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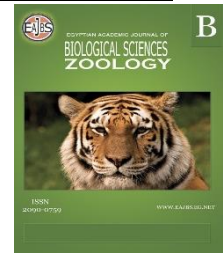


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Evaluation of Heavy Metal Residues in Poultry Farms in Ismailia Province

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ABSTRACT

Heavy metals pollution, along with environmental pollution, is increasing every day. It contaminates foodstuff. Moreover, in the last few years, the frequency of renal failure and liver cirrhosis in a human was markedly increased which could be related to heavy metal pollution in Egypt. The present study was designed to investigate the presence of heavy metals (lead, cadmium and iron) as well as macro elements (sodium, calcium and manganese) in drinking water (tap and well water), feed and tissue residues in 5 different broiler's farms (A,B,C,D and E) in Ismailia Governorate. Farm A was in Fayed area, farm B was in El Tall El Kebir area, farm C was in Abu Khalifa area, farm D was in Taie area, and farm E was in Sarabium area. Both farms A and B used tap water while farms C, D and E used well water. Levels of lead (Pb) were non-detectable or within the permissible limits stated by the World Health Organization (WHO) and Egyptian Organization of Standardization (EOS) in all water types, feed and tissues. The levels of cadmium (Cd) were non-detectable or within permissible limits in both water types, while it was detected in the feed of all farms and exceeded the permissible limit in farms B and C with increased tissue residue in farm B. Iron (Fe) water level was non-detectable in farm A, while it was detected in the other farms and were within the permissible limit. Iron feed and tissues levels were considerably high in broilers in all tested farms. Sodium was detected in higher levels in water and feed of farms C and D with exceeding permissible limits in the water. The sodium (Na) level was significantly higher in kidney samples of farm C than in other farms. Calcium (Ca) levels were higher in the water of farms C and D than permissible limits with significantly higher kidney residue in farm D than others. Magnesium (Mg) was detected in water within the permissible limit in all farms with more values in Farms C and D that were reflected as significant elevations in tissue residues of the former farms. Levels of heavy metal are variable between different farms under investigation with high levels of lead and cadmium in farms that used tap water for broilers farms that were attributed to the nearness of human activities to such farms.

INTRODUCTION

Poultry is the largest supplier of animal protein and a good source of essential amino acids, vitamins and minerals for humans especially in developing countries where chicken is relatively cheap (Taha, 2003; Hassanin *et al.*, 2014). The commercial poultry industry is well developed all over the world due to its easy digestibility, availability, acceptance and low price compared with other meat. Unfortunately, Chickens, either free-range or under intensive rearing systems, are exposed to a massive array of heavy metals through contaminated feed, drinking water and litter (Ahmed *et al.*, 2017; Hu *et al.*, 2018). Where feed contamination can affect livestock health, reproduction and productivity, at the same time, may reduce the safety of animal-derived food products such as milk, meat and eggs (Peng *et al.*, 2018). Therefore, metal residues may concentrate in their meat and eggs (Abdulkhaliq *et al.*, 2012). Finally, They reach consumers not only by the polluted environment but also by ingestion of contaminated food that cannot be tasted, smelled, or seen be hidden in meat and offal (Ragab *et al.*, 2014; Darwish *et al.*, 2018). Heavy metals are characterized by their long biological half-life, endurance, and non-biodegradability (they cannot be metabolized by the body) and can bio-accumulate in living tissues and have toxic effects on the nervous system, gastrointestinal tract, genital system, hepatic toxicity, immune system and carcinogenesis (Burak *et al.*, 2010; Jaishankar *et al.*, 2014; Devi and Yadav, 2018; Ahmed *et al.*, 2019). On the other hand, the accumulation of heavy metals varies significantly from one organ to another within an animal depending on quantity of element consumed, the interval of exposure, as well as animal breed and age, and varies also between one animal and another. The determination of heavy metals in tissue and organs of poultry has therefore received serious attention (Iwegbue *et al.*, 2008). Many metals are essential for human and animal nutrition including Fe and Cu, as they constitute a part of the structure of enzymes and vitamins. However, when ingested and reaching a certain level, they may be harmful to humans (Filazi *et al.*, 2003; Niedziółka *et al.*, 2010). The most environmental heavy metal pollutants that have been recorded are Pb and Cd (Lenntech, 2012); they able to cause toxicity, even at very low concentrations (Abbas *et al.*, 2019). Heavy metals such as copper, iron, manganese and zinc are basic elements that are standardized in poultry feeding and may be accumulated in tissues and organs of the birds if added in feed more than required levels (Smulikowska and Rutkowski, 2005; Alkhalaf *et al.*, 2010). Also, minerals such as sodium (Na), calcium (Ca) and magnesium (Mg) have important biological and physiological functions so they must be supplemented at sufficient amounts in poultry feeds (Driver *et al.*, 2005; Halle, 2009). The human health risk assessment requires identification, collection, and integration of information on hazardous chemicals and also the relationship between exposure, dose, and adverse health effects (Sobhan Ardakani, 2017).

MATERIALS AND METHODS

Area of Study:

The present study was conducted in the Forensic Medicine Department Faculty of Veterinary Medicine, Suez Canal University. It was carried out on five broiler poultry farms (Fayed, El Tall El Kebir, Abu Khalifa, Taie and Sarabium) Ismailia Governorate at different localities from December 2017 till December 2018. The housing system was a deep litter system in all farms except Sarabium (closed and deep litter system). Sources of water and feed were shown in Table (1).

Table 1. Topographical examination of investigated poultry farms.

Farm	Location	Housing	Type of breeding and strain	Capacity of the farm	Source of water	Source of feed
A	Fayed	Deep litter system	Broiler (cup strain)	5000 birds	Tape water	Formulated by farm's owner
B	El Tall El Kebir	Deep litter system	Broiler (Ross strain)	10000 birds	Tape water	El-Salah feed company
C	Abu Khalifa	Deep litter system	Broiler (I R strain)	6000 birds	Driven well (Depth:43m)	El-Dkahlia feed company
D	Taie	Deep litter system	Broiler (Ross strain)	4500 birds	Driven well	El-Obour feed company
E	Sarabium	Closed & Deep litter system	Broiler (Cup strain)	12000 birds	Driven well (Depth:24m)	Formulated by farm's owner

Sampling:

Fifty samples of drinking water, feed and tissue samples were collected from five broiler farms in different areas at Ismailia (10 samples from each farm were collected during 2018 & 2019). Water samples were taken in identified clean washed screw-capped glass bottles. The required volume (100 ml) of the filtrate was measured and preserved by 0.3 ml of nitric acid and kept in the refrigerator to avoid evaporation according to (APHA, 1995). Feed samples were kept in labeled air-sealed plastic bags and kept in the refrigerator till the analysis according to (AOAC, 2006). Broilers were slaughtered, picked up and eviscerated and from each broiler carcass, muscle (breast and thigh) and organs (gizzard, liver and kