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REGULAR PAPER **Experimental Research**

Heavy metal concentrations in duck eggs and potential human health risk via consumption

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Abstract

Heavy metals commonly found in environmental matrices are from natural or anthropogenic activities. Their contamination effects especially on human health from non-degradable properties are of major concern. The aims of this study, thus, were to determine Cd, Cu, Fe, Mn, Ni, Pb and Zn residues in duck eggshells, yolk and albumin, and to investigate the correlations of these metal residues with the surrounding environmental media (soil, water, and feed). Target hazard quotient (THQ) of individual heavy metal was used to evaluate potential human health risk via egg consumption. Thirty duck egg samples were randomly collected from each free range laying duck farm (total of 8 farms). The samples collected were analyzed for heavy metal concentrations using the flame atomic absorption spectrometry (FAAS). Results showed that average concentrations of Fe (71.96±1.33 μg/g dw) in yolk and Pb (6.76±0.21 μg/g dw) in albumin were significantly highest when compared to other investigated metals, whereas Cu concentration was mostly found in egg shells. In addition, the predominantly found metal in soil samples was Fe, whereas in water and feed samples were Ni and Pb, respectively. Principle component analysis (PCA) revealed that the concentrations of heavy metals found in eggshells and egg contents are highly correlated with their concentrations in the surrounding water and soil matrices as well as feed. The THQs for Pb, which were greater than 1, indicated that there could be potential for human health risks upon consumption of contaminated duck eggs.

Key Words: environmental matrices, duck egg, heavy metals, human health risk, THQ

Introduction

Metals can either be beneficial or harmful to living organisms. Some metals (copper/Cu, iron/Fe, manganese/Mn, and zinc/Zn) are considered as necessary at trace amounts but toxic when exceeded. For example, excesses in the amount of Cu can cause Wilson's disease, whereas Zn can result in eating disorders and immunocompromised condition, respectively[20,32]. On the other hand, some other metals (lead/Pb, nickel/Ni, cadmium/Cd) are highly toxic and can only pose negative health effects on living organisms[4]. The potential toxicities of this metal

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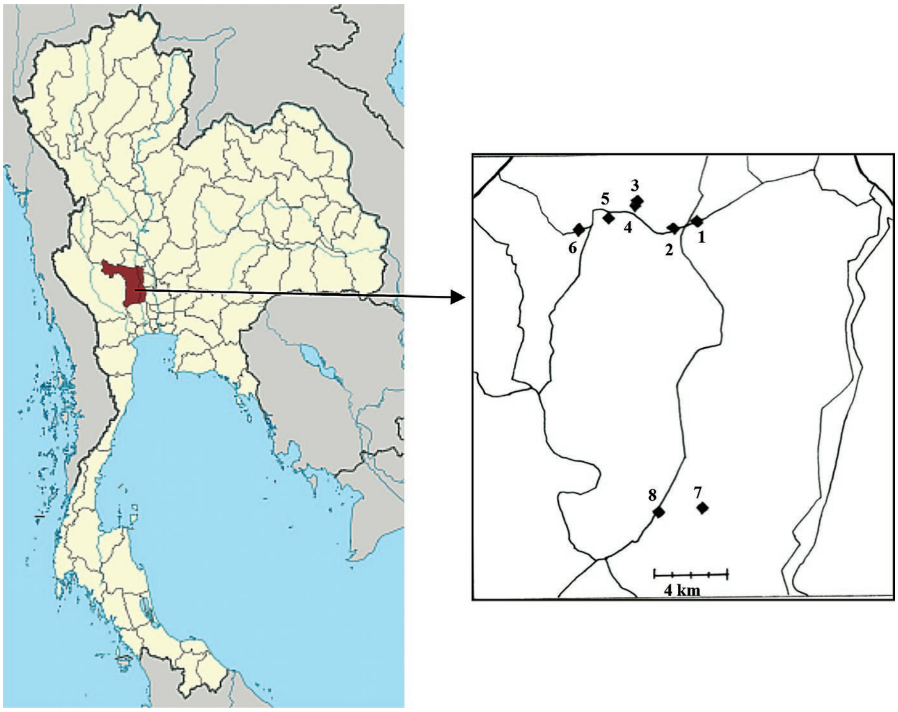

Fig. 1
Map of the study area showing 8 sampling locations (farm # 1-8) locations in Song Phi Nong district, Suphanburi province, Thailand. Duck egg (n = 30), water (n=5), feed (n=5), and soil (n=5) samples were collected from each location.

group range from acute to chronic depending on the concentration and period of exposure. Health deterioration can be found in renal, hepatic, gastrointestinal, skeletal, neurological as well as other systems[9,11,29,34].

Once metals are released into the environment, they accumulate into different matrices. Increases in industries as well as agricultures have lately contributed to more heavy metal contaminations in the surrounding environments due to their non-degradable properties. The increased heavy metals can be transferred to various organisms within the food chain.

Appropriate bioindicators, especially the non-invasive ones, are useful for investigating heavy metal contaminations in the environments. They live and feed in the contamination area, and also coexist with other animals. The heavy metal contamination levels in these indicators, thus, can be used to estimate risks to other animals as well as humans. A large number of environmental studies have been conducted by using birds[7,13,27,30,38] since they are ubiquitous, have been studied extensively, and are at high level of the food chain[12]. In addition, they are ecologically versatile and live in various habitats as herbivores, carnivores, or omnivores[17]. Besides, they are more sensitive to environmental contaminants than other vertebrates[13]. Birds, therefore, can appropriately be utilized as environmental bioindicators.

Several parts of avian body can be collected for investigation of environmental contaminated toxicants. Among those, egg is a technically non-invasive mean. Eggs can accumulate a large number of toxicants and can also be harmed by them[3,6,15,21]. Thus, they have extensively been used to investigate environmental pollutions, especially heavy metals[12].

Chicken and duck eggs are nutritious food in daily diet since they provide high-quality, bioavailable protein with minute total fat33). Eggs can also be used as an indicator for estimating human health risk following

Table 1. Limit of detection (LOD) and spiked recovery percentage of spiked standard metals in egg samples.

Metals	Cd	Cu	Fe	Mn	Ni	Pb	Zn
LOD (μg/g)	0.002	0.009	0.007	0.008	0.015	0.019	0.001
LOQ (μg/g)	0.006	0.030	0.023	0.026	0.050	0.062	0.002
Spiked concentration (μg/g)	1	5	10	1	5	10	10
Recovery percentage (%)	96.42	93.01	83.27	81.14	96.96	97.49	95.37

consumption. Moreover, they are popularly consumed by Thai people.

In central region of Thailand, Suphanburi is an agricultural province where farmers routinely use pesticides as well as fertilizers. Moreover, farmers in that area also raise free range laying ducks for extra income which have yielded high duck egg production. Free range duck usually fed themselves in the rice field, and have freely access to soil and water sources nearby. These ducks could likely be exposed to heavy metals found in pesticides and fertilizers. Since eggs can bioaccumulate heavy metals and is highly consumed by the people of Thai, thus, they fit for this study.

The aim of this study was to determine the concentrations of Cd, Cu, Fe, Mn, Ni, Pb and Zn in duck eggs collected from farms in Suphanburi province, Thailand. We also investigated the associations of heavy metals levels found in eggs with their surrounding matrices; i.e., water, soil, and feed to find out the major environmental source(s) of heavy metals that could be transferred to duck eggs by using correlation analysis. In addition, the Target hazard quotient (THQ) values were used to evaluate human health risks following the consumption of these contaminated duck eggs.

Materials and Methods

Sample collection

Thirty duck egg samples were randomly collected from the egg-laying area of each duck farm (total of 8 farms) in Song Phi Nong district, Suphanburi province, Thailand (Figure 1). All selected farms had the duck ponds that received water from the adjacent areas. All ducks were fed with both commercial feed and free feeding. Soil (500 g), feed (500 g), and water (300 mL) samples within the husbandry and the surrounding area were systematically collected into polyethylene bags and bottles. Egg samples were cleaned in the laboratory with distilled water. Egg contents were removed and separated into albumin and yolk whereas eggshells were cleaned with distilled water then air-dried. Both egg content and eggshells were kept in polyethylene bags. All samples were stored at -20ºC before analysis.

Sample preparation

Albumin and yolk were oven dried at 65°C. Soil and feed samples were dried at 65°C in the oven and grinded with mortar and pestle whereas water samples were filtered to remove debris using No. 4 filter paper (Whatman®). One hundred microlitres (100 μL) of 70% HNO3 were added to 100 mL filtered water samples just prior to the analysis[2]. Eggshell (0.8 g), feed (0.8 g) and soil samples (0.5 g) were added with 5 mL concentrated nitric acid (70%), whereas, egg contents (0.5 g) were added with 5mL 70% HNO_3/H_2O_2 (1:1 v/v) mixture. All samples were then placed under hood at 25°C overnight for digestion. Further digestion was performed in 150-180ºC water bath for three hours2). All digested samples were diluted to a volume of 25 mL with deionized water (Milli-Q), seven metals (Cd, Cu, Fe, Mn, Ni, Pb, and Zn) were analyzed in each sample using flame atomic absorption spectrometry (FAAS; 240B: Agilent Technologies).

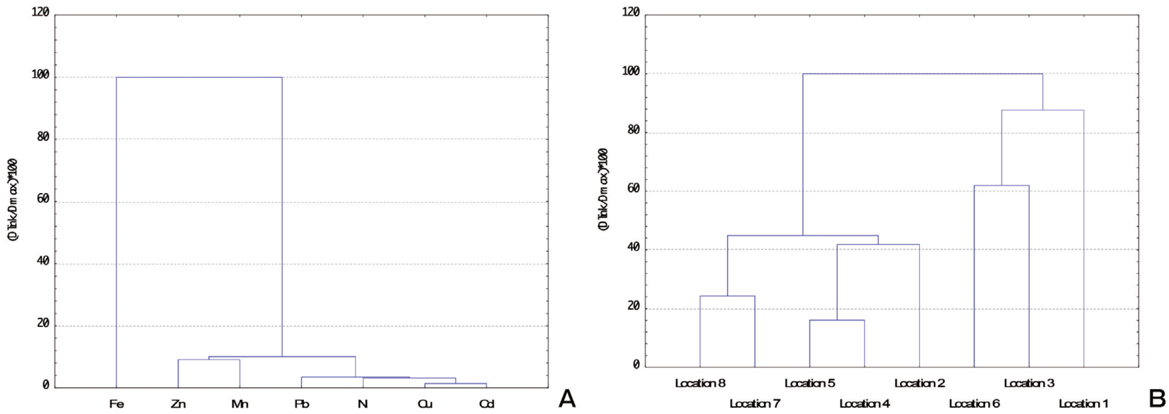

Fig. 2
Hierarchical cluster analysis (CA) of heavy metal concentrations in environmental matrices based on analyzed parameters (A) and different sampling locations (B).

Heavy metal analysis

Heavy metal standard solutions (1000 mg/L) were purchased from Merck (Germany). All working solutions were prepared in deionized water (ranged from 0.05 – 2.00 mg/L for Cd and Zn; 0.50 – 5.00 mg/L for Cu and Fe; 0.02 - 4.00 mg/L for Mn; 0.50 – 10.00 mg/L for Ni; and 0.50 – 20.00 mg/L for Pb). Concentrations of heavy metals were determined using corresponding calibration curves.

