



# Metal contamination in quail meat: residues, sources, molecular biomarkers, and human health risk assessment

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## Abstract

Quail meat is an emerging source of high-quality animal protein. Quails are exposed to a wide range of xenobiotics such as heavy metals. In this study, residual concentrations of four toxic metals, of significant public health importance, including cadmium (Cd), lead (Pb), arsenic (As), and nickel (Ni), were determined in edible tissues of quails. In addition, metal loads were measured in water, feed, and litter samples collected from same quail farms as possible sources for quail exposure to heavy metals. The possible use of metallothionein (MT) and heat shock protein 70 (Hsp70) as molecular biomarkers of exposure to heavy metals was further investigated. Furthermore, the dietary intake and the potential risk assessment of the examined heavy metals among children and adults were calculated. The edible tissues of quails contained high concentrations of four heavy metals (contents (ppm/ww) ranging from 0.02 to 0.32 in Cd, 0.05 to 1.96 in Pb, 0.002 to 0.32 in As, and 1.17 to 3.94 in Ni), which corresponded to the high contents of these metals in the feeds, water, and litter. MT and Hsp70 mRNA expressions showed positive correlations with the concentrations of heavy metals in tissues indicating the possibility to use these proteins as biomarkers for quail's exposure to toxic metals. Dietary intake of quail meat and risk assessment revealed potential risks especially for children after prolonged exposure to the examined metals. Thus, legislations should be established and continuous screening of metal residues should be adopted in order to reduce the toxic metal concentrations in feeds and drinking water for quails. Reduction of exposure to heavy metals subsequently would lead to minimization of exposure of such toxicants through consumption of quail meat.

**Keywords** Heavy metals · Quail · Biomarkers · Risk assessment

## Introduction

Quails, an important source of meat and eggs production worldwide, are used as a source of meat in Egypt since the ancient times. The bird is characterized by the short production cycles, the use of small areas of space, and low production costs due to their small size and resistance to diseases, compared to chicken. Quails can solve the problem of the shortage

of red meat in many parts of the world and can partly provide humans with part of their needs of proteins, fats, vitamin, and minerals (FAO 2003).

Quails are exposed to a vast array of xenobiotics such as heavy metals, either in free-range birds or in birds under intensive rearing systems, in which the birds can be exposed to heavy metals via the animal feed, drinking water, and litter, derived from the harvested agricultural crops (Ahmed et al. 2017). These metals find their way to the human food chain via the consumption of contaminated meat. However, information on the load of heavy metals, either in such sources or in the meat and giblets of quail, is limited.

Like other organisms, birds try to detoxify their bodies from environmental contaminants via the different groups of metabolizing enzymes and transporters (Darwish et al. 2010). However, information about the biological responses of quails to heavy metals is limited.

Metallothioneins (MTs) are a group of proteins that regulate the cellular levels of metals and essential elements. For instances, upon exposure to heavy metals,

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MTs are upregulated in the liver cell lines of humans and rats, the liver tissues of penguins, and kidney tissues of ducks (Darwish et al. 2014; Kehrig et al. 2015; Darwish et al. 2016; Shi et al. 2017). The heat shock proteins (Hsps) are a family of conserved proteins, responsible for the folding, protection, degradation, and translocation of other proteins. Hsp70 is essential in preventing protein degradation, especially under the conditions of stress. In the duckweed (*Lemna minor*), Hsp70 is induced upon exposure to lead (Pb) and cadmium (Cd) (Tukaj et al. 2011). Therefore, the possible use of MTs and Hsp70 as molecular biomarkers for the exposure of quails to heavy metals is investigated in this study.

Lead (Pb), cadmium (Cd), arsenic (As), and nickel (Ni) are the major toxic metals that can find their way into the human body, mainly via the consumption of contaminated food, leading to several toxicological implications (US EPA 1991; EC 2006; Loutfy et al., 2006; FAO/WHO 2010). The exposure of humans to Pb is linked to severe complications in the nervous system and red blood cells. In addition, the exposure of children to the high levels of Pb is correlated with a significant reduction in their cognitive development and intellectual performance (EC 2006). The intake of Cd is associated with renal tubular dysfunction, osteomalacia, and osteoporosis. In addition, there is sufficient evidence that Cd-intake is associated with a high risk of lung and breast cancers (FAO/WHO 2010). The dietary intake of the elevated concentrations of As is associated with dermatological, respiratory, nervous, mutagenic, and carcinogenic effects (Lin et al. 2013). A chronic dietary exposure of humans to Ni is also associated with dermatotoxicity, lower body weight, and fetotoxicity among pregnant women (US EPA 1991).

The major task of the food safety, environmental hygiene, and poultry medicine sectors is to ensure the safety of foods, introduced to humans and to estimate the health hazards in humans if such contaminated foods are consumed. Therefore, this study was undertaken to estimate the residual concentrations of the four elements of high significance to public health (Cd, Pb, As, and Ni) in the edible tissues of quails (meat, liver, and kidney). Secondly, the concentrations of the heavy metals were measured in the feed, drinking water, and litter samples collected from the same quail farms, in order to investigate the sources of the exposure of quails to these heavy metals. Thirdly, the possible use of MTs and Hsp70 as the molecular biomarkers for the exposure of quails to the measured metals was investigated via the quantitative estimation of their mRNA expressions and determining their correlations with the metals, screened in the liver and kidney tissues. Finally, the correlations among the accumulated metals were analyzed and health risks to humans were assessed and discussed.

## Materials and methods

All experiments, using animals were conducted according to the animal use ethics of Zagazig University, Egypt.

### Chemicals and reagents

Nitric acid and perchloric acids were purchased from Merck, Darmstadt, Germany. The Revert Aid™ First-Strand complementary DNA (cDNA) Synthesis Kit was purchased from MBI Fermentas, Germany. The SYBR Premix Ex TaqII was purchased from TaKaRa, Biotech. Co. Ltd., Germany. The metal standards and other reagents were of analytical grade and purchased from Merck, Darmstadt, Germany.

### The collection and preparation of samples

All samples were collected randomly and equally from four different quail farms that depended on the battery rearing system at Sharkia Governorate, Egypt. The average flock size was  $1075.0 \pm 298.6$ . The geographical coordinates (latitude and longitude) for these farms are  $30^{\circ}43'39.1''\text{N } 31^{\circ}47'06.9''\text{E}$ ,  $30^{\circ}40'15.1''\text{N } 31^{\circ}34'25.6''\text{E}$ ,  $30^{\circ}35'25.8''\text{N } 31^{\circ}28'49.4''\text{E}$ , and  $30^{\circ}30'19.3''\text{N } 31^{\circ}19'55.4''\text{E}$ . The quail farms are located in the rural areas far from the industrial zones of Egypt. The present study was conducted from July to October 2016. The collected samples included drinking water ( $n = 20$ ; 50 mL/each sample); feed ( $n = 20$ ; 50 g/each sample); litter, including both wood dust and bird's excreta ( $n = 20$ ; 50 g/each sample); and domesticated, living male Japanese quails (*Coturnix coturnix japonica*) ( $n = 20$ ;  $5.00 \pm 1.00$  weeks of age). The birds were bled out by the severing of blood vessels in the neck, using a sharp knife without stunning specifically for this research. The Terrestrial Animal Health Code approved this method for research purposes (OIE 2017). After sacrifice, an amount of 10 g of each of the liver, kidney, and breast muscle tissues ( $n = 20$ /tissue) were collected and transferred cold to the Food Control Laboratory, Faculty of Veterinary Medicine, Zagazig University, Egypt. Samples were stored at  $-20^{\circ}\text{C}$  until the extraction and measurement of the heavy metals. Subsequently, small portions of the liver and kidney tissue samples were kept and stored at  $-20^{\circ}\text{C}$  for the extraction of total RNA.

### Analytical procedures

An amount of 1 g of each tissue sample or a volume of 1 mL of each water sample was digested in 5 mL volume of the acid digestion mixture, containing 3 mL of 65% nitric acid and 2 mL of 70% perchloric acid (Tekin-Ozan 2008; Darwish et al. 2015). The contents were left to stand overnight at room temperature in nitric acid-washed-falcon tubes. Then, these tubes were incubated at  $70^{\circ}\text{C}$  for 3 h in a water bath with

swirling at 30-min intervals during the heating period. The tubes were then left to cool at room temperature, diluted with 20 mL of deionized water, and filtered using Whatman Grade No.42 Quantitative Filter Paper. The filtrates were kept at room temperature until further analysis of the contents of the heavy metals.

### Instrumentation

Levels of As were measured by hydride generation/cold vapor atomic absorption spectroscopy and graphite furnace in case of Pb, Cd, and Ni (Perkin Elmer®, PinAAcle™ 900 T Atomic Absorption Spectrophotometer, Shelton, CT, USA).

### Quality assurance and quality control

All measurements were done in triplicate. The reference material DORM–3 (Fish protein, the National Research Council, Canada) was used to ensure the accuracy and validity of the analytical procedures of the heavy metals. The recovery rates ranged from 90 to 105%. The instrumental limits of detection (LOD), calculated against the calibration curves for the analyzed metals, were found to be 0.1, 0.005, 0.002, and 0.2 µg/g for Pb, Cd, As, and Ni, respectively. The registered values for Pb, Cd, As, and Ni were expressed as µg/g wet weight (ppm).

### RNA extraction, cDNA synthesis, and qPCR

Using the TRIzol reagent and following the [guanidinium thiocyanate-phenol-chloroform extraction](#) method, total RNA was extracted. The concentration and quality of the extracted RNA were spectrophotometrically assessed at 260/280 nm, and using electrophoresis on a 1.0% agarose gel, the integrity of the extracted RNA was visualized. cDNA was synthesized from the extracted RNA using Revert Aid™ First-Strand cDNA Synthesis Kit (MBI Fermentas, Germany) according to the manufacturer's directions. Primers were purchased from Invitrogen, Germany. The sequences of the primers for the B-actin housekeeping gene, the metallothionein (MT) gene, and the heat shock protein 70 (Hsp70) gene with their amplicon sizes are given in Table 1. Using the comparative cycle threshold (ΔΔCT) method, quantitative real-time PCR (qPCR) for MT and Hsp70 genes were normalized to the B-actin gene (Darwish et al. 2016). The qPCR reaction mixture contained 4 µL of cDNA as a template, mixed with 10 µL of 1× SYBR Premix Ex TaqII (TaKaRa, Biotech. Co. Ltd., Germany), 1 µL of the forward primer (0.5 µM), 1 µL of the reverse primer (0.5 µM), and 4 µL of distilled water. The cycle conditions were optimized as follows: 95 °C for 20 s as initial holding stage, 40 denaturation cycles at 95 °C for 3 s and annealing at 60 °C for 30 s and 95 °C extension for 15 s. Amplification of a single amplicon of the expected size was confirmed using melting curve

analysis and agarose gel electrophoresis. The experiments were repeated at least three times on different occasions. Three different samples containing the lowest levels of metal contamination were assigned as the controls in this study.

### Health risk assessment

The health risks of humans to the heavy metals were assessed for the Egyptian adults and children on the basis of the calculation of estimated daily intake (EDI), hazard quotient (HQ), and hazard index (HI) of the metals examined in the edible tissues of quails as follows:

Using the following equation, described by the Human Health Evaluation Manual, the values of EDI (µg/kg bwt/day) for the heavy metals was obtained (US EPA 2010):

$$EDI = \frac{C_m \times F_{IR}}{BW}$$

where  $C_m$  is the concentration of the metal in the sample (µg/g wet weight), and  $F_{IR}$  is the rate of the ingestion of food (meat) in Egypt, which was estimated to be 70.14 g/day of poultry muscle tissue and 8 g/day of kidney tissue (FAO 2013). The Egyptian population, especially children, commonly consumes poultry liver, including that of the quails as popular food and so, for an accurate estimation of health risks, the rate of the ingestion of liver tissue was estimated to be 70.14 g/day, similar to that of muscle tissue. BW is the body weight of the Egyptians, which was estimated to be 70 kg for adults and 30 kg for children.

The assessment of non-cancer risk followed the guidelines recommended by the US Environmental Protection Agency (US EPA 1989). As stated in the following equation, for non-carcinogenic effects, the EDI values were compared to the recommended reference doses (RfD) (4E–03, 1E–03, 3E–04, 5E–04 mg/kg/bwt/day for Pb, Cd, As, and Ni, respectively) (US EPA 2010):

$$\text{Hazard Quotient (HQ)} = \text{EDI/RfD}$$

In order to investigate the total risk of the mixed metals, a hazard index (HI) was generated using the following equation:

$$HI = \sum HQ_i$$

where  $i$  represents each metal.

A value of HQ and/or HI of > 1 indicated a potential risk to human health, whereas a value ≤ 1 indicated no risk.

### Statistical analysis

Using the Tukey-Kramer honestly significant difference tests, statistical significance was evaluated considering  $p < 0.05$  as significant. Multivariate correlations and principle component

**Table 1** Primer information used in quantitative real-time PCR analysis

Target	Forward primer (5'-3')	Reverse primer (5'-3')	Amplicon size	Reference
MT	TCAGGACTGCACTT GTGCTGC	ACCTGGGACAGGAA AGAAGC	102	Maxfield et al. (2005)
Hsp70	ATGAAACTGAGTCG CTCGCA	CAGTCTGTTGCACC TTTGCC	158	Mehaisen et al. (2017)
B-actin	GGATGCAGAAGGAG ATCACTG	CAAGTACTCCGTGT GGATCG	90	Mehaisen et al. (2017)

MT metallothionein, Hsp70 heat shock protein 70

analysis (PCA) were performed using JMP program (SAS Institute, Cary, NC, USA).

## Results and discussion

### The levels of heavy metals and metalloid

#### Cadmium

The mean concentrations of Cd (ppm/ww) were in the range of 0.02–0.78 in all the samples examined. The kidney and liver tissue samples had significantly ( $p < 0.05$ ) higher concentrations of Cd ( $0.21 \pm 0.05$  and  $0.17 \pm 0.05$ , respectively), compared to the muscle tissue samples ( $0.07 \pm 0.02$ ) (Table 2). Such a high load of Cd in the liver and kidney tissue samples may be attributed to the fact that the liver is the organ of detoxification and the kidney is the

organ of excretion (Jarup and Akesson 2009). In 70, 95, and 25% of the muscle, water, and feed samples examined, the concentrations of Cd exceeded the maximum permissible limits (MPL) (EC 2006) (Table 2). The findings of the present study are in agreement with another study that recorded high concentrations of Cd in the liver and kidney samples of free-range chicken collected from Tarkwa, Ghana (Bortey-Sam et al. 2015). Higher concentrations of Cd were recorded in the liver and kidney samples of broilers, free-range chicken, and ducks, collected from Pakistan, Turkey, Zambia, and Nigeria (Mariam et al. 2004; Uluozlu et al. 2009; Yabe et al. 2013; Ogbomida et al. 2018) (Table 3). The litter and feed samples in the present investigation had significantly higher residual concentrations of Cd, compared to the other samples (Table 2). In the current study, the concentrations of Cd, recorded in the water and feed samples, were lower than that reported in Ahmed et al. (2017).

**Table 2** Heavy metal residues in the edible tissues of quails, water, feed, litter, and percentage of samples exceeding maximum permissible limits of the tested metals

	Cd			Pb			As			Ni		
	Range (Median)	Mean $\pm$ SD	MPL %	Range Median	Mean $\pm$ SD	MPL %	Range Median	Mean $\pm$ SD	MPL %	Range Median	Mean $\pm$ SD	MPL %
Liver	0.08–0.32 (0.17)	0.17 $\pm$ 0.05 <sup>cd</sup>	0.5 0	0.66–1.11 (0.96)	0.91 $\pm$ 0.14 <sup>bc</sup>	0.5 100	0.06–0.15 (0.09)	0.09 $\pm$ 0.02 <sup>cd</sup>	0.05 100	2.88–3.94 (3.31)	3.33 $\pm$ 0.31 <sup>c</sup>	1.0 100
Kidney	0.11–0.31 (0.21)	0.21 $\pm$ 0.05 <sup>c</sup>	0.5 0	0.88–1.96 (1.22)	1.31 $\pm$ 0.37 <sup>b</sup>	0.5 100	0.12–0.32 (0.17)	0.17 $\pm$ 0.05 <sup>bc</sup>	0.05 100	2.56–3.89 (3.01)	3.04 $\pm$ 0.31 <sup>c</sup>	1.0 100
Muscle	0.02–0.12 (0.07)	0.07 $\pm$ 0.02 <sup>d</sup>	0.05 70	0.05–0.81 (0.17)	0.18 $\pm$ 0.16 <sup>d</sup>	0.1 40	0.002–0.009 (0.006)	0.006 $\pm$ 0.002 <sup>e</sup>	0.01 0	1.17–1.99 (1.45)	1.55 $\pm$ 0.23 <sup>d</sup>	0.5 100
Water	0.06–0.54 (0.29)	0.31 $\pm$ 0.15 <sup>bc</sup>	0.05 95	0.43–0.62 (0.51)	0.52 $\pm$ 0.06 <sup>cd</sup>	0.2 100	0.003–0.151 (0.011)	0.05 $\pm$ 0.02 <sup>de</sup>	0.01 30	1.65–3.76 (2.23)	2.37 $\pm$ 0.59 <sup>cd</sup>	0.07 100
Feed	0.08–0.78 (0.36)	0.37 $\pm$ 0.18 <sup>b</sup>	0.5 25	0.88–1.98 (1.11)	1.29 $\pm$ 0.42 <sup>b</sup>	1.0 60	0.17–0.33 (0.26)	0.24 $\pm$ 0.05 <sup>b</sup>	0.2 70	2.11–7.86 (4.39)	4.78 $\pm$ 1.85 <sup>b</sup>	5.0 45
Litter	0.28–1.16 (0.71)	0.68 $\pm$ 0.29 <sup>a</sup>	NA	3.76–9.87 (7.21)	6.95 $\pm$ 1.75 <sup>a</sup>	NA	0.11–0.87 (0.65)	0.63 $\pm$ 0.22 <sup>a</sup>	NA	2.21–12.31 (6.05)	6.84 $\pm$ 3.15 <sup>a</sup>	NA

Mean values in the same column that carry different superscript letter are statistically different at  $p < 0.05$

MPL maximum permissible limits according to EC (2006), % percentages of samples exceeding MPL set by EC (2006), NA not available

**Table 3** Elemental concentrations (ppm/ww) in poultry edible tissues from literature cited in the current study

Species	Sample type	Tissue	Country	Cd	Pb	As	Ni	Reference
Chicken	Broiler	Muscle	Pakistan	0.31	3.10	44.09	NA	Mariam et al. (2004)
Chicken	Broiler	Liver	Philippines	0.03	0.08	NA	NA	Villar et al. (2005)
Chicken	Market	Liver	Turkey	2.24	0.12	0.06	0.01	Uluozlu et al. (2009)
Chicken	Market	Liver	India	0.17	0.95	NA	NA	Kumar et al. (2007)
Chicken	Market	Kidney	India	0.39	1.18	NA	NA	Kumar et al. (2007)
Chicken	Market	Muscle	India	0.18	0.41	NA	NA	Kumar et al. (2007)
Chicken	Broiler	Liver	China	0.01	3.62	NA	NA	Zhuang et al. (2009)
Chicken	Free-range	Muscle	Zambia	0.01	0.23	NA	0.02	Yabe et al. (2013)
Chicken	Free-range	Liver	Zambia	1.60	4.15	NA	0.07	Yabe et al. (2013)
Chicken	Free-range	Kidney	Zambia	3.50	7.62	NA	0.08	Yabe et al. (2013)
Chicken	Broiler	Liver	Zambia	0.00	0.06	NA	0.03	Yabe et al. (2013)
Chicken	Free-range	Kidney	Ghana	0.72	0.25	0.14	0.07	Bortey-Sam et al. (2015)
Chicken	Free-range	Liver	Ghana	0.22	0.13	0.07	0.01	Bortey-Sam et al. (2015)
Chicken	Free-range	Muscle	Ghana	ND	0.01	0.04	0.55	Bortey-Sam et al. (2015)
Chicken	Market	Muscle	Bangladesh	0.03	0.17	0.03	0.39	Islam et al. (2015)
Chicken	Market	Muscle	Bangladesh	0.23	0.37	0.43	1.48	Shaheen et al. (2016)
Chicken	Market	Muscle	Argentina	NA	NA	0.003	NA	Sigrist et al. (2016)
Chicken	Market	Muscle	China	0.002	0.07	0.02	0.05	Hu et al. (2017)
Chicken	Market	Liver	China	0.004	0.09	0.04	0.06	Hu et al. (2017)
Chicken	Market	Kidney	China	0.02	0.07	0.03	0.05	Hu et al. (2017)
Chicken	Free-range	Liver	Nigeria	0.29	0.17	0.07	0.02	Ogbomida et al. (2018)
Chicken	Free-range	Kidney	Nigeria	0.89	0.59	0.08	0.02	Ogbomida et al. (2018)
Chicken	Free-range	Muscle	Nigeria	0.003	0.02	0.02	0.04	Ogbomida et al. (2018)
Duck	Market	Muscle	Poland	0.002	0.02	NA	0.01	Kalisinska et al. (2004)
Duck	Market	Muscle	Taiwan	0.04	0.05	0.01	NA	Chen et al. (2013)
Duck	Market	Muscle	Bangladesh	NA	0.005	0.03	NA	Islam et al. (2014)
Duck	Market	Muscle	Bangladesh	0.16	0.15	0.16	1.37	Shaheen et al. (2016)

