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Health risk assessment of Cd, Cr, Cu, Ni and Pb in the muscle, liver and gizzard of hen's marketed in East of Iran



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ABSTRACT

The present study aimed to determine the average concentration of some metals, including cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni) and lead (Pb) in the chicken, hen's liver, and gizzard in the east of Iran. Estimated daily intake (EDI), target hazard quotient (THQ), hazard index (HI) and carcinogenic risk (CR) were calculated. In this cross-sectional study, fifty one samples including chicken, hen's liver and gizzard were obtained from Birjand, Iran. Measurement of Cd, Cr, Cu, Ni, and Pb was carried out by using an Inductively Coupled Plasma-Optic Emission Spectroscopy (ICP-OES). All of the measured metals were detected in 100 % of the samples while the Cr and Ni had the highest levels in the chicken. Pb concentration of Cd and Cu was in the liver samples while the Cr and Ni had the highest levels in the chicken. Pb concentration was at the highest level in the gizzard. The least amount of Cr, Ni, and Pb was found in the liver while Cu had the least content in the muscle. EDI had an acceptable level, but the highest daily intake of all studied metals was through muscle. Cr had the highest THQ and it was more than one in the meat. HI in chicken was more than one. Liver and gizzard of hens had a neglectable HI. CR was neglectable in the case of both Cd and Pb, but it was considerable for Cr and Ni. The consumption of chicken in both adults and children may pose a significant health risk for consumers.

1. Introduction

Nowadays, the consumer demand for more qualified food products has increased. Everybody has searched for healthy and safe foods. Due to the uniqueness of the food supply in any country, accordance of food to the developed standards to ensure consumer's safety is very vital [1]. Metal residues are considered as major contaminants of foods. Environmental pollution resulted from natural or human activity is among the most important sources of heavy metals in foods [2,3]. According to the World Health Organization report, 25 % of the diseases occurred to human is through long-time exposure to environmental contamination [4]. The poultry industry develops quickly due to the increase of demand for poultry meat. Moreover, lower price and more content of protein are the main factors of higher amount of poultry meat's consumption. Nowadays, chicken is the second consuming meat after pork throughout the world. The risk assessment studies accelerate the process of ensuring safer food production [5-8]. Chicken, hen's liver, and gizzard constitute the usual part of popular diet of people.

Some metals including copper [9] have essential roles like coenzymes [9–12]. It must be considered that these essential metals have a level of adequacy for the body (Cu 0.9 mg/day) and toxic effects have been exhibited in the higher amounts. The excess amount of Cu was associated with the liver damage [13]. Other metals such as cadmium (Cd), and lead (Pb) even with the lower content have been considered as toxic metals [14]. Unfortunately, absorption of these toxic metals easily happens from the atmospheric air and the digestive tract [15–17]. Pb has a significant role in the formation of the renal tumors, decrease of the cognitive development, increase of the blood pressure and cardiovascular diseases risk for adults. Cd may cause kidney dysfunctions, prostate and breast cancer, osteomalacia and reproductive deficiencies

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Abbreviations: Cd, cadmium; Cr, chromium; Cu, copper; Ni, nickel; Pb, lead; EDI, Estimated daily intake; THQ, target hazard quotient; HI, hazard index; CR, carcinogenic risk; ICP-OES, Inductively Coupled Plasma-Optic Emission Spectroscopy; TDI, Tolerable daily intakes; PCA, Principal component analysis; ANOVA, A one- way analysis of variance; ML, Maximum Limit; EFSA, European Food Safety Agency.

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[13]. The problem of heavy metal is severe in developing countries due to the uncontrolled level of pollution in the environment and they were reported from different foodstuffs, including meat, aquatic animals, processed foods, pistachio and honey [2,9,18–23].