Quality control and assurance

FAAS methods for seven heavy metals was validated for limit of detection (LOD), limit of quantification (LOQ) based on sample replication measurement on blank solution. The LOD and LOQ were calculated using the linear regression of the calibration curve for each metal. They are computed as LOD = 3SD/b and LOQ = 10SD/b, where SD is the standard deviation of the response and b is the slope of calibration curve28). The precision and accuracy of FAAS were analyzed by spiking standard solution of each heavy metal into egg samples (1 µg/g for Cd and Mn; 5 µg/g for Cu and Ni, 10 µg/g for Fe, Pb and Zn). In this present study, LOD, LOQ and recovery percentages of seven heavy metals were shown in Table 1. LODs and LOQs of all seven heavy metals were between 0.001-0.019 µg/g and 0.002-0.062 µg/g, respectively. The precision in termed of recovery percentages ranged from 81.14%-97.49%, whereas, the average accuracy of selected heavy metals were 99.95% – 99.99%.

Health risk estimation

The THQ was used to estimate human health risk from consuming heavy metals contaminated duck eggs[36)]. It was calculated by using the following equation:

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

where, EF is exposure frequency (132 times/year)[5)]; ED is exposure duration (70 years); FIR is food ingestion rate (50 g/person/time)[5)]; C is heavy metal concentration (mg/kg); RFD is oral reference dose (µg/kg/day) (Cd 1 µg/kg/day, Cu 500 µg/kg/day, Fe 800 µg/kg/day, Mn 140 µg/kg/day, Ni 20 µg/kg/day, Pb 0.6 µg/kg/day and Zn 300 µg/kg/day)[36,37)]; WAB is the average body weight (60 kg)[31)];TA is averaging exposure time for non-carcinogens (365 day/year x ED). If THQ is more than 1.00, there could be a potential risk associated with the metal[18)]. The total THQ (tTHQ) or the sum of THQs of all seven heavy metals in duck egg were also calculated.

Statistical analyses

One-way analysis of variance (ANOVA) was used to analyze the differences among heavy metals found in environmental matrices,

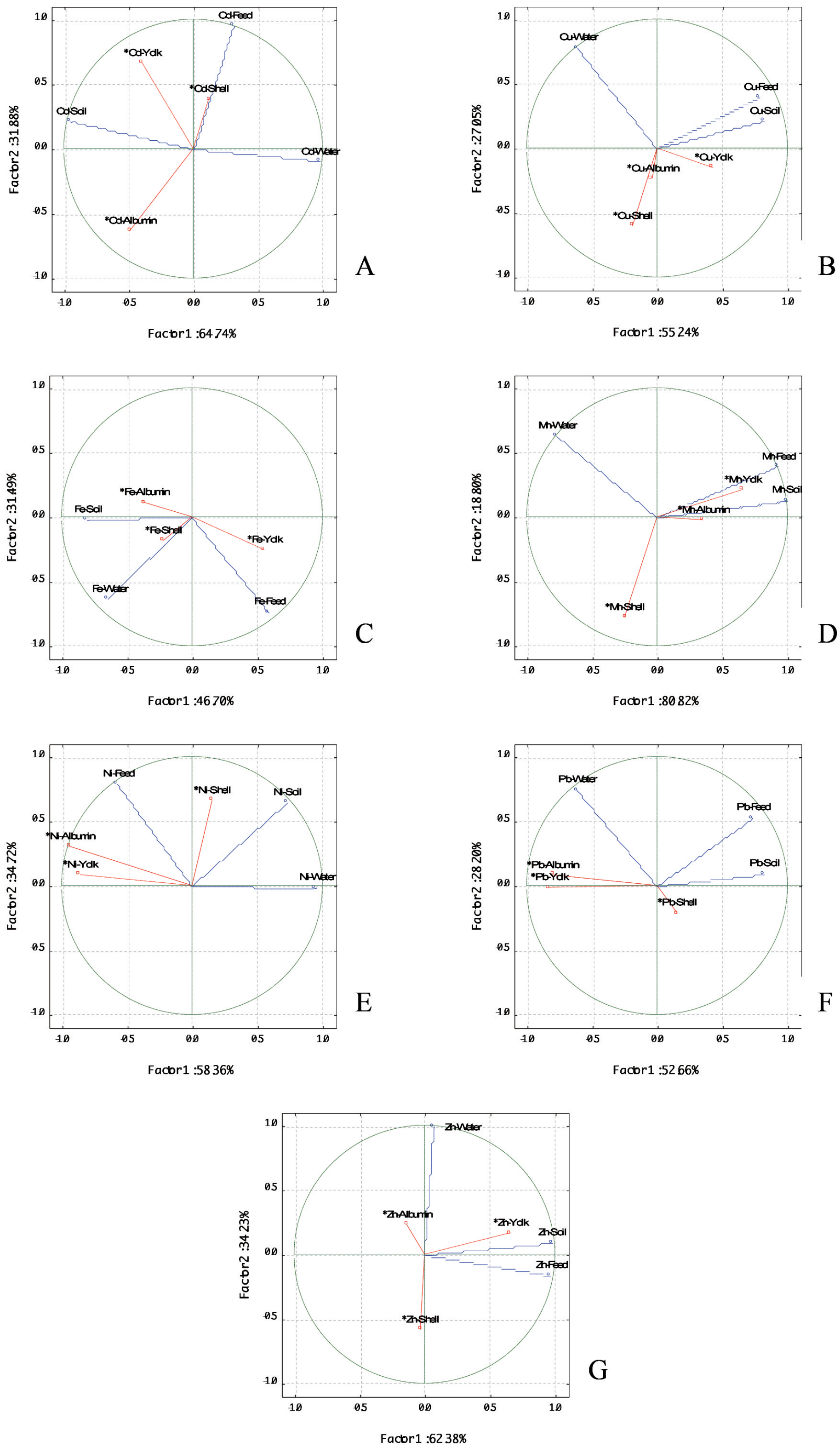

Fig. 3
PCA plots of seven heavy metals Cd (A), Cu (B), Fe (C), Mn (D), Ni (E), Pb (F) and Zn (G) concentration observed in eggs and surrounding environmental matrices.

Table 2. Heavy metals concentrations (mean ± SEM; μg/g) in albumin, yolk and shell collected from eight duck farm in Suphanburi province

Metal	Sample	Location 1	2	3	4	5	6	7	8
Cd	Shell	1.52±0.04^{b}	1.55±0.03^{bc}	1.66±0.03^{de}	1.60±0.01^{cd}	1.63±0.02^{cd}	1.65±0.02^{d}	1.74±0.02^{e}	1.41±0.03^{a}
	Albumin	1.52±0.02^{f}	0.79±0.09^{e}	0.66±0.03^{d}	0.18±0.02^{a}	0.51±0.02^{c}	0.34±0.01^{b}	0.30±0.02^{b}	0.33±0.01^{b}
	Yolk	0.35±0.02^{bc}	1.49±0.01^{f}	0.72±0.06^{d}	0.25±0.02^{ab}	0.44±0.02^{c}	1.08±0.08^{e}	0.21±0.01^{a}	0.26±0.01^{ab}
Cu	Shell	11.03±0.53^{b}	5.95±0.51^{a}	16.76±1.03^{e}	7.27±0.61^{a}	13.41±0.38^{cd}	14.85±0.43^{d}	24.18±0.64^{f}	12.80±0.39^{c}
	Albumin	2.42±0.08^{b}	1.54±0.06^{a}	4.02±0.13^{c}	2.34±0.08^{b}	1.71±0.18^{a}	2.22±0.06^{b}	2.13±0.09^{b}	1.62±0.07^{a}
	Yolk	1.53±0.03^{d}	1.42±0.03^{c}	2.66±0.04^{e}	0.89±0.03^{b}	0.77±0.03^{a}	1.51±0.04^{d}	0.91±0.02^{b}	0.88±0.02^{b}
Fe	Shell	7.80±0.32^{a}	8.41±0.19^{a}	9.51±0.26^{bc}	9.12±0.11^{b}	9.32±0.20^{bc}	10.34±0.16^{d}	9.89±0.33^{cd}	8.35±0.19^{a}
	Albumin	3.87±1.67^{a}	3.20±0.63^{a}	2.64±0.30^{a}	4.67±0.26^{a}	11.01±1.76^{b}	6.84±2.93^{a}	4.18±1.16^{a}	3.50±0.14^{a}
	Yolk	100.73±3.35^{d}	80.97±2.18^{a}	75.39±1.98^{c}	75.55±2.27^{c}	50.53±2.94^{a}	62.57±2.92^{b}	56.90±2.72^{ab}	73.09±2.65^{c}
Mn	Shell	3.98±0.11^{bc}	4.04±0.06^{c}	3.98±0.06^{bc}	3.83±0.04^{ab}	4.32±0.06^{d}	4.23±0.04^{d}	3.85±0.06^{abc}	3.72±0.05^{a}
	Albumin	0.18±0.04^{ab}	0.49±0.08^{d}	0.35±0.03^{c}	0.18±0.02^{ab}	0.22±0.03^{b}	0.93±0.02^{e}	0.10±0.02^{a}	0.95±0.01^{e}
	Yolk	1.66±0.06^{b}	2.08±0.09^{c}	1.59±0.08^{b}	1.35±0.06^{a}	1.33±0.07^{a}	1.23±0.05^{a}	1.56±0.06^{b}	2.86±0.06^{d}
Ni	Shell	4.99±0.35^{a}	4.71±0.21^{a}	7.18±0.22^{c}	5.00±0.13^{a}	6.01±0.21^{b}	6.75±0.14^{c}	7.97±0.21^{d}	4.82±0.20^{a}
	Albumin	1.24±0.11^{a}	1.79±0.24^{b}	2.53±0.13^{c}	1.83±0.12^{b}	1.63±0.09^{b}	1.88±0.12^{b}	1.73±0.13^{b}	2.57±0.12^{c}
	Yolk	1.24±0.13^{b}	1.14±0.11^{ab}	3.31±0.13^{d}	1.71±0.12^{c}	1.59±0.11^{c}	0.87±0.09^{a}	1.81±0.09^{c}	3.13±0.10^{d}
Pb	Shell	9.43±0.41^{a}	9.72±0.31^{a}	12.87±0.36^{c}	9.64±0.21^{a}	12.27±0.27^{bc}	14.12±0.43^{d}	14.93±0.32^{d}	11.36±0.35^{b}
	Albumin	8.42±0.13^{e}	6.26±0.33^{cd}	9.55±0.31^{f}	10.96±0.67^{g}	3.37±0.22^{b}	6.08±0.16^{c}	2.39±0.14^{a}	7.02±0.11^{d}
	Yolk	13.83±1.07^{d}	9.63±0.10^{c}	14.29±0.30^{d}	17.75±0.17^{e}	4.57±0.12^{a}	7.73±0.36^{b}	5.29±0.18^{a}	10.04±0.09^{c}
Zn	Shell	2.96±0.12^{b}	2.76±0.12^{ab}	2.45±0.18^{a}	2.41±0.08^{a}	3.48±0.16^{c}	3.65±0.15^{c}	3.60±0.14^{c}	2.88±0.08^{b}
	Albumin	3.19±1.24^{abc}	3.61±0.65^{abc}	3.14±0.20^{abc}	2.00±0.12^{a}	5.35±1.09^{c}	4.09±0.28^{abc}	2.47±0.73^{ab}	4.25±0.31^{bc}
	Yolk	50.23±0.88^{d}	54.27±0.99^{e}	40.79±0.79^{a}	41.92±0.86^{ab}	44.55±0.81^{c}	43.80±0.84^{bc}	42.56±0.75^{abc}	52.46±0.71^{de}

Concentration in the same row with the same superscript did not differ at $P<0.05$

Table 3. Heavy metals concentrations (mean ± SEM; µg/g or µg/mL) in soil, water and feed collected from eight duck farm in Suphanburi province

Metal	Sample	Location 1	2	3	4	5	6	7	8
Cd	Water	$(1.20)\text{x}10^{-3\,a}$	$(0.33\pm0.15)\text{x}10^{-3\,a}$	$(0.90\pm0.00)\text{x}10^{-3\,a}$	$(1.80\pm0.36)\text{x}10^{-3\,a}$	$(5.68\pm1.57)\text{x}10^{-3\,b}$	Nd	Nd	Nd
	Soil	2.42 ± 0.26^{b}	2.6446 ± 0.2123^{b}	3.03 ± 0.41^{b}	2.71 ± 0.21^{b}	1.61 ± 0.23^{a}	2.36 ± 0.42^{ab}	2.57 ± 0.18^{b}	2.60 ± 0.11^{b}
	Feed	0.3091 ± 0.1510^{a}	0.52 ± 0.18^{ab}	0.48 ± 0.17^{ab}	0.53 ± 0.19^{b}	0.45 ± 0.21^{a}	0.2576 ± 0.1195^{a}	0.45 ± 0.21^{ab}	0.49 ± 0.16^{ab}
Cu	Water	$(3.00\pm0.84)\text{x}10^{-3\,ab}$	$(2.80\pm1.56)\text{x}10^{-3\,ab}$	$(1.67\pm0.67)\text{x}10^{-3\,a}$	$(5.60\pm0.51)\text{x}10^{-3\,b}$	$(6.50\pm1.50)\text{x}10^{-3\,b}$	Nd	$(2.00\pm0.00)\text{x}10^{-3\,a}$	Nd
	Soil	16.9158 ± 2.3066^{b}	29.88 ± 1.82^{c}	3.4596 ± 1.3285^{a}	19.20 ± 2.37^{b}	1.50 ± 0.62^{a}	30.44 ± 5.47^{c}	20.81 ± 1.3684^{b}	34.73 ± 0.72^{c}
	Feed	5.9824 ± 3.4327^{a}	10.13 ± 0.81^{a}	7.3276 ± 0.5395^{a}	11.40 ± 2.06^{a}	5.92 ± 0.22^{a}	3.26 ± 1.55^{a}	6.87 ± 0.30^{a}	8.55 ± 0.76^{a}
Fe	Water	$(16.46\pm5.76)\text{x}10^{-3\,a}$	$(10.32\pm2.12)\text{x}10^{-3\,a}$	$(16.32\pm0.83)\text{x}10^{-3\,a}$	$(14.92\pm2.52)\text{x}10^{-3\,a}$	$(6.93\pm3.06)\text{x}10^{-3\,a}$	$(22.02\pm1.62)\text{x}10^{-3\,a}$	$(22.94\pm3.81)\text{x}10^{-3\,a}$	$(14.28\pm1.75)\text{x}10^{-3\,a}$
	Soil	$2445.7991\pm778.1084^{ab}$	1202.48 ± 190.39^{a}	4317.7145 ± 673.0387^{b}	704.98 ± 403.38^{a}	631.44 ± 192.38^{a}	3570.49 ± 2422.35^{b}	58.93 ± 20.95^{a}	65.48 ± 21.30^{a}
	Feed	149.7505 ± 26.3293^{a}	128.89 ± 22.82^{a}	145.7425 ± 20.2105^{a}	125.43 ± 21.35^{a}	140.34 ± 19.34^{a}	144.73 ± 26.25^{a}	131.41 ± 20.96^{a}	123.79 ± 23.02^{a}
Mn	Water	$(7.64\pm0.51)\text{x}10^{-3\,a}$	$(4.70\pm1.06)\text{x}10^{-3\,a}$	$(8.00\pm0.47)\text{x}10^{-3\,a}$	$(7.80\pm1.10)\text{x}10^{-3\,a}$	$(12.90\pm3.97)\text{x}10^{-3\,a}$	$(8.22\pm0.61)\text{x}10^{-3\,a}$	$(7.76\pm0.21)\text{x}10^{-3\,a}$	$(7.74\pm0.57)\text{x}10^{-3\,a}$
	Soil	343.2846 ± 33.0103^{c}	487.41 ± 24.48^{d}	229.2723 ± 53.2970^{ab}	308.19 ± 23.36^{bc}	194.15 ± 5.26^{a}	460.0849 ± 39.0840^{d}	289.79 ± 15.44^{bc}	458.09 ± 7.14^{d}
	Feed	125.8959 ± 9.0580^{a}	172.91 ± 19.19^{b}	100.1350 ± 3.1019^{a}	181.69 ± 12.57^{b}	130.39 ± 22.41^{a}	124.0378 ± 6.7043^{a}	89.57 ± 5.58^{a}	182.17 ± 8.95^{b}
Ni	Water	$(96.96\pm3.68)\text{x}10^{-3\,c}$	$(90.96\pm3.18)\text{x}10^{-3\,c}$	$(95.00\pm2.99)\text{x}10^{-3\,c}$	$(98.80\pm2.89)\text{x}10^{-3\,c}$	$(92.44\pm3.06)\text{x}10^{-3\,c}$	$(82.96\pm17.72)\text{x}10^{-3\,c}$	$(6.04\pm0.93)\text{x}10^{-3\,b}$	$(1.80)\text{x}10^{-3\,a}$
	Soil	2.4941 ± 0.0000^{a}	1.64 ± 0.63^{a}	7.5962 ± 0.9772^{b}	5.98 ± 1.20^{b}	Nd	8.6031 ± 1.7535^{b}	1.54 ± 0.30^{a}	0.25 ± 0.25^{a}
	Feed	2.3075 ± 0.1875^{a}	3.73 ± 0.48^{a}	4.0800 ± 0.2343^{a}	3.67 ± 0.01^{a}	3.82 ± 0.02^{a}	2.5491 ± 0.1817^{a}	3.76 ± 0.69^{a}	3.97 ± 0.07^{a}
Pb	Water	$(15.65\pm15.55)\text{x}10^{-3\,a}$	$(20.18\pm5.60)\text{x}10^{-3\,a}$	$(16.04\pm5.09)\text{x}10^{-3\,a}$	$(29.74\pm7.13)\text{x}10^{-3\,a}$	$(69.78\pm17.34)\text{x}10^{-3\,b}$	$(33.78\pm13.75)\text{x}10^{-3\,a}$	$(35.77\pm6.69)\text{x}10^{-3\,a}$	$(22.30\pm8.85)\text{x}10^{-3\,a}$
	Soil	10.6524 ± 1.0827^{ab}	16.68 ± 0.85^{ab}	38.0129 ± 13.5961^{c}	15.46 ± 1.30^{ab}	4.80 ± 0.47^{a}	22.8589 ± 1.6576^{b}	12.16 ± 0.43^{ab}	17.36 ± 1.08^{ab}
	Feed	127.5377 ± 3.0564^{a}	134.91 ± 6.62^{a}	125.1362 ± 3.8771^{a}	127.83 ± 1.45^{a}	128.59 ± 1.15^{a}	122.3386 ± 3.2435^{a}	130.94 ± 5.17^{a}	135.41 ± 5.94^{a}
Zn	Water	$(11.32\pm1.59)\text{x}10^{-3\,a}$	$(10.50\pm1.90)\text{x}10^{-3\,a}$	$(10.64\pm1.90)\text{x}10^{-3\,a}$	$(10.70\pm1.75)\text{x}10^{-3\,a}$	$(11.10\pm2.30)\text{x}10^{-3\,a}$	$(8.18\pm1.56)\text{x}10^{-3\,a}$	$(13.62\pm2.20)\text{x}10^{-3\,a}$	$(14.56\pm1.44)\text{x}10^{-3\,a}$
	Soil	171.2706 ± 44.3714^{de}	295.07 ± 17.04^{f}	61.8299 ± 12.5357^{a}	159.41 ± 16.18^{bc}	113.16 ± 4.22^{b}	248.5248 ± 36.6174^{ef}	190.18 ± 9.96^{cd}	285.25 ± 3.78^{f}
		107.6087 ± 7.0975^{b}	135.49 ± 8.16^{c}	70.2943 ± 4.8680^{a}	141.46 ± 6.02^{c}	74.60 ± 3.98^{a}	81.6206 ± 2.2394^{a}	69.05 ± 4.66^{a}	144.47 ± 10.45^{c}

Concentration in the same row with the same superscript did not differ at $P<0.05$
Nd is non-detectable

albumin, yolk, and egg shells. A value of $p < 0.05$ was chosen as the level of statistically significant difference. In addition, multivariate analysis including principle component analysis (PCA) and hierarchical cluster analysis (CA) was performed to explore the possible sources of heavy metals found in egg samples. In this study, PCA using data with eigenvalue greater than 1 was performed to determine a relationship of each heavy metal levels in egg contents with its presence in the environmental matrices. In addition, the number of significant component was selected on the basis of verimax rotation. Difference heavy metal concentrations and different sampling locations were grouped on the basic of similarities within a class dissimilarities among different class by using Ward's CA. All data processes were performed using the statistical software SPSS 16.0 and STATISTICA 8.0.

Results

Heavy metal concentrations in eggshells, albumin and yolk

Mean concentrations of detected seven heavy metals on dry weight basis in eggshells, albumin and yolk of each farm were shown in Table 2. Concentrations of Cd, Cu, Mn, Ni, and Pb found in eggshells of all sites were significantly the highest when comparing to albumin and yolk, whereas the highest concentrations of Fe and Zn were found in yolk. The highest concentrations of total studied heavy metals in eggshells, albumin and yolk collected from Location 3, 5 and 1, respectively. The ranked concentrations of heavy metals in eggshells were Cu (13.28±0.40 µg/g dw) followed by Pb (11.79±0.17 µg/g dw), Fe (9.09±0.10 µg/g dw), Ni (5.93±0.11 µg/g dw), Mn (3.99±0.03 µg/g dw), Zn (3.02±0.06 µg/g dw), and Cd (1.60±0.01 µg/g dw). On the contrary, the lowest concentrations of most heavy metals were found in albumin except for Cu and Zn which their lowest ones were found in yolk and eggshell, respectively.