NA not analyzed, ND not detectable

The rural areas in Egypt, where the quail farms are located, cultivate rice extensively, followed by burning of the rice straw that may lead to the release of Cd and Pb into the environment, where they can find their way into the body of quails via inhalation of the contaminated air

## Lead

In the present study, Pb was detectable in all the examined samples. The mean residual concentrations of Pb (ppm/ww) in the examined samples are shown in Table 2. The litter samples had significantly higher mean concentrations of Pb ( $6.95 \pm 1.75$ ), while the muscle tissue samples had the lowest residual concentrations of Pb ( $0.18 \pm 0.16$ ). All of the liver, kidney, and water samples, as well as 40% of the muscle and 60% of the feed samples, had Pb levels higher than the established MPL (EC 2006) (Table 2). The residual concentrations of Pb recorded in the liver, kidney, and muscle tissue samples of quails were in agreement with that reported in the

edible tissues of broilers in India (Kumar et al. 2007). Compared to the present study, higher contents of Pb were recorded in the liver and kidney samples of broilers and free-range chicken, collected from Pakistan, China, and Zambia (Mariam et al. 2004; Zhuang et al. 2009; Yabe et al. 2013). However, Villar et al. (2005) recorded lower concentrations of Pb in the muscles of broilers, collected from Philippines. In addition, Bortey-Sam et al. (2015) detected lower concentrations of Pb in the giblets of chicken collected from Tarkwa, Ghana (Table 3). The mean concentrations of Pb, recorded in the water and feed samples, were low, compared to that collected from the industrial areas of West Bengal, India, but corresponded to that reported previously (Ahmed et al. 2017; Kar et al. 2017).

Lead can reach quails via the inhalation of polluted air, and the drinking water, as Pb is commonly used in the manufacturing of water pipes (Renner 2009). It might also be present in the animal feed that contains bone and fishmeal (Ishii et al. 2017).



**Table 4** Correlation analysis of different heavy metals in the examined samples

Samples		Cd		Pb		As		Ni	
		Spearman	<i>P</i> value	Spearman	<i>P</i> value	Spearman	<i>P</i> value	Spearman	<i>P</i> value
Kidney	Liver	<i>0.54</i>	<i>0.014*</i>	0.04	0.86	<i>0.79</i>	<i>&lt; 0.001*</i>	0.34	0.14
Muscle	Liver	0.35	0.14	0.28	0.22	<i>0.58</i>	<i>0.007*</i>	-0.006	0.98
Muscle	Kidney	0.33	0.16	-0.08	0.73	<i>0.57</i>	<i>0.008*</i>	-0.099	0.68
Water	Liver	-0.35	0.13	0.38	0.10	<i>0.58</i>	<i>0.007*</i>	0.09	0.69
Water	Kidney	-0.58	<i>0.007*</i>	0.38	0.10	<i>0.64</i>	<i>0.002*</i>	-0.02	0.93
Water	Muscle	-0.09	0.74	0.10	0.66	<i>0.65</i>	<i>0.002*</i>	0.09	0.70
Feed	Liver	0.39	0.08	<i>0.61</i>	<i>0.004*</i>	<i>0.59</i>	<i>0.005*</i>	-0.04	0.86
Feed	Kidney	<i>0.61</i>	<i>0.004*</i>	0.003	0.99	<i>0.61</i>	<i>0.005*</i>	-0.23	0.33
Feed	Muscle	<i>0.74</i>	<i>0.001*</i>	0.08	0.75	0.43	0.06	0.351	0.13
Feed	Water	-0.05	0.82	0.12	0.61	0.38	0.10	-0.05	0.83
Litter	Liver	0.38	0.103	0.26	0.27	0.39	0.09	-0.16	0.51
Litter	Kidney	<i>0.71</i>	<i>0.001*</i>	-0.02	0.93	<i>0.64</i>	<i>0.003*</i>	-0.12	0.61
Litter	Muscle	<i>0.53</i>	<i>0.016*</i>	<i>0.62</i>	<i>0.003*</i>	<i>0.57</i>	<i>0.008*</i>	-0.03	0.90
Litter	Water	-0.48	<i>0.03*</i>	-0.05	0.84	<i>0.50</i>	<i>0.02*</i>	-0.06	0.81
Litter	Feed	<i>0.64</i>	<i>0.003*</i>	0.24	0.29	0.39	0.09	<i>0.68</i>	<i>0.001*</i>

Values in italics are those with significant correlations (*p* < 0.05)

**Arsenic**

The results, obtained in Table 2, show the residual concentrations of As in the giblets and surroundings of quails. It is clear from the recorded results that the litter samples had significantly higher residual concentrations of As ( $0.63 \pm 0.23$  ppm/ww), followed by the feed samples ( $0.24 \pm 0.05$  ppm/ww), the kidney tissue samples ( $0.17 \pm 0.05$  ppm/ww), the liver tissue samples ( $0.09 \pm 0.02$  ppm/ww), the water samples ( $0.05 \pm 0.05$  ppm/ww), and finally the muscle tissue samples ( $0.005 \pm 0.002$  ppm/ww). The levels of As in the samples, examined in the present study, corresponding to those reported earlier (Bortey-Sam et al. 2015). However, Hu et al. (2017) reported higher concentrations of As in the edible tissues of chicken and in the animal feed samples, collected from Guangdong Province, China. In addition, Mariam et al. (2004) recorded higher concentrations of As in the muscles of broiler muscles in Pakistan (Table 3). Unlikely, lower concentrations of As were reported in the muscles of ducks and chicken, collected from Bangladesh and Argentina (Islam et al. 2014, 2015; Sigrist et al. 2016). In the present study, the contents of Arsenic exceeded the established MPL in 100% of the liver and kidney tissue samples and 70% of the animal feed samples (EC 2006) (Table 2).

The reason for this high load of As in the tissue samples in accordance with the feed samples is probably due to the use of As-based feed additives in the formula of quail feed, which is similar in many parts of the world (Hu et al. 2017).

**Nickel**

Nickel is one of the trace elements found in the environment at low concentrations. In the samples analyzed, the residual concentrations of Ni followed a descending order (ppm/ww) as follows: the litter samples ( $6.84 \pm 3.14$  ppm/ww) > the feed samples ( $4.78 \pm 1.85$  ppm/ww) > the liver tissue samples ( $3.33 \pm 0.31$  ppm/ww) > the kidney tissue samples ( $3.04 \pm 0.31$  ppm/ww) > the water samples ( $2.37 \pm 0.59$  ppm/ww) > the muscle tissue samples ( $1.55 \pm 0.23$  ppm/ww) (Table 2). The concentrations of Ni in the muscle samples of quails were in agreement with that reported in muscle samples of chicken, collected from Bangladesh (Shaheen et al. 2016), but were higher than that reported in ducks, collected from Poland (Kalisinska et al. 2004), and in the muscles of chicken, collected from Turkey, Zambia, and Nigeria (Uluozlu et al. 2009; Yabe et al. 2013; Ogbomida et al. 2018). Comparing the recorded concentrations of Ni in this study with the MPL of Ni revealed that all the examined tissue samples and 45% of the feed samples exceeded the allowed MPL (Table 2). The high load of Ni in the edible tissues of quails may be attributed to the addition of mineral mixtures, containing Ni as feed supplements to the birds, to improve their productivity and prevent nutritional diseases (Shaheen et al. 2016).

It is noteworthy to confirm that the concentrations of the examined toxic metals, in the edible tissues of quails in the current study, were comparable to the levels of the metals in free-range chicken, despite being reared under controlled conditions, possibly indicating the high susceptibility of quails to bio-accumulate and bio-magnify metals, compared to the

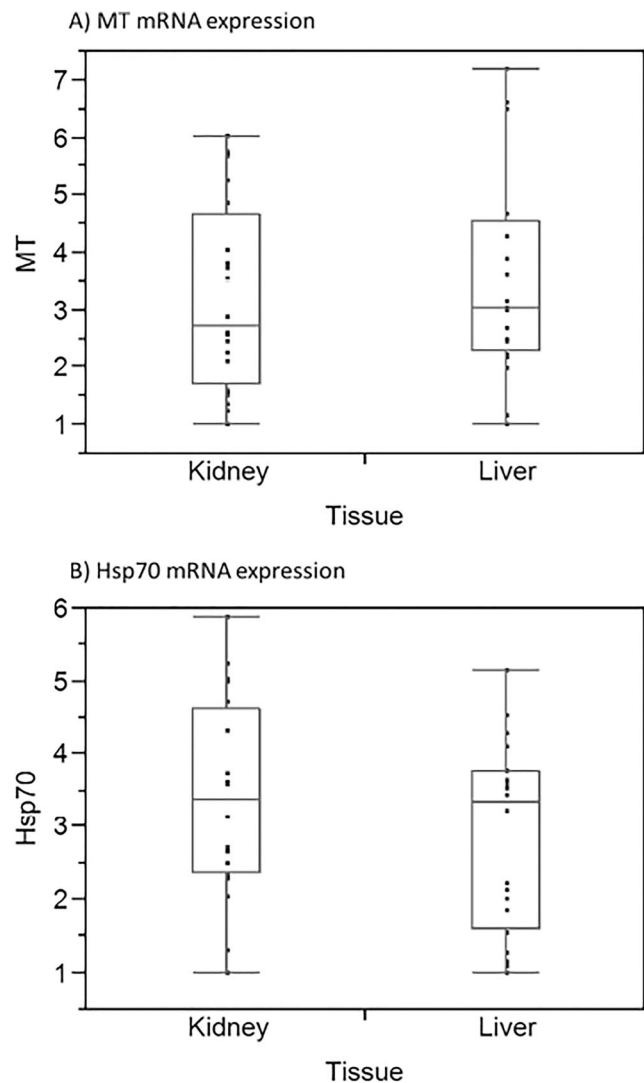
other bird species. Therefore, future studies are needed to confirm this interesting observation via the investigation of differences in the accumulation of metals among species and the mechanisms behind this phenomenon.

### The possible sources of the exposure of quails to heavy metals

In order to investigate the possible sources of the exposure of quails to the toxic metals, detected in this study, Spearman's correlation analysis was conducted (Table 4). The recorded results showed that the residual concentrations of Cd had significant ( $p < 0.05$ ) positive correlations in case of kidney vs liver, feed vs kidney, feed vs muscle, litter vs kidney, litter vs muscle, and litter vs feed. In addition, Pb showed significant positive correlations in case of feed vs liver and litter vs muscle. Interestingly, significantly positive correlations in the accumulation pattern of As were observed in all the tissue, feed, and water samples examined. On the other hand, Ni had significant positive correlation in the litter and feed samples only (Table 4). From these results, it may be suggested that bird feed, drinking water, and litter may contribute to the exposure of quails to toxic metals. The tendency of metal accumulation in the liver and kidney tissues is reasonable as the liver and kidney are the major organs of xenobiotic metabolism and detoxification (Abou-Arab 2001). Such positive correlations in the accumulation of heavy metals in the liver and kidney have also been reported in free-range chicken, sheep, goats, and cattle (Bortey-Sam et al. 2015; Darwish et al. 2015).

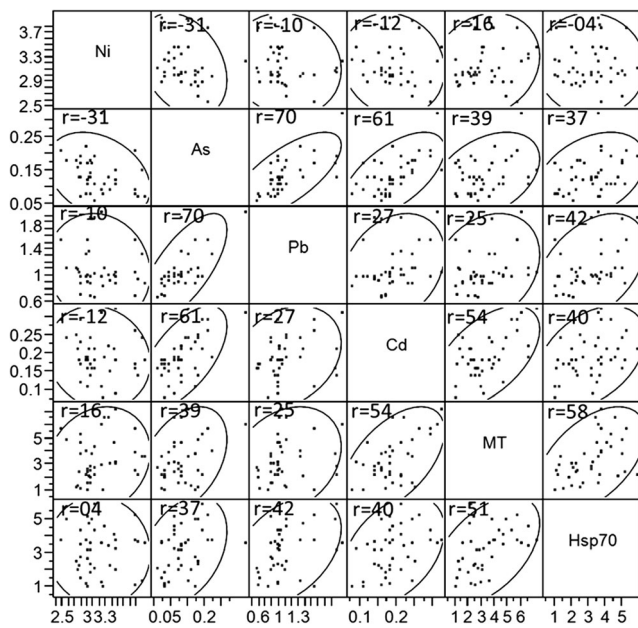
### Molecular biomarkers for the exposure of quails to heavy metals

Like other birds, quails have developed xenobiotic metabolizing systems to be protected from the adverse effects of various environmental contaminants. MTs are considered one of the best-characterized heavy metal-binding proteins (Cobbett and Goldsbrough 2002). Heavy metals like Pb can induce oxidative stress, leading to severe biological implications in many organisms (Darwish et al. 2016). The Hsps are reported to be one of the major biomarkers of oxidative stress in several species (Tukaj et al. 2011). Thus, inductions of MTs and Hsps, induced in the liver and kidney tissues of quails, are considered molecular biomarkers for the exposure of quails to heavy metals. In this study, the achieved results showed a clear induction of both MT and Hsp70 mRNA expressions in the liver and kidney tissues of quails, with significantly positive correlations with the levels of Cd, Pb, and As in these tissues (Figs. 1 and 2). The induction of MTs in the tissues of quails reflects the metal-detoxification trials in quails. In addition, the induction of Hsp70 in the liver and kidney tissues of quails indicates the exposure of quails to oxidative stress



**Fig. 1** mRNA expression of MT and Hsp70 in the liver and kidney tissues of quails. Data represent mean  $\pm$  standard errors ( $n = 20$  each) for the mRNA expressions of **a** MT and **b** Hsp70 in the liver and kidney tissues of quails based on the real-time PCR quantification

and their bio-adaptive trials. From the aforementioned results, it is possible to consider MTs and Hsp70 as possible biomarkers for the exposure of quails to the heavy metals such as Cd, Pb, and As. In agreement with this finding, Kehrig et al. (2015) reported the involvement of MTs and selenium (Se) in the detoxification of Cd, Pb, and mercury in the liver and kidney of the Magellanic penguin (*Spheniscus magellanicus*), found stranded on the Southern Brazilian coast. Furthermore, Shi et al. (2017) showed oxidative damage and kidney apoptosis in ducks in addition to a clear induction of MTs, after exposure to Cd and molybdenum. Hence, in order to establish other biomarkers for the exposure, effects, and susceptibility of birds to heavy metals and to confirm the roles of MTs and Hsp70 in responding to other metals, future investigations are needed.



