Quantitative analysis of some heavy metals and trace elements residues in chicken breast fillets: levels and health implications

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Abstract

Chicken are important source for human diet because they provide a great part of nutrients, including the necessary trace elements. Heavy metals from manmade pollution sources are continuously released into aquatic and terrestrial ecosystems and therefore, the concern about the effect of anthropogenic pollution on the ecosystems is growing. As contamination with heavy metals is a serious threat because of their toxicity risk, bioaccumulation and biomagnifications in food chain, this study was conducted to determine heavy metals (lead, cadmium, arsenic, cupper, selenium and zinc) concentration in chicken breast muscle fillet using two different methods for sample digestion named wet digestion method (method 1) and ultrasonic assisted acid leaching method (method 2).

Appropriate quality assurance procedures and precautions were carried out to ensure reliability of the results. In order to determine the reliability of instruments, a blank and known standard were run after every 5 samples. In addition, a recovery study of the total analytical procedure was carried out for metals in selected samples by spiking analyzed samples with aliquots of metal standards and then reanalyzing the samples. A recovery was achieved for each element the standard curve of each element and the standards curve and regression (r^2) of each element was more than 0.99. A comparison between the results of the two methods was performed to assist the performance of each digested technique for analyzing the studied heavy metals and it was found that the ultrasonic assisted acid leaching method (method 2) was more effective when analyzing the cadmium element while the wet digestion method was more effective when analyzing copper and zinc, meanwhile the two methods had no significant difference in their results for the rest of the other tested elements . The concentration of the elements were ranged from: not detected (ND) to less than the permissible limits set by many global authorities such as WHO/FAO, ANZFA (Australia New Zealand Food Authority) and the Commission Directive (EC) of the European Union that could be attributed to contamination during secondary manufacturing or sample preparation.

Key words:

Quantitative analysis, wet digestion, ultarasonic assisted acid leaching, heavy metal, trace elements, chicken breast fillets: levels and health implications.

Introduction

Poultry meat is nowadays considered a major component of diet and source of protein. Therefore it is important to keep this source of food away as possible from contamination by any kind of contaminants. The risk associated with the exposure to heavy metals present in food product had aroused widespread concern in human health. Improvements in the food production and processing technology had in-

creased the chances of contamination of food with various environmental pollutants, especially heavy metals. Ingestion of these contaminants by animals causes deposition of residues in meat **(Sabir *et al.,* 2003).** Hence contamination with heavy metals is a serious threat because of their toxicity, bioaccumulation and biomagnifications in the food chain **(Demirezen and Uruç, 2006).** Although contamination of animal feed by toxic metals cannot be entirely avoided given the prevalence of these pollutants in the environment, there is a clear need for such contamination to be minimized, with the aim of reducing both direct effects on animal health and indirect effects on human health **(Scan, 2003).**

Therefore the determination of heavy metals in tissue and organs of poultry animals has received a great attention **(Surtipanti *et al.,* 1990; Malak *et al.,* 2007 and Iwegbue *et al.,* 2008)** due its serious effects on human health of all ages. Among the more toxic metals which accumulate in food chains and have a cumulative effect are lead, cadmium and arsenic **(Cunningham and Saigo, 1997).**

Because heavy metals are persistent contaminants in the environment that can cause serious environmental and health hazards where they are released into the environment from natural as well as man-made activities. Some heavy metals (like Cu and Fe) are essential to maintain proper metabolic activity in living organisms; others (like Pb and Cd) are non-essential and have no biological role **(Ayar *et al.,* 2009).**

Materials and Methods

1-Determination of moisture content:

Moisture content was determined by drying samples to a constant weight in an electric oven at 120±5 ^{0}C, and was calculated as percent of water loss. (**Ubesena,2010**).

2-Analyticalprotocols:

A-Washing procedure:

Washing of the glassware and plastic filmis an important process to avoid any source of contamination especially when trace elements or heavy metals are analyzed. The test tubes, polyethylene tubes and glassware were soaked in water and soap for 2 hours then rinsed several times with tap water. After that glassware has been rinsed once with distilled water, once with mixture (consisting of 520 mL deionized water, 200 mL concentrated HCl and 80 mL H_2O_2), and once with washing acid(10% HNO_3). Finally, they were washed with deionized water then air-dried inincubator away from contamination ordust (**El-Mowafi, 1995**).

B-Digestion methods:

- **Method 1** (wet digestion method) according to **(Al Ghais, 1995):**

The muscle samples were weighted and placed in polyethylene bags and stored at – 20 °C till the time of analysis. At the time of analysis 2.00g of each muscle sample were weighed into a 100mL digestion bottle. 10mL of a digestion mixture (4:1 65%v/v HNO_3 and 70%v/v $HCIO_4$) were added to the meat samples **(AlGhais, 1995).** The bottles were tightly closed and the contents were gently swirled and allowed to initial digestion at room temperature for 3-4 hours followed by carful heating to 40-45 °C for one hour to prevent frothing. The digestion temperature was raised to 70 – 80 °C with gentle shacking until digestion process was completed. At that point all the organic matrixes must have been destroyed. Finally, the digest was allowed to cool and then transferred into a 20mL standard flask rinsing with de-ionized water and later made up to mark with de-ionized water. The solutions were transferred into acid-leached polyethylene bottles and kept at room temperature until analysis with AAS.

Sample blanks were prepared by taking 10mL of the reagents mixture through the same procedure.

- **Method 2** (ultrasonic assisted acid leaching method) according to **(Manutsewee *et al.,* 2007):**

An ultrasound-assisted solid–liquid extraction procedures using diluted mixed acid solution was used for determination of the concerned heavy metals in chicken muscle samples. A 30-min sonication, 56°C operating temperature and 6mL of 1:1:1 HNO_3 (4M):HCl(4M):H_2O_2 (0.5M) were used for 0.5g of dried sample.

The mixture was centrifuged at 3000 rpm for 10 min. The liquid phase was transferred to a 10 mL volumetric flask. The solid residue was washed with 2 mL of de-ionized water, then centrifuged at 3000 rpm for 15 min, transferred to the same volumetric flask and the volume was adjusted to 10 mL with de-ionized water. This solution was kept in a refrigerator at 4 °C before measurement. The metals in the leachates were determined by AAS techniques (flame atomic absorption spectrometry for cadmium, lead, copper and zinc while the graphite flame atomic absorption spectrometry (GFAAS) techniques were used for the determination of arsenic and selenium in the digested samples.

A nickel solution containing 2.5 g/l Ni, used as chemical modifier, was prepared by dissolving an appropriate amount of Ni $(NO_3)2$ in 0.1 mol/l HNO_3 for selenium determination using graphite furnace atomic absorption spectrometry.

C- Determination of recovery rates according to (Nick *et al*., 2012):

Muscle samples were spiked with 1,2or 3mL of the various standard solutions of the heavy metals under study. Triplicate spiked sample were carried through the digestion reaction. The percentage recovery was calculated as:

% Recovery = (X-Y) x 100/Z

Where X = Concentration of the spiked sample

Y = Concentration of the un-spiked sample

Z = Concentration of the metal ion added

Standard curves for the metal analytes (As, Cd, Pb, Cu, Zn and Se) were prepared from stock solutions (standard concentrations of 1000mg/ l) of metal analytes. To cover the optimum emission working range (0.01 to 5.00 mg/ml) further serial dilutions was prepared. Usually freshly stored standard curves in the system software where available and was used. Blank solutions were be also prepared accordingly **(Mohammed *et al.*, 2013).**

Statistical Analysis:

The data collected were presented as mean and standard deviation and were subjected to one way analysis of variance (ANOVA) (P≤0-05) to assess whether heavy metals varied significantly. All statistical calculations were performed with SPSS 9.0 for Windows **(Ozdamar, 1991).**

Results and Discussion

Table (1). Descriptive statistics of dry matter %.

	N	Minimum	Maximum	Mean	
				Statistic	Std. Error
Dry matter %	50	23.630	26.347	25.15032	0.114713

(mg/kg, origin matter)

Table (2). mean recovery of each method in relation to each tested heavy metal.

	Recovery Rate %					
	Arsenic	Cadmium	Lead	Cupper	Zinc	Selenium
wet weight method 1	98.12	101.085	106.16	90.88*	98.78*	92.08
dry weight method 2	98.39	**99.679***	99.59	83.97	92.42	91.97

*Methods have significance difference in the obtained results of corresponding heavy metal concentration in relation to the opposite method.

*statistical significance at P≤0.05

Table (3). Statistical analysis of heavy metals determinations using two different digesed methods after correction of results to be in wet weight basis.

The metal	N° of samples	Mean	Standard. Deviation	Minimum	Maximum	*Significance
Lead wet weight method	50	0.02554	0.181	0.000	1.277	0.980
dry weight method	50	0.02465	0.174	0.000	1.232	
Cadmium wet weight method	50	0.0000400	0.00028284	0.00000	0.00200	0.000
dry weight method	50	0.0032004	.00288047	0.00000	0.00791	
Arsenic wet weight method	50	0.37808	0.216857	0.000	0.771	0.990
dry weight method	50	0.37862	.227935	0.000	0.801	
Cupper wet weight method	50	0.025689	0.0070909	0.0169	0.0382	0.000
dry weight method	50	0.020937	.0041256	0.0116	0.0256	
Zinc wet weight method	50	0.617586	0.0709996	0.5665	0.7155	0.000
dry weight method	50	0.479993	.0419196	0.4194	0.5601	
Selenium wet weight method	50	0.202540	0.0652022	0.0665	0.3155	0.745
dry weight method	50	0.206524	.0565285	0.1261	0.3493	

n = number of samples; min = minimal levels; max = maximal levels; sd = standard deviation
* statistical significance at P≤0.05

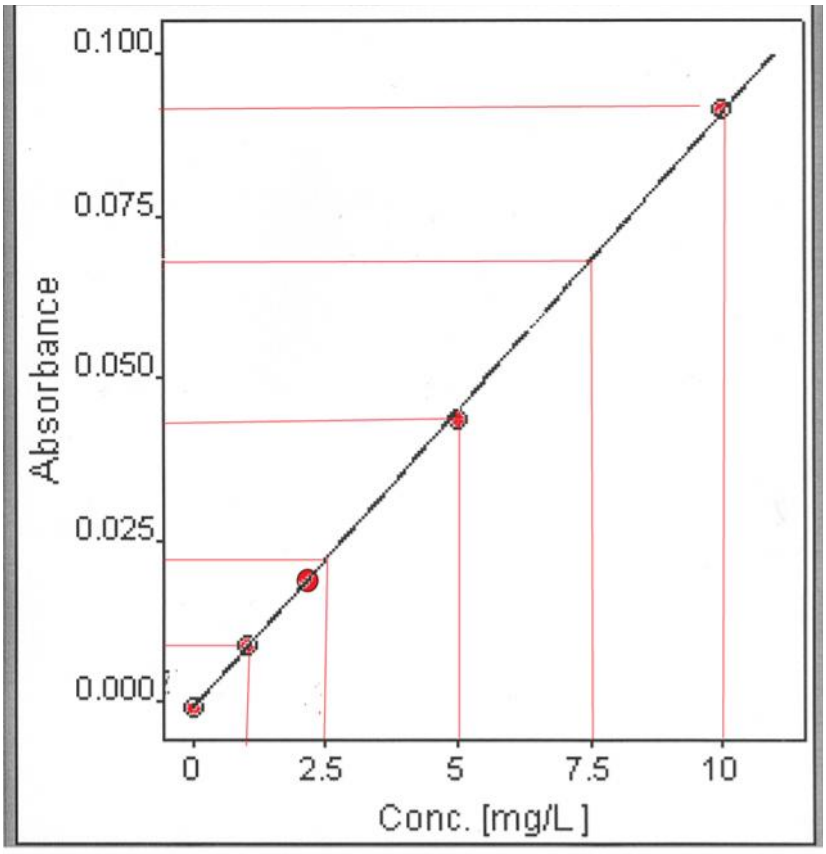

Figure (1). Standard calibration curve of arsenic (r2 = 0.993).

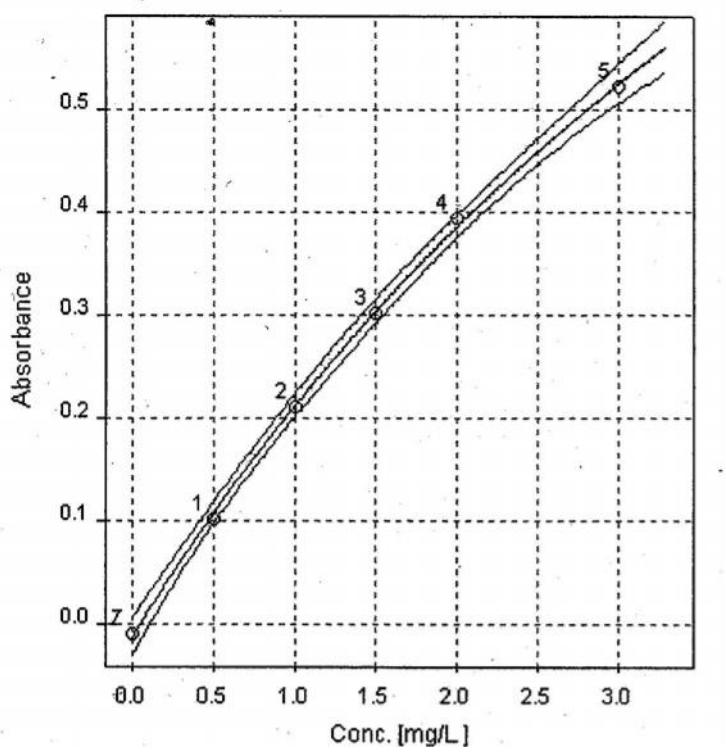

Figure (2). Standard calibration curve of cadmium (r2 = 0.9985).

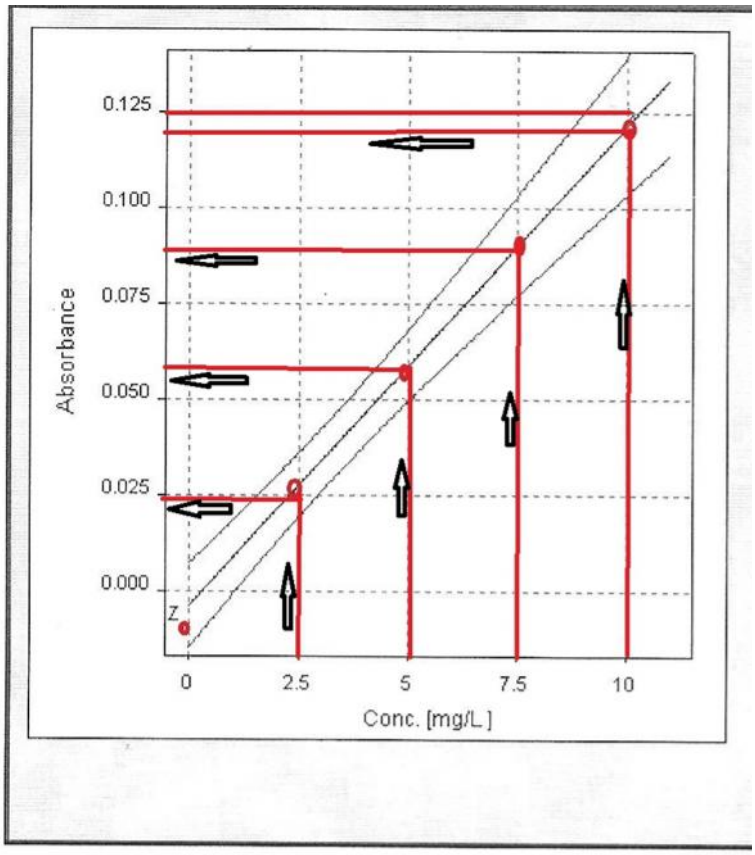

Figure (3). Standard calibration curve of lead (r^2 = 0.9932).

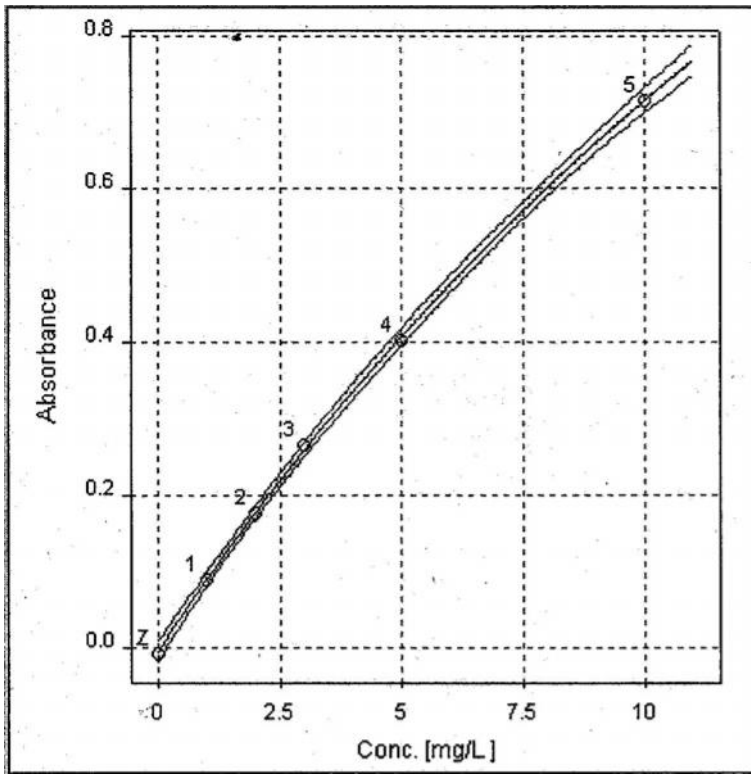

Figure (4). Standard calibration curve of cupper ($r^2 = 0.9994$).

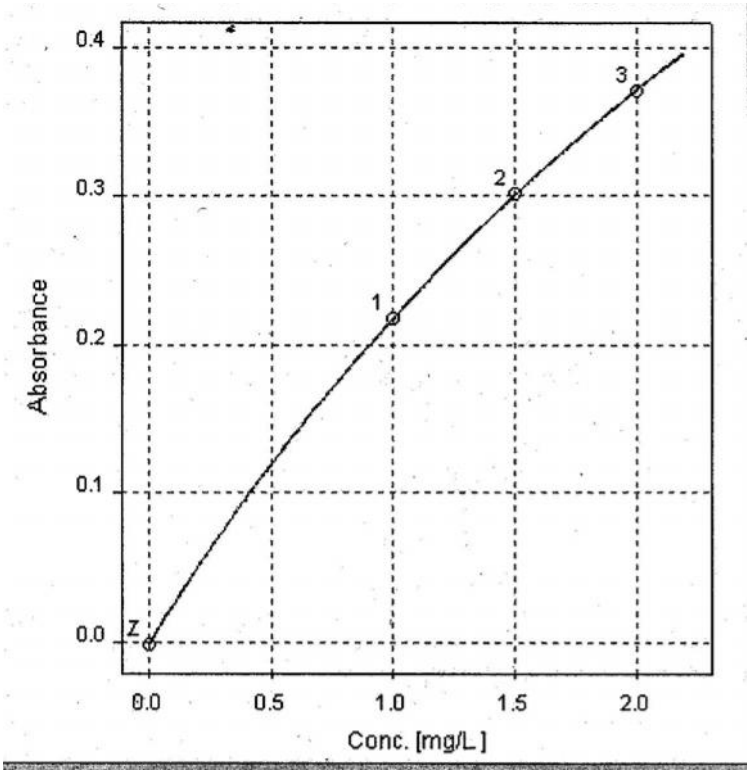

Figure (5). Standard calibration curve of zinc ($r^2 = 0.9999$).

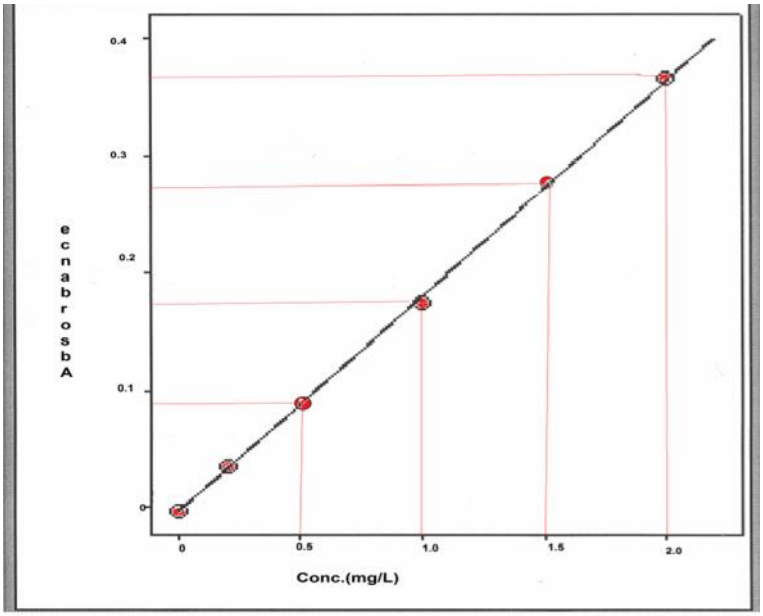

Figure (6). Standard calibration curve of selenium ($r^2 = 0.9975$).

The pollutants often have direct physiological toxic effect becausethey stored or incorporated in tissues, sometimes permanently **(Mariam *et al.* 2004 and Demirezen and Uruc, 2006).** So, the risks of heavy metals are potential environmental contaminants with the capability of causing human health problems if present to excess in the food we eat. They are given special attention throughout the world due to their toxic effects even at very low of concentrations **(Das, 1990).** Several cases of human disease, disorders, malfunction and malformation of organs due to metal toxicity have been reported **(Jarup, 2003).**

Trace heavy metals are significant in nutrition, either for their essential nature or their toxicity. Copper and zinc are known to be essential and may enter the food materials from soil through mineralization by crops or environmental contamination with metal-based pesticides. Excessive intake of trace elements has been reported to be toxic **(Fairweather-Tait, 1988).**

Table (1) represented the moisture contents in chicken breast muscle fillets with a mean per cent value ± standard error of 25.15032 ± 0.11% where the results of the method using dry weight were corrected for the purpose of comparison with the results of method that used wet weight in analyzing heavy metals.