Heavy metal contamination can lead to adverse health effects on human, so it is essential to determine the level of chemical contaminants in foods especially chicken due to high level of consumption to determine their potential health hazard. According to the best of our knowledge, there are no data about the heavy metal concentration in poultry and edible organs which were marketed in this region and health risk assessment was not carried out for consumers of these products. Moreover, there is no report about chromium (Cr) levels in chicken, hen's liver, and gizzard. The present study aimed to measure the mean concentration of heavy metals (Pb, Cd, Cu, Cr and nickel (Ni)) in the muscle, liver, and gizzard of hens marketed in the east of Iran. Moreover, estimated daily intake (EDI), target hazard quotient (THQ), hazard index (HI) and carcinogenic risk (CR) of chicken and edible organs were assessed.

2. Material and methods

2.1. Sampling

A total of 51 samples, including liver (17), gizzard (17) and muscle (17) of hen were obtained from three to five different retails (one was taken from each retail) in five different regions of Birjand, east of Iran (Fig. 1) (January to September 2017). Samples were labeled and placed in cooler boxes. All samples were held at -20 °C until analysis.

2.2. Sample preparation and metal analysis

All laboratory equipment and containers were washed with Nitric acid 10 % (*Merck KGaA*, Darmstadt, Germany) to ensure cleaning. After washing with deionized water, five ml of HNO₃ (65 %) and 15 mL of HCl (*Merck KGaA*, Darmstadt, Germany) were mixed with one gram (g) of the sample. Samples were held at room temperature overnight. For full acid digestion, sample was heated on heater block at 105 °C during 2–3 h. Then, the digested sample was cooled, and filtered by Whatman (Ashless no. 42) paper, and diluted with deionized water up to 50 mL. Quantification of Pb, Cr, Ni, Cu, and Cd were done through the use of an Inductively Coupled Plasma-Optic emission spectroscopy (ICP-OES) (Spectro Arcos, Germany). A standard solution mixture of studied metals

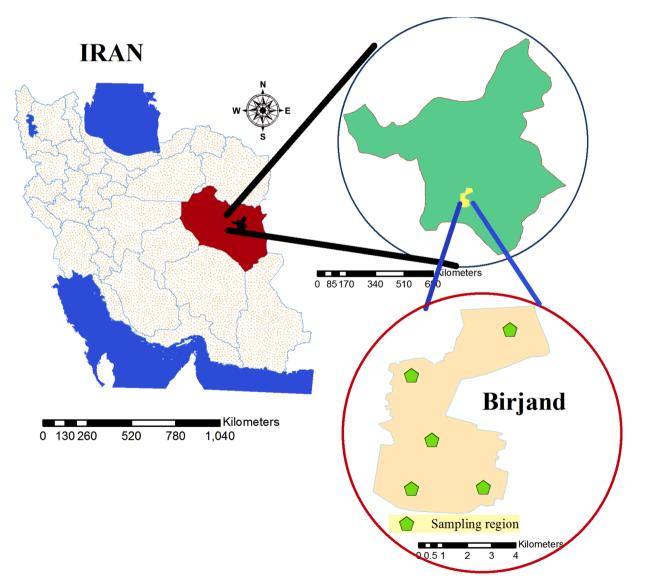


Fig. 1. Sampling map of the study.

was used to calibrate the instrument (to establish standard curves before metal analysis). In order to obtain the standard curve, the injection of standards and blank solution into ICP-OES was performed according to the protocol of the instrument. Levels of metals were stated in mg/kg (ppm) of wet weight (mg/kg WW).

2.3. Quality control

The detection limit of Cd, Cr, Cu, Ni, and Pb were 1, 1, 2, 3 and 3 ppb in this study, respectively. To perform a recovery study, different concentration of a multi-element standard solution was added to the analyzed samples and the sample was re-measured. Also, certified reference material, fish protein DORM-3 (National Research Council, Canada) was used. Recovery rates were in the range of 95 %–103 % for the studied metals. All measurements were replicated three times. The reliability of instrument was determined by injection of a blank and some standards between the working runs (relative standard deviation, RSD \leq 3%).

2.4. Estimated daily intake (EDI)

The following equation was used to calculate the EDI of toxic metals (Cd, Cr, Cu, Ni and Pb):

$$EDI = \frac{MC \times FDC}{BW}$$

Where: MC is the average level of specific metal in the food (mg/kg, ww); FDC corresponds to the average consumption of offal and muscle in a day, 3 and 32 g/day for adult (60 kg in BW) and children (30 kg in BW) respectively, (g/person/d) [24–26]; BW is the average of body weight. Tolerable daily intakes (TDI) of metals [24,27] were compared with the EDI of studied metals.

2.5. Target hazard quotient (THQ)

The THQ was calculated with the use of U.S.EPA [4] and Wang et al. [28]. Oral reference dose (RfD) for Cd, Cr, Cu, Ni and Pb is, 0.001, 0.003, 0.04, 0.02 and 0.004 (mg/kg BW/day), respectively [4,9].

$$THQ = \frac{EFr \times ED \times FIR \times MC}{RfD \times BW \times AT} \times E - 3$$

Where: EFr is the time of exposure (365 days/year); ED is the period of exposure (72 years); FIR is the food ingestion rate (g/person/d); MC is the average level of metal in the food (mg/kg, ww); RfD relates to the oral reference dose (mg/kg/d); BW expresses the average of body weight, adult (60 kg); children (30 kg); AT is the average exposure time (365 days/year × number of exposure years, assuming 72 years in this study). If the value is less than one, it means there is no health risk for consumer and if it is equal or more than one, consumers might expose non-carcinogenic health risk.

2.6. Hazard index (HI)

The HI evaluates the risk of exposure to more than one metal and measured by summation of the THQs:

$$HI = \Sigma THQ = THQ Cd + THQ Cr + THQ Cu + THQ Ni + THQ Pb$$

Where Σ THQ: sum of target hazard quotients of metals and THQ Cd, THQ Cr, THQ Cu, THQ Ni and THQ Pb are the target hazard quotients for cadmium, chromium, copper, nickel, and lead, respectively. HI value more than one represents a potential non-carcinogenic adverse effect [29].

2.7. Carcinogenic risk (CR)

CR is the possibility of developing cancer throughout the life of a person because of digestion of a potential carcinogen. Cancer risk of Pb, Cd, Cr and Ni was determined by the following equation:

$$CR = CSF \times EDI$$

Where CSF relates to the carcinogenic slope factor of 0.0085 (mg/kg/day)⁻¹ for Pb, 0.38 (mg/kg/day)⁻¹ for Cd recommended by USPEA [27], 0.5 for Cr and 0.84 for Ni according to Nduka et al. and Xu et al. [30,31]. The range of acceptable risk value is E-4 to E-6.

2.8. Statistical analysis

SPSS (version16) was used to analyze the data. Kolmogorov-Smirnov test was used to test the normal distribution of the data. One sample *t*-test was performed to compare the concentration of Cd and Pb with ML which was set by WHO and EU. The mean concentrations of metals in two seasons were compared by using Independent *t*-test. A one- way analysis of variance (ANOVA) (Tukey's test) was carried out to assess the metals in different tissues of chicken. A statistically significant difference was considered as p < 0.05. Principal component analysis (PCA) was used to measure the pattern distribution of metals and diagramed by R software (version 3.5).

3. Results

All samples were polluted with the studied metals. Table 1 shows the metal concentration in the tissues of hen. The highest content of Cd and Cu were in the liver samples of hen, while the highest levels of Cr and Ni were in the chicken (Table 1). The highest concentration of Pb was in the gizzard (0.11 mg/kg) (Table 1). Content of Cd in the chicken, hen's liver and the gizzard was significantly (p < .001) lower than ML which was set by codex as 0.05, and 0.5, respectively for meat and the edible organ of poultry [32]. Pb in the liver and gizzard was significantly (p < .001) lower than ML which was recommended for meat and edible organ of poultry as 0.1, and 0.5, respectively [32]. Chicken content of Pb was lower than ML (p > 0.05). The value of Cd, Cr, Cu, Ni and Pb in warm (summer) and cold (winter) seasons didn't have any statistically significant difference (p > 0.05) (Fig. 2). In the three different tissues, value of Cr, Cu, Ni and Pb had a significant difference, while Cd content didn't have any significant difference (Table 1).

Table 1

Mean (\pm SE) and range of metal concentrations (mg/kg ww) in the muscle, liver and gizzard of hen.

Sample type (number)		Cd	Cr	Cu	Ni	Pb
muscle (n = 17)	Mean ± SE Range	$\begin{array}{c} 0.021 \\ \pm \ 0.00 \\ 0.019 \\ - \\ 0.027 \end{array}$	$2.98 \pm 0.50^{a} \ 0.726 - 8.686$	$\begin{array}{c} 0.94 \pm \\ 0.10^{a} \\ 0.504 - \\ 2.202 \end{array}$	$\begin{array}{r} 1.82 \pm \\ 0.40^{a} \\ 0.166 \\ \textbf{-7.166} \end{array}$	$\begin{array}{l} 0.091 \pm \\ 0.01^a \\ 0.021 {-} 0.294 \end{array}$
Liver (n = 17)	Mean ± SE Range	$\begin{array}{c} 0.021 \\ \pm \ 0.00 \\ 0.016 \\ - \\ 0.027 \end{array}$	$\begin{array}{c} 0.71 \pm \\ 0.05^{ m b} \\ 0.437 \\ - \\ 1.119 \end{array}$	$\begin{array}{l} 3.30 \pm \\ 0.20^{\rm b} \\ 2.739 - \\ 6.311 \end{array}$	$\begin{array}{l} 0.10 \pm \\ 0.02^{\rm b} \\ 0.010 - \\ 0.321 \end{array}$	$\begin{array}{l} 0.04 \pm 0.00^{b} \\ 0.008 {-} 0.051 \end{array}$
Gizzard (n = 17)	Mean ± SE Range	$\begin{array}{c} 0.02 \pm \\ 0.00 \\ 0.013 - \\ 0.032 \end{array}$	1.76 ± 0.39 0.604 - 7.591	$\begin{array}{l} 1.24 \pm \\ 0.07^a \\ 0.916 - \\ 1.765 \end{array}$	$1.04 \pm 0.23^{a} \\ 0.203 - 4.284$	$\begin{array}{l} 0.11 \pm 0.00^a \\ 0.050 {-} 0.186 \end{array}$

Superscript 'a' letter in each column means significant difference with liver (p < 0.05).

Superscript 'b' letter in each column means significant difference with muscle (p < 0.05).

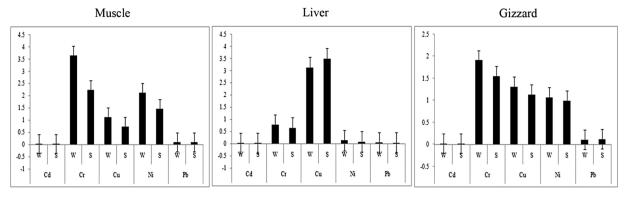


Fig. 2. Mean concentration of metals (mg/kg ww) in the winter (W) and summer (S).

Table 2 shows the EDI of metals. Daily intake of all metals was below the provisional tolerable daily intake (PTDI) recommended by US EPA [27] and ISIRI [24]. The metal concentration in the different tissues was separated (Fig. 3). The mean of Cd, Cr and Ni was correlated. Pb was highly correlated with Cu than Cd, Cr and Ni concentration. Cu level was negatively correlated with Cd, Cr, and Ni (Fig. 3). THQs of studied metals for children and adults were calculated (Fig. 4). THQ of all metals in the adults was lower than one. THQs of the liver, and gizzard had the same value. In adults, Cr in the muscle had the highest THQs, while it was more than one in children. HI was also determined (Fig. 5). According to HI, consumption of chicken in both adults and children may pose a risk. Moreover, children who consume liver may expose some health problems. The CR of exposure to Cd, Pb, Cr, and Ni through consumption of chicken, liver and gizzard was evaluated (Table 3). The CR values of Cd and Pb were acceptable just exposure to Cd through chicken may lead to some carcinogenic problems, but the CR due to Cr and Ni in chicken and edible organs was unacceptable.