**Fig. 2** Multivariate correlation analysis between the molecular biomarkers and heavy metals in the liver and kidney tissues of quails. Correlation analysis between the mRNA expressions of the molecular biomarkers (MT and Hsp70) and the measured heavy metals in the liver and kidney tissues of quails

The elevation of certain trace elements such as Se and zinc is a well-known protection phenomenon against the rise of other metals in many species (Yang et al. 2008). In this study, although the essential elements were not estimated, certain correlations were observed among the metals, recorded in the giblets of quails (Fig. 2). Interestingly, Ni had negative correlations with the tested metals as in Ni–As ( $r = -0.31$ ), Ni–Pb ( $r = -0.10$ ), and Ni–Cd ( $r = -0.12$ ). Unlikely, As had positive correlations with both Cd and Pb as in As–Cd ( $r = 0.61$ ) and As–Pb ( $r = 0.70$ ), respectively. Similarly, positive correlations between As–Hg and Cd–Pb have been reported previously (Cui et al. 2005; Darwish et al. 2015; Maia et al. 2017). An explanation to the correlation data for biological samples is not simple due to differences in the sources and metabolism of metals. However, the induction of MTs, the

enzymes linked to metal-detoxification, might be considered a possible reason (Komsta-Szumaska and Chmielnicka 1983).

**Dietary intake of metals and health risk assessment**

The values of EDI for Cd ( $\mu\text{g/kg bwt/day}$ ) are in the range of 0.03 to 0.27 in adults and from 0.06 to 0.61 in children. On the other hand, EDI values for Pb ranged from 0.18 to 1.28 in adults and from 0.35 to 2.91 in children. EDI values for As are in the range of 0.01 to 0.13 in adults and 0.01 to 0.29 in children. The highest EDIs were reported for Ni, while these values were in the range of 0.43 to 5.32 in adults and 0.81 to 12.21 in children (Table 5). The calculated EDIs for Cd, Pb, and As in both adults and children were low, compared with tolerable daily intake (TDI) values for Cd (1.00), Pb (3.57), As (2.00), and Ni (0.13) (EC 2006; FAO/WHO 2010). Furthermore, we calculated the values of HQ and HI for the metals examined (Table 5). HQ values in all tested metals did not exceed one. HI values for the four toxic metals examined (Pb, Cd, As, and Ni) were found to be higher in liver samples than one if consumed by children (Table 5). All these values were in agreement with those obtained previously (Bortey-Sam et al. 2015; Darwish et al. 2015). The values of EDI, HQ, and HI did not show high risks and hazards. However, the chronic exposure of humans to these toxic metals via the consumption of quail products and other foods may reveal potential hazards.

At many locations across Nigeria, China, and Zambia, Pb is reported to be the cause of many poisoning cases, especially in children in many locations in Nigeria (Ajumobi et al. 2014; Xu et al. 2014; Yabe et al. 2015). In addition, Pb has been reported to cause a significant cytotoxicity in the human HepG2 cells (Darwish et al. 2016). Human exposure to elevated concentrations of Cd might result in pulmonary effects such as bronchitis and pneumonia. Further, renal effects might result due to sub-chronic exposure to Cd (Young 2005). Moreover, chronic human exposure to As and Ni is associated with cardiovascular, neurological, and dermatological effects (Feng et al. 2013; US EPA 1991).

**Table 5** Estimated daily intake ( $\mu\text{g/kg BW/day}$ ) and hazard analysis of Cd, Pb, As, and Ni due to consumption of edible tissues of quails

	Adults				Children				Adults				Children									
	EDI				HQ				HI				HQ				HI					
	Cd	Pb	As	Ni	Cd	Pb	As	Ni	Cd	Pb	As	Ni	Cd	Pb	As	Ni	Cd	Pb	As	Ni		
Liver	0.17	0.91	0.09	3.34	0.39	2.13	0.23	7.78	0.17	0.23	0.32	0.17	0.89	0.39	0.53	0.76	0.38	2.07				
Kidney	0.03	0.19	0.02	0.43	0.06	0.35	0.05	0.81	0.03	0.05	0.08	0.02	0.18	0.06	0.09	0.15	0.04	0.34				
Muscle	0.07	0.18	0.01	1.55	0.16	0.43	0.01	3.62	0.07	0.05	0.01	0.08	0.19	0.16	0.11	0.01	0.18	0.46				
Sum	0.27	1.28	0.13	5.32	0.61	2.91	0.29	12.21	0.27	0.32	0.41	0.27	1.26	0.61	0.73	0.92	0.61	2.87				

EDI estimated daily intake, HQ hazard quotient, HI hazard index



## Conclusion

The present study revealed high residual concentrations of the four toxic metals (Pb, Cd, As, and Ni) in the edible tissues of quails. Quails may be exposed to these toxicants during their lifetime via contaminated feed, water, and litter. MTs and Hsp70 may be considered possible biomarkers for the exposure of quails to heavy metals. However, in order to establish other possible biomarkers for the exposure, susceptibility, and effects of quails to heavy metals, future approaches are still needed. A prolonged consumption of metal-contaminated quail products by humans may lead to several adverse effects. Therefore, there is a need to control the exposure of quails to heavy metals and, subsequently, to save people from the potential health risks.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that there is no conflict of interest.

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