Most of the inorganic arsenic in chicken comes from an arsenic-based drug called roxarsone, which is added to chicken feed as a growth promoter and as an antimicrobial, although some arsenic exists in drinking water. Technically, the FDA requires chickens to be fed a roxarsone-free diet for five days before slaughter to allow any residues of the drug to pass through chickens' systems. Roxarsone, and a chemically similar drug, nitarsone, remain the last approved uses of arsenic in food production. Roxarsone, known by its brand name 3-Nitro, kills poultry intestinal parasites, promotes growth and makes meat look pinker. It contains organic arsenic, which is far less toxic than its inorganic counterpart.

In this study, the range of accumulation of arsenic (As) in chicken breast fillets was in the range of (not detected (ND) – 0.771 ppm (method 1) and of ND – 0.801 ppm (method 2) with no significant difference between the two methods table (3). Even though the results-were less than the permissible limit set for As (2.0 ppm) in tissue of poultry **(ANZFA, 2001). Ubesena *et al.*, (2010)** found As in chicken breast muscles and the concentration was ranged from ND to 2.60 ppm on dry basis.

However, arsenic levels in broiler meat did not violate the tolerance levels for human consumption (2,000 µg/kg for liver and kidney; 500 µg/ kg for muscle) set by the US FDA (**FDA Regulations, 1992)**. This result suggests that arsenic contaminated drinking water and feed can play a vital role in the elevation of arsenic in broiler tissues. Although relatively small amounts of arsenic was obtained in tissues, but it must be taken into account due to its detrimental effects on consumers and the tolerance level for arsenic residue in edible tissues must be changed in the purpose of avoiding human health from arsenic toxic effects.

Cadmium compounds are used as color pigment and in re-chargeable nickel-cadmium batteries. It is also present as a pollutant in phosphate fertilizers. **Jarup *et al.,* (1998)** pointed out that cadmium is present in most foodstuffs, but concentrations vary greatly. Cadmium exposure may cause kidney damage and/or skeletal damage **(WHO, 1992; and Jarup *et al.,* 1998).**

Cadmium (Cd) in chicken breast fillet samples of this study are below the highest permissible hygienic limits for Cd (muscle 0.1mg/kg; Codex 1996, FAO/WHO, 2000)and the maximum levels of cadmium in meat of chickens allowed by the Commission Directive (EC) 466/2001 of 0.05 mg/kg of fresh weight, **(Anonymous, 2001).** The findings of this study were similar to that reported in **(Skalická, *et al.,* 2002 and Akan *et al.,* 2010)** from the safety level point of view as the results in table (3) showed that maximum concentration of cadmium using method (1) was 0.002 ppm while it was 0.0079 ppm when using method (2) with a significant difference between the two methods that could give a chance to use the method (2) named (ultrasonic assisted acid leaching method) when analyzing sample for cadmium. Method (2) had recovery rate of **99.679**% while method (1) had 101.085%, so these figures might indicate that the measurement of digestion efficiency may not indicated through the calculation of the recovery rate of spiked samples only but the real sample may play a role in differentiating between two methods of digestion as there may be many other factors in the real samples that does not present in the spiked sample and can the digestion process.

Airborne lead can be deposited on soil, water and plants thus reaching human via the food chain. Lead is accumulated in the skeleton and cause renal tubular damage and may also give rise to kidney damage **(WHO, 1995)**. International Agency for Research on Cancer (IARC) classified cadmium and lead as human carcinogen **(IARC, 1993).**

Lead (Pb) was not detected in any of the samples analyzed except in one sample out of the fifty analyzed samples when using the two method of digestion where the concentration of lead in this only sample was 1.277 and 1.232 ppm in method (1) and method (2) respectively. The authors suggested that this sample might be contaminated during processing through utensels, packaging or water supply. Pb occurs in tap water and ground water to a degree, but existing data suggest that Pb concentration is rather low with a maximum permissible contaminant level of 0.01 mg/L. **(EOSQS, 1993 and WHO, 2011).** In other studies, Pb was detected in poultry feedstuff **(Okoye *et al.,* 2011)** and tissue samples including poultry meat **(Iwegbue *et al.,* 2008).** It is a favorable condition that Pb was not detected in all the samples because Pb a potentially toxic substance with no known physiological functions. Pb toxicity affects the hematologic, renal and neurologic systems and there is no evidence for a threshold below which Pb has no adverse effects, especially in children health **(Needleman *et al*, 1979; Goyer, 1993 and Salwa *et al.,* 2012).**

