyzed by one-way analysis of variance followed by the Tukey honestly significant difference test. Differences were considered significant at the p<0.05 level.

RESULTS

Metal analysis in eggs: Result demonstrated that disparity in metals concentrations in eggs among four bird's species, among all the heavy metals, Zn concentration was maximum and Cd was minimum in four species eggs, the highest concentration of Zn were occurred in quail eggs, followed by chicken and guinea fowl however, their level does not significantly differ among species. Al concentration was significantly (p<0.05) highest in quail eggs followed by those of chicken and pigeon. Mean while, Cr was occurred in analogous values in chicken and guinea fowl eggs (3.2 and 3.6 μg g^{-1}), respectively followed by those of guinea fowl and pigeon. The same situation was observed in nickel concentration (Table 2). As and Cd levels in eggs of birds were found in less values compared to those of other metals. For As chicken showed the highest value. While for Cd concentration, quail presented the highest once. As far as, the highest values for Pb were obtained in Quail eggs while the lowest value found in guinea fowl eggs.

Health risk estimation: According to Norimah *et al*. (2008) that daily consumption of chicken egg for one person in Malaysia about one egg (medium) daily, which equivalent (without eggshell) average (37.2 g/person/day), we suggest that daily consumption of other birds eggs was in the same rate. Metals (As, Cd and Pb) were choose to estimate the THQ due these metals may inadvertently enter the food chain and pose health risk to human (Zhuang *et al*., 2009) and Zn because it has high concentration among diagnosed metals in current study. Target hazard quotient and cancer risk of As were presented in Table 3. In order to estimate the human health risk from consuming metal-contaminated egg, we used the reference dose of metals RFD and estimated metal exposure dose EED (mg/kg/day). The THQ values of As in egg of domestic bird were ranged from 0.5 to 0.03 for people who eat eggs 7 times a week, from 0.3 to 0.02 for people who eat eggs 4 times a week and from 0.2 to 0.005 for people who consume eggs one time a week (Table 3). For Zn, TQH values ranged from 0.07 to 0.05 for people who eat eggs 7 times a week, from 0.06-to 0.03 for people who consume eggs 4 times a week and from 0.03 to 0.007 when human eat eggs one time a week (Table 4). For Cd the values of THQ reached from 0.12 to 0.063 for eggs consumed in 7 times a week, from 0.07 to 0.04 in 4 time a week and 0.025-0.008 in one time a week of egg consumption (Table 5). While THQ values of Pb have been ranged from 0.2 to 0.04, 0.1-0.02 and 0.03-0.004 for

Table 2: Mean concentration of heavy metals (μg g^{-1} dry weight±SD) in egg content of domestic avian species

Metals	Chicken	Guinea fowl	Quail	Pigeon
Al	17.11±1.49b	12.291±5.20b	65.72±19.0a	16.56±6.27b
Cr	3.24±2.45a	0.87±0.34b	3.60±1.22a	0.70±0.19b
Ni	1.11±2.45	0.13±0.02	1.02±0.02	0.08±0.01
Zn	34.22±3.4	34.20±7.4	48.47±11.6	25.29±3.06
As	0.30±0.14a	0.03±0.01b	0.10±0.04b	0.02±0.009b
Cd	0.054±0.02	0.075±0.006	0.10±0.03	0.058±0.05
Pb	0.420±0.10b	0.101±0.06b	0.75±0.20a	0.156±0.05b

Values with different letters within arrow are significantly (p<0.05) differ

Table 3: Health risk estimate for As ingestion from domestic birds eggs

Bird species	Level of exposure day/week	Mean metal conc. (μg g^{-1})	HQRFD = 0.003 (mg/kg/day)	Cancer risk SF = 1.5 (mg/kg/day)
Gallus gallus	7	0.30±0.14	0.578	2.5×10^{-4}
domesticus	4		0.374	1.6×10^{-4}
	1		0.083	3.6×10^{-5}
Coturnix	7	0.10±0.04	0.23	8.7×10^{-5}
coturnix	4		0.125	5.6×10^{-5}
japonica	1		0.028	1.2×10^{-5}
Numida	7	0.03±0.01	0.058	2.6×10^{-5}
meleagris	4		0.0374	1.6×10^{-5}
	1		0.0082	3.7×10^{-6}
Columba	7	0.02±0.009	0.0387	1.7×10^{-5}
livia	4		0.0249	1.1×10^{-5}
	1		0.0055	2.4×10^{-6}

Table 4: Health risk estimate for Zn ingestion from domestic birds egg

Bird species	Level of exposure day/week	Mean metal conc. (μg g^{-1})	HQ RFD = 0.3 (mg/kg/day)
Gallus gallus	7	34.22±3.4	0.07
domesticus	4		0.04
	1		0.03
Coturnix coturnix	7	48.47±11.6	0.09
japonica	4		0.06
	1		0.01
Numida meleagris	7	34.20±7.4	0.07
	4		0.04
	1		0.03
Columba livia	7	25.29±3.06	0.05
	4		0.03
	1		0.007

Table 5: Health risk estimate for Cd ingestion from domestic birds egg

Bird species	Level of exposure day/week	Mean metal conc. (μg g^{-1})	HQ RFD = 0.0005 (mg/kg/day)
Gallus gallus domesticus	7	0.054±0.02	0.063
	4		0.040
	1		0.008
Coturnix coturnix japonica	7	0.10±0.03	0.120
	4		0.075
	1		0.025
Numida meleagris	7	0.075±0.006	0.090
	4		0.056
	1		0.012
Columba livia	7	0.058±0.05	0.074
	4		0.043
	1		0.010

Table 6: Health risk estimate for Pb ingestion from domestic birds egg

Bird species	Level of exposure day/week	Mean metal conc. (μg g^{-1})	HQ RFD=0.002 (mg/kg/day)
Gallus gallus domesticus	7	0.420±0.10	0.122
	4		0.085
	1		0.023
Coturnix coturnix japonica	7	0.75±0.20	0.22
	4		0.14
	1		0.031
Numida meleagris	7	0.101±0.06	0.03
	4		0.02
	1		0.004
Columba livia	7	0.156±0.05	0.043
	4		0.03
	1		0.0064

Table 7: HQ (hazard quotient) values when the consumption of eggs more than one eggs/person/day

Metals	HQ (consumption 2 eggs/day)	HQ (consumption 3 egg/day)
As	1.20	1.70
Zn	0.13	0.30
Cd	0.23	0.36
Pb	0.23	0.46

people who eat egg 7, 4 and 1 time a week respectively (Table 6). Risk to human health from the intake of metal contaminated eggs was characterized using hazard quotient HQ is a ratio of determined dose of a pollutant to the dose level (a reference dose or RfD). If the ratio is less than 1 like in Zn, Cd and Pb, there will not be any noticeable risk (Table 7). Conversely, an exposed population of concern will experience health risks if the dose is equal to or greater than the RfD (Khan *et al*., 2008).

DISCUSSION

Metals concentrations in avian eggs: Demonstration of trace elements in eggs may be an important instrument for nutritionists and environmentalists. Thus, current study provide a base information of the composition of domestic avian eggs in trace elements, such information was rare before in Malaysia. Present study confirmed that a large variation in metals concentrations among domestic avian eggs, this differences may be resulted from feeding behavior and different ability for birds to ingest soil and grass and the metabolic activities among different bird species (Nisianakis *et al*., 2009). In this study, diet, geographical location, or environmental pollution could not have contributed to such differences due all birds were fed a common diet and kept at the same range area free from any local pollution. Concentration of Zn and Ni in chicken egg content in present study reached 34.22 and 1.11 μg g^{-1}, respectively were higher than values previously recorded by Van Overmeire *et al*. (2006), Meanwhile, Pb and Cd concentration in current study in chicken eggs content get to (0.42, 0.05 μg g^{-1}, respectively) were less than values reported by Fakayode and Olu-Owolabi (2003). Arsenic burden was found in high concentration in chicken egg content than pigeon egg content, similar observation was also reported by Nisianakis *et al*. (2009). According to Malaysian Food Act (1983) concentration of Cd, Pb and Zn were lower than the recommended legal limit (1, 2 and 100 μg g^{-1}, respectively). As concentration in chicken egg was 0.3 μg g^{-1} and these results are obviously lower than the permitted limits 2.0 ppm (ANZFA, 2001).

Health risk estimation: Some of the heavy metals such as Zn, Cr, Ni and Cu act as micro-nutrients at lower concentrations, they become toxic at higher concentrations. Small amounts of these elements are common in our environment and diet and are actually necessary for good health but large amounts of any of them may cause acute or chronic toxicity (poisoning). Heavy metal toxicity can result in damaged or reduced mental and central nervous function, lower energy levels and damage to blood composition, lungs, kidneys, liver and other vital organs. Long-term exposure may result in slowly progressing physical, muscular and neurological degenerative processes that mimic Alzheimer's disease, Parkinson's disease, muscular dystrophy and multiple sclerosis. Repeated long-term contact with some metals or their compounds may even cause cancer (IOSHIC, 1999).

The estimated THQ values in present study seem to indicate that consumption of these egg does not implicate an appreciable human health risk for the consumer due THQ values of (As, Zn, Cd and Pb) were less than 1, cancer risk SF for arsenic in this study ranged 10^{-4}-0^{-6} (Table 3) the US EPA considers cancer risk that are below about 1 chance in 1,000,000 (1×10^{-6}) to be so small as to be negligible and risks above 1×10-4 to be sufficiently large that some sort of remediation is desirable. Excess cancer risks that range between 1×10-6 and 1×10-4 are generally considered to be acceptable (USEPA, 1991). If we suggest that consumption of chicken eggs will be increase from 1 egg to 2 or 3 eggs/day/person. Then values of THQ of Zn, Cd and Pb were still in permission level except for Arsenic value which it value exceed to 1.2 and 1.7 respectively (Table 6) this indicates that human health risk might be of concern in case of increased eggs consumption.

CONCLUSION

We conclude that the concentration of Al, Cr, Ni, Zn, As, Cd and Pb were varied among the egg of domestic bird. The eggs of quail were accumulated high levels of Al, Zn and Pb compared to those in other birds eggs, meanwhile, Cd was occur in less concentration in the egg of chicken, guinea fowl and pigeon . From the standpoint of human health As, Zn, Cd and Pb. THQ values (<1) show a situation without risk to the consumer in the area of study. Further ecotoxicological studies are needed on the eggs of species in different area of Malaysia.

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