In egg contents (yolk and albumin), most heavy metals were predominantly found in yolk except for Cu. The ranked concentrations of heavy metals found in yolk were Fe (71.96±1.33 µg/g dw) followed by Zn (46.30±0.43 µg/g dw), Pb (10.39±0.32 µg/g dw), Ni (1.85±0.07 µg/g dw), Mn (1.70±0.04 µg/g dw), Cu (1.32±0.04 µg/g dw), and Cd (0.60±0.03 µg/g dw). On the other hand, the ranked concentrations of heavy metals in albumin were Pb (6.76±0.21 µg/g dw) followed by Fe (4.99±0.53 µg/g dw), Zn (3.51±0.25 µg/g dw), Cu (2.25±0.06 µg/g dw), Ni (1.90±0.06 µg/g dw), Cd (0.58±0.03 µg/g dw), and Mn (0.43±0.03 µg/g dw).

Heavy metal concentrations in water, feed, and soil

Concentrations of all heavy metals found in water samples were relatively low (µg/mL) when comparing to soil and feed (Table 3). The highest concentration of total studied heavy metals in water, feed and soil were observed in Location 4, 1 and 3, respectively. The ranked concentrations were in the following order; Ni [(78.27±7.14) $\times10^{-3}$ µg/mL] > Pb [(32.28±5.32)$\times10^{-3}$ µg/mL] > Fe [(15.74±1.54)$\times10^{-3}$ µg/mL] > Zn [(11.33±0.82) $\times10^{-3}$ µg/mL] > Mn [(8.10±0.75)$\times10^{-3}$ µg/mL] > Cu [(3.59±0.49)$\times10^{-3}$ µg/mL] > Cd [(2.49±0.56)x10-3 µg/mL].

Most heavy metal concentrations in soil were significantly higher than those found in water and feed except for Pb which its greater concentrations were in feed (Table 3). Ranked heavy metal concentrations in soil were in the following order: Fe (1547.94±341.52 µg/g) > Mn (340.85±18.20 µg/g) > Zn (186.98±13.56 µg/g) > Cu (19.76±1.87 µg/g) > Pb (16.99±2.06 µg/g) > Ni (4.49±0.67 µg/g) > Cd (2.51±0.10 µg/g).

All investigated heavy metals were also found in feed samples. The metallic element with the highest concentrations in feed was Pb, whereas the lowest concentration one was Cd (Table 3). Ranked detected concentrations of heavy metals were in the following order: Mn (126.71±10.94 µg/g dw) > Pb (126.29±7.530 µg/g dw) > Fe (124.95±8.50 µg/g dw) > Zn (93.87±9.42 µg/g dw) >

Cu (7.62±0.65 μg/g dw) > Ni (4.88±0.63 μg/g dw) > Cd (0.82±0.21 μg/g dw).

Cluster analysis

Heavy metal concentrations found in environmental matrices from different locations were grouped with their similarities of contaminations by hierarchical cluster analysis (CA). According to the average contaminations in environmental matrices, Fe level was the highest concentration found in all locations followed by Zn, Mn, Pb, Ni, Cu, and Cd. Heavy metal contaminations in the environmental matrices were generally grouped into two categories (Figure 2A). Cluster 1 included only Fe and cluster 2 included Cu, Mn, Ni, Pb and Zn. The analysis of spatial similarities of contaminated heavy metals in environmental matrices divided all locations based on heavy metal concentrations into three clusters; i.e., 1, 2 and 3. Cluster 1 locations were 8 and 7, whereas cluster 2 locations were 5, 4, and 2. The remaining cluster 3 consisted of locations 6, 3 and 1 (Figure 2B).

Principle component analysis (PCA) of heavy metals concentrations in eggshells, albumin and yolk in correlation to environmental matrices (water, feed and soil)

Plots of principle component analysis (PCA) with eigenvalues greater than 1.00 showed that the first two components of each heavy metal residue in egg samples (albumin, yolk and eggshell) are correlated to their concentrations in the surrounding environmental matrices (Figures 3A-G). Percentages of total variances of six heavy metals were higher than 80%, except Fe which was 78.19% (Figure 3C). The PCA indicated that most heavy metal residues in yolk were correlated to their concentrations found in feed except for Ni and Pb (Figure 3C and 3F, respectively). In addition, Cu, Mn, and Zn residues in yolk were also correlated to their concentrations found in the surrounding soils (Figure 3B, 3D and 3G, respectively) whereas, Pb concentrations in yolk and albumin were correlated to its level found in water (28.08%; Figure 3F). However, Cd and Fe concentrations in eggshell were correlated to their concentration found in feed and water (Figure 3A and 3C), respectively. Whereas, the other heavy metals found in eggshell were not correlated with their concentrations found in environmental matrices (Figure 3B, 3D-3G).

Health risk estimation

Human health risk from the intake of heavy metal contaminated duck eggs was determined using the THQ values. Average egg consumption of Thai people is 132 of 50g egg/capita[5]. The Pb THQ was the highest (ranged from 0.63 to 2.28), whereas the remainders were below 1. This indicated that Pb can potentially pose human health risk. However, the total THQ (tTHQ) additionally revealed that intakes of multiple heavy metals contaminated duck eggs should be of concern since human health risk can likely be increased (Figure 4).