It should be highlighted that food is a principal source of copper (Cu) as an essential element for humans. In this study, the average Cu levels ranged between 0.017–0.038 ppm with a mean ± SD of 0.026 ± 0.007 when using the method (1) while the concentration range was 0.012 – 0.026 ppm and a mean ± SD of 0.021 ± 0.004 ppm for when using method (2) respectively. It was found that there was a significant difference in Cu levels between the results of the two methods as method (1)

higher concentrations than that of method (2). **Mariam *et al.*, (2004)** reported higher concentration of copper in mutton, beef and poultry meat from Lahore region.

Although Cu is an essential element, it is toxic and the maximum limit of intake was set from 1 to 10 mg/day. Our, results indicated that chicken breast fillets were below the permissible limit of 200 ppm **(ANZFA, 2001)** but we recommended that chicken ration should supplemented with adequate amount of cupper that does not the chicken health but give a reasonable amount of cupper in their tissues after slaughter to share in the overall requirements of consumer need.The addition of copper sulfate (125 or 250 mg kg $^{-1}$) to broiler chicken diet enhances weight gain and feed efficiency **(Miller *et al.*, 1986)** while Fe and Zn are supplemented to balance the ratio **(Tazisong *et al.*, 2005).**

Zinc is an essential element in our diet, but too little or too much can be harmful. Zinc concentrations were found to be highest in meat, liver, fish and eggs **(Janet and Carl, 1994).**

In this study, concentrations of zinc (Zn) ranged between 0.057–0. 72 ppm with a mean ±SD of 0.63±0.071 and 0.42 – 0.56 ppm with a mean ±SD of 0.48±0.042 in chicken fillets when using method (1) and (2) respectively and chicken gizzard, respectively. The concentration of Zinc in samples hada significant difference between the two methods, so it is preferable to use the wet digestion method (method 1) when analyzing zinc in chicken filets. **Mariam *et al.*, (2004)** reported mean levels of 28.53 ± 3.39 ppm Zn in lean meat of poultry in Lahore. It was observed that all the values in the studied samples were below the permissible limit (150 mg.kg-1) set by the Australia-New Zealand Food Authority **(ANZFA, 2001).**

The potential toxicity of selenium was first suspected over 50 years ago, and through the years, well defined syndromes of toxicity have been described in animals and humans living in somniferous areas when the soil content is relatively rich in selenium, contributing to relatively high selenium in vegetation. Many of these plants are consumed by livestock and within a few weeks cause a disease syndrome **(Hogberg and Alexander, 1986).** Although the permissible levels of selenium for most animal species ranges between 0.1 and 0.2 mg Se/ kg diet, it is clear that excess level over requirement reflects the toxicologic potency of the metal. Multiple roles played by selenium in the maintenance of the homeostatic condition in animals are still being discovered. However, the need for adequate selenium nutrition in both humans and food production animals appears to be less than optimal in many parts of the world **(Edens and Brake 2006).**

In case of selenium (Se) it was found that concentrations of Seranged between 0.06 –0. 32 ppm with a mean ±SD of 0.2±0.07 and 0.12 – 0.35 ppm with a mean ±SD of 0.21±0.06 in chicken filets when using method (1) and (2) respectively. The concentration of Zinc in samples had no significant difference between the two methods. **Salwa (2012)** found selenium in the pectoral muscle with a mean concentration of 0.60 ± 0.21 when analyzed in dry basis that could be nearly the same as our results when converted to wet weight basis.

Selenium is recognized as an essential micronutrient in animal and humans, playing important biological roles as antioxidant, as a regulator of thyroid hormone metabolism or as anticarcinogenic agent. Our results were below the maximum value of Se 2 μg g-1 recommended by **(ANZFA, 2001).**

Conclusion

It was concluded that although contamination of animal feed by toxic metals cannot be entirely avoided given the prevalence of these pollutants in the environment, there is a clear need for such contamination to be minimized, with the aim of reducing both direct effects on animal health and indirect effects on human health. Feeds supplement added to chicken diet should be measured and calculated its residues in meat to avoid undesirable increase in their amounts.

We recommend the need to identify sources of pollution in the local places of raising poultry and reduction of these sources through direct

supervision, as well as activating the role of censorship on imported foodstuffs and the detection of heavy metals due to their influence on human health.