4. Discussion

4.1. Level of measured metals

The mean content of Cd, Cu, and Pb in chicken was 0.21, 0.94 and 0.09, respectively. Alturigi and Albedair (2012) reported the mean level of Cd, Cu, and Pb as 5.92, 5.4 and 8.77, respectively, in dry weight of chicken in Saudi Arabia [33] which was higher than the results of the current study. It can be due to the measurement of metal in wet weight in the current study. European Food Safety Agency (EFSA) reported that poultry didn't contain the considerable amount of Cd [34]. Kidney and liver samples of poultry had higher concentration of Cd and Pb than the muscle samples [35], which was not seen in the present study. Pagan-Rodriguez et al. (2007) did not report any Cd or Pb contamination in the muscle of poultry higher than ML set by the international institution [35] which was compatible with the present study. Mahmood et al. (2015) reported a higher amount of Ni (4.1), Pb (2.75) and Cd (0.66) in the liver samples [5]. Values of heavy metal (mg/kg dry weight) in the muscle samples of poultry were as follows: Ni (4.78 \pm 3.3), Pb (2.8 \pm 0.9) and Cd (1.6 \pm 1.5) [5], which were also greater than the present study. In the current study, Cd concentration was higher in the liver than muscle which was not in agreement with another study

[5]. Among the analyzed metals, Cu had the highest level, and Cd had the lowest level in the edible organs, but Cr had the highest content in the muscle samples. In other studies, Cd was at the lowest level in poultry edibles [5]. It seems that metals aggregate in poultry muscle than the liver or gizzard except for Cu and Pb, respectively [5,36]. Results of the current study disagreed with other studies which showed the accumulation of Pb in the liver [37,38]. In the present study, Pb had higher content in the gizzard, which was proved in a previous study [25]. Storage of Pb in the edible organs of chicken can relate to pollution of soil, feeds and water. It was mentioned that Pb could be retained in gizzard [25]. The mean concentration of Cu (mg/kg) in sausage and ham samples was 1.88 and 1.48, respectively [23], which were higher than the results of chicken and gizzard in the present study. Moreover, the mean of Pb was 0.35 mg/kg in sausage samples and 0.32 mg/kg in ham samples [23] which were also higher than the results of present study. It seems that the content of metals were higher in processed foods than unprocessed ones. The mean contents (mg/kg) of Pb, Cd, Cr, and Cu in the commercial egg samples were 0.29, 0.18, 0.31, and 2.8, respectively [39] that all of the metals except Cr were higher than content of metals in chicken and offal's in the present study.

Tropism of contaminant to a specific organ in the living organisms is not uniform [40]. The content of heavy metal residue in the organ correlates with the period of exposure, the amount of ingestion, age, and breed of animal [5]. However, the route of exposure mainly affect the toxicity of the metals [5]. Some metals had lower concentration in the muscle, although the measurement of metals in the muscle is important due to the higher amount of consumption of poultry [5]. Bortey-Sam et al. (2015) reported the higher level of Pb in liver, and gizzard than the level of Pb in the present study. But they observed the lower level of contamination with Cr, and Ni in all of the samples, and also Cu and Cd had a lower content in muscle samples [25].

Moreover, in the current study Cd and Pb contaminated all of the samples but in the Bortey-Sam et al. (2015), Cd was measured in 14 % of muscles, 100 % of the liver samples and 90 % of the gizzards, and Pb was aggregated in all of the liver samples, 80 % of the gizzards and 38 % of the muscles [25]. The lower level of Pb in comparison with other detected metals may relate to major deposition of Pb in the bone and positive correlation with age [41]. In the present study, the chicken had the highest level of Ni and Cr while Ogbomida et al. (2018) observed the highest level of the mentioned metals in the gizzard [26]. As gizzard is

Table 2

EDI of metals through	h the consumption of	chicken, liver and	gizzard (µg	/kg bw/day).

Comula	Cd		Cr		Cu		Ni		РЬ	
Sample	Adults/ children									
muscle	0.011232	0.022464	1.591936	3.183872	0.503184	1.006368	0.968816	1.937632	0.048597	0.097195
Liver	0.001065	0.002129	0.035653	0.071306	0.165153	0.330306	0.005483	0.010965	0.001927	0.003853
Gizzard	0.001018	0.002035	0.088215	0.176429	0.061812	0.123624	0.051894	0.103788	0.005644	0.011288
TDI ((USEPA 2010), ISIRI, 2010)	1		1500		40		20		3.6	

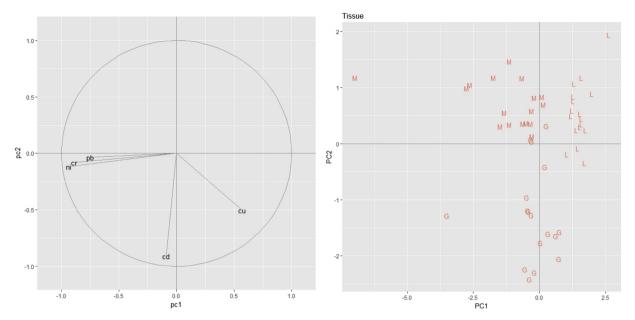


Fig. 3. Distribution of the metals in the tissues of hen.

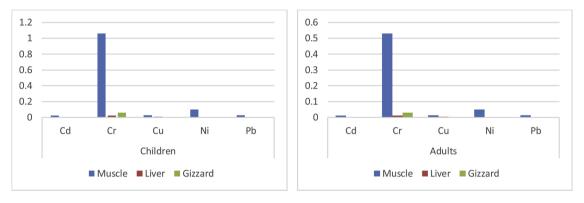


Fig. 4. Target hazard quotients (THQs) of Cd, Cr, Cu, Ni and Pb in the children and adults.

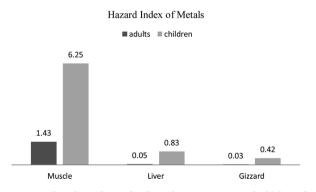


Fig. 5. Hazard Index of metals through consumption of chicken, liver and gizzard.

Table 3

Carcinogenic risk of Pb, Cd, Cr and Ni.

•				
Sample Name	Cd	Pb	Cr	Ni
Muscle	4.3E-3	4.13E-4	7.96E-1	8.10E-1
Liver	4.05E-4	1.64E-5	1.80E-2	4.60E-3
Gizzard	3.87E-4	4.8E-5	4.40E-2	4.30E-2

the main organ for grinding up ingested food, it may have the higher level of metals. Diet is considered as a major pathway of intake of heavy metal and the accumulation of metal in the animal tissue correlates with their feeds [42]. Offal indicates short time exposure to metals and is a good target organ to test the metals [26].

4.2. Lead and cadmium

Pb and Cd were categorized as number two and seven, respectively, out of the 275 most hazardous materials in the environment for human [26]. They don't have any biological role in the body, but a few levels of them can be tolerated. Higher amounts of these metals were down regulating the metabolic pathways [43,44]. Exposure of animals to these toxic metals mainly occurs through diet [26]. The main sources of Pb in the environment can relate to vast implementation of Pb in batteries, oils and fats, industry sewage, high ways traffic, and mines [5]. Moreover, the aggregation of trace amounts during a long period in humans raise a particular concern for children that may be exposed to neurotoxic effects or abnormal neural development in the long-time Pb intake [43]. The major environmental sources of Cd are oils, fertilizers and rubber car tires [5]. Animals can absorb Cd through inhalation, and ingestion route; they can also act as an indicator for Pb and Cd pollution of water, air, and soil [45]. Calcium metabolism is influenced by chronic Cd intoxication and leads to fracture of bones and skeletal deformations. Foods containing Cd are the major route of exposure in the general

non-smoking population in the most countries [15]. The mean contents of Cu, Cd, and Pb were less than 0.5 mg/kg in honey samples of Iran and the lowest one was found for lead, at a concentration of 0.11 mg/kg [21], which was higher than the present study.

4.3. Copper, chromium and nickel

Cu, Cr and Ni have been categorized as essential elements in the trace amounts, but higher levels of them have been considered as carcinogenic or toxic and influence kidneys, liver, bones or teeth [26,46]. Cu is a coenzyme and has some roles in the synthesis of collagen and connective tissues, nerves and immune system [26]. The mean value of Cu was the highest in the liver in the current study and other studies [26]. It may be due to the acting of liver as a primary storage organ [26]. Cr integrates into the metabolism of carbohydrate, fat, and protein in the body [26]. In the present study, Cr was in the range of 0.7 in the liver to 2.99 in the muscle which was higher than other studies [26,36]. In the region of present study, there are some mines from which chromite, Cu and Granit are extracted. It may be the reason for the higher level of Cr and Cu in the meat and offal of hens. The ground water of region also contains Cr in the range of 0.28–132.34 g/l [47]. Ni has essential roles in animal biology. Intake of the higher amount of Ni can lead to the reduction of body weight, damage of heart and liver, and skin irritation [26]. The release of Ni in the environment mainly occurs through fuel oil, refinery products, and effluents from mining and refining operations [48]. Ni is not a cumulative toxin but is a known toxin for blood, immune system, liver, lung and kidney [49]. Ni is one of the agents that can cause oxidative stress and leads to genotoxicity and cancer [50]. Results of the current study showed that the range of Ni is 0.1-1.8 (mg/kg WW) in the liver and muscle, respectively which was higher than the range of Ogbomida et al. [26]. The mean value of Ni in the muscle and gizzard samples of the present study was greater than 0.5 mg/kg recommended by WHO [15]. The mean of metals ($\mu g/g$ wet weight) in canned fish was 2.66 for Cr, 0.92 for Cu, and 0.22 for Ni [51], which were lower than the present study.

4.4. EDI, THQ, HI and CR

Toxicity of metals is observed at different concentrations. The comparison of EDI of metals in this study with PTDI recommended by national and international regulations showed that EDI is lower than TDI. Fig. 4 shows the THQs of consumption of chicken, liver, and gizzard. As the THQ level more than one can cause some problems, the consumption of Cr through chicken may cause some health problems for any consumer. The THQ of studied metals is lower than one indicating that people would not expose significant health hazards from the consumption of individual metals through poultry. HI level lower than one represents no hazard; one to ten relates to moderate hazard while greater than ten corresponds to the higher hazard or risk for the consumer [52]. Chicken consumers may be exposed moderate hazard as HI is more than one. HI of liver consumption in children is also close to one and may be considered [29], due to the additive effect of consumption of more than one metal. CR of Cd in muscle and for Cr and Ni in all samples were greater than E-4 which shows a potential risk of cancer through consumption of them.

5. Conclusions

Toxic heavy metals can have serious adverse health impacts on human. Metals were detected in chicken and edible organs in various concentrations. The EDI was lower than the PTDI recommended by the international organization, but THQ of Cr was noticeable which was shown the significant health hazard in the chicken. Moreover, HI of chicken should be considered in both adults and children. Also, consumption of chicken may pose some health issues due to Cd level for consumers. It showed that continuous monitoring is needed for status of metals in meat and edible organs; control of metals content during the whole production process of poultry foods; and research on the effects of supplementation of hen diet with various metal contents.

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Availability of data and materials

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study protocol was approved by the Research Institutional Review Board of Birjand University of Medical Science.

Consent for publication

Not applicable.

Authors' statement

KN designed the study, and drafted the manuscript. FS provided the overall statistical analysis of the data and drafted the manuscript. MZ drafted the manuscript and critical language editing of the manuscript. TZ participated in the design, collection of samples, and critical analysis of the data and drafted the manuscript. All authors reviewed and approved the final version of the manuscript.

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Declaration of Competing Interest

The authors report no declarations of interest.

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