Discussion

Avian eggs have extensively been used for investigation of environmental pollution, especially heavy metals[10,16,19,39]. This study used duck eggs to investigate seven heavy metals contaminations and their correlations with the environmental matrices (water, soil as well as feed). From cluster analysis (CA), the studied locations were divided into 3 clusters. The resulted indicated that the nearby sampling locations have similar heavy metal profiles. In addition, CA finding also provided the evidence that the toxic metal concentrations (Cd, Pb, and Ni) found in environmental matrices were correlated to some trace metals (Cu, Mn and Zn) but not Fe. The residues of Cu, Mn and Zn in yolk were statistically correlated to those levels found in feed and soil by PCA (Figure 3B, D and G). These findings also indicated that feed and surrounding soil contaminations were potential sources of residues in yolk. In addition, the

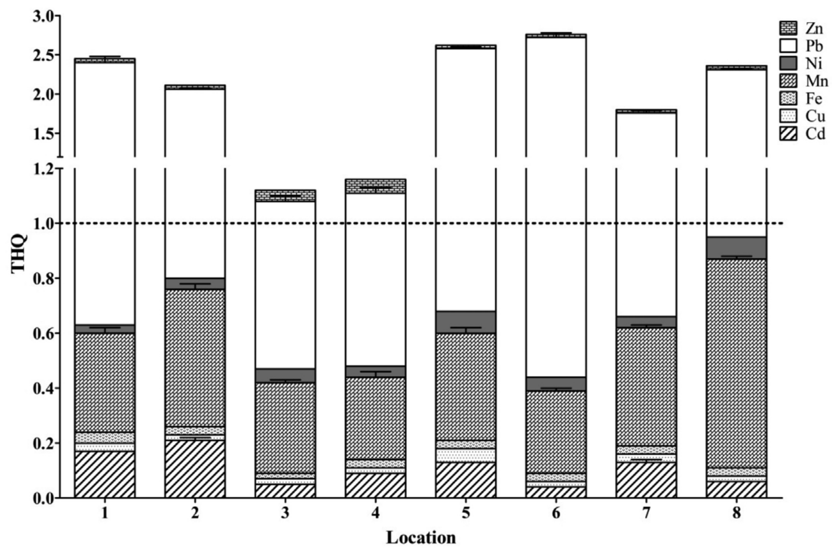

Fig. 4
THQs values of seven heavy metals in eggs (values above line indicated the potential health risk via consumption THQ>1).

deposition of Fe in eggs is associated with its level in feed. This is well related to farmer practice which uses commercial diets supplemented with essential nutrients, especially Fe, required for egg hardness, and normal growth and development of avian embryo[26]. However, in this study, all essential nutrient concentrations in were below the maximum tolerable levels of minerals in feed according to NRC (Cd 10 μg/g, Cu 100 μg/g , Fe 500 μg/g, Mn 2000 μg/g and Zn 500 μg/g)[22]. From this study, not only essential elements but also toxic metals (Cd and Ni) were detected in feed. They were positively correlated to the levels found in yolk but were lower than the NRC maximum tolerable concentrations allowance[22]. However, only Pb concentration in feed were higher than the NRC allowance limit (10 μg/g)[22]. The improvement of surrounding environments is recommended to help reducing the levels of Cu, Fe, Mn, and Zn in yolk.

Fe contaminations in egg contents and in soil samples were the highest. Moreover, the detected soil Fe concentration (4317 μg/g) was higher than a previous report (53.86 μg/g)[1]. However, all toxic heavy metal concentrations (Cd, Pb, Mn, and Ni) detected in soils were lower than the limitation of the Pollution Control Department of Thailand/PCD[25]. In addition, the average Cu concentration in eggshells was the highest among investigated heavy metals and positively correlated to its concentration in water. This finding indicated that duck eggs might accumulate Cu from contaminated water. The source of Cu probably is the copper sulfate commonly used for algae control in the agricultural areas[8,40]. In addition, only Pb concentrations in water was higher than the PCD limit of water quality[24]. This is possible since one of the major waterways that flow through Suphanburi province is the Tha Chin River where the Pb contamination report was higher than the PCD standard limit[23,24]. This indicated that uses of Pb contaminated water for agricultural purposes can ultimately deteriorate human health via duck egg consumption.

Heavy metal contaminated matrices in the surrounding environments have had impacts on residues in ducks and eggs. These metals can eventually be transferred to humans via consumption of contaminated duck eggs. Duck egg normally contains metallic elements including Fe (3.85 mg/egg), and Zn (1.41 mg/egg)[35]. Different studied locations had quite different heavy metal profiles in all matrices. Moreover, the concentrations of heavy metals detected in environmental matrices were also different among studied locations. The apparent differences among these all locations were their farm management as well as the surrounding areas, either paddy fields or industries. Good Agricultural Practice (GAP) could be beneficial for the reduction of heavy metal contaminations.

The metal with the lowest levels in egg content was Cd. This finding is in agreement with another study which Cd levels in eggs of colonially nesting waterbirds were much lower than other metals in all studied areas and species[14]. Environmental conditions and diets of the birds are expected to contribute to such differences. The significantly positive correlations of heavy metal concentrations found in environmental matrices and free range duck eggs are most likely due to common polluted source in the surrounding environments coupled with their feeding and swimming habits[6]. In addition, human health risks from ingestion of metal contaminated duck eggs is of concern. This can be indicated by using the THQ values. The THQs of most investigated heavy metals except Pb were < 1. However, the sum of all metals THQs were > 1 (the highest THQ was 2.28). Therefore, the consumption of multiple metals contaminated duck eggs could also potentially cause human health risks.

Conclusion

This findings from this study suggested that high levels of metals were detected in duck eggs and feed was the potential source of metal exposure. This study also indicated that the consumption of these contaminated eggs can pose potential human health risk base on the THQ determination. It also showed that heavy metals can be accumulated in eggshells. Thus, eggshells can be utilized as a non-invasive biomonitor for heavy metal exposure. In addition, not only environmental matrices have had impact on heavy metal residues in duck eggshells but individual farm management is also important.

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Conflict of interest

The authors declare that there are no conflicts of interest, including any financial, personal or other relationship with other people or organizations.

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