This study set out to elucidate the concentration distribution of this study was conducted to determine heavy metals (lead, cadmium, arsenic, cupper, selenium and zinc) concentration in chicken breast muscle fillet. A generally comparative assessment of the result shows that are within safe limits for consumption. These levels of heavy and trace elements in analyzed samples could result from contamination of the feed, water source, the environment, the processing and packing area.

The information provided herein will be essential to frame guidelines and standards for heavy metals in feedstuff and meat products in Egypt. We recommend the need to identify sources of pollution in the local places of raising poultry and reduction of these sources through direct supervision, as well as activating the role of censorship on imported food and feedstuffs and the detection of heavy metals in food of animal origin due to their influence on human health.

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التحليل الكميى لمتبقيات بعض المعادن الثقيلة والعناصر النادره فى فيليه صدور الدجاج : المستويات والاثار الصحية

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الملخص العربى

لحوم الدجاج مهمة لغذاء الانسان لانها توفر جزاءا كبيرا من المواد الغذائية بما فى ذلك العناصر النادرة الضرورية للوظائف الحيوية، يتم اطلاق المعادن الثقيلة من مصادر التلوث من صنع الانسان باستمرار فى المصدر المائية والبرية وبالتالى فان القلق حول تأثير حجم التلوث البشرى على المصادر المائية اخذ فى الازدياد كما ان التلوث بالمعادن الثقيلة بشكل تهديدا خطيرا لما لها من السمية، والتراكم الحيوى فى السلسلة الغذائية لذلك تم اجراء هذه الدراسة لتحديد تركيز المعادن الثقيلة (الرصاص – الكادميوم – الزرنيخ) والعناصر النادره (النحاس ، السيلينيوم والزنك) فى عضلات فيليه الدجاج وقد اجريت باستخدام طريقتين مختلفتين لهضم العينات طريقة الهضم الرطب (التجربة 1) واستخدام لموجات فوق الصوتية بمساعدة طريقة الترشيح بالحامض (التجربة 2) وتمت اجراءات ضمان الجودة المناسبة والاحتياطات الى ضمان موثوقية النتائج فى الطريقتين المستخدمتين فى هذه الدراسه، تم غسيل وتنظيف جميع الاوانى الزجاجية بعناية فائقة الحامض والماء المقطر من اجل تحديد توثيق النتائج تم قياس المحاليل القياسية الضابطه بعد كل 5 عينات بالاضافة الى ذلك اجريت دراسة معامل الاستخراج من اجمالى الاجراءات التحليلية لاستخلاص المعادن فى العينات المختارة من قبل فى العينات التى تم تحليلها مع مراعة عدد من معايير المعادن القياسية ثم اعادة تحليل العينات وكان معدل التصحيح الذى تم تحقيقة لكل عنصر و (R2) لمنحنى مستوى كل عنصر اطثر من 99 تم اجراء مقارنة بين نتائج الطريقتين لمساعدة اداء كل طريقة الهضم لتحليل المعادن الثقيلة التى تم دراستها وتبين ان الموجات فوق الصوتية بمساعدة طريقة الرشح بالحمض (تجربة 2) كان اكثر فعالية عند تحليل عنصر الكادميوم فى حين ان كان اسلوب الهضم الرطب اكثر فعالية عند تحليل النحاس والزنك وفى الوقت نفسه تبين من اجراء الطريقتين انه لا يوجد فرق كبير فى نتائجها فى بقية العناصر الاخرى المختبرة طبقا للمواصفة القياسية المصرية وقد تراوح تركيز العناصر من لم يتم الاستدلال علية الى اقل من الحدود المسموح بها التى وضعتها العديد من السلطات العالمية مثل منظمة الصحة العالمية / منظمة الاغذية والزراعة ANZFA (الهيئة العامة للغذاء – نيوزيلندا) والمفوضية (EC) من الاتحاد الاوروبى الا فى عينة واحدة من عينات تم العثور على خمسين من 2،1 جزء فى المليون تركيز الذى يعزى الى التلوث اثناء المعالجة وخلص الى انه على الرغم من تلوث الاعلاف الحيوانية المعادن السامة لا يمكن تجنبها تماما نظرا لانتشار هذه الملوثات فى البيئة وهناك حاجة واضحة لمثل هذا التلوث على ان يكون الحد الادنى وذلك بهدف الحد من الاثار المباشرة على الصحة الحيوانية والاثار غير المباشرة على صحة الانسان.

الكلمات الدالة: التحليل الكمى – طريقة الهضم الرطب واستخدام الموجات فوق الصوتية بمساعدة طريقة الترشيح بالحامض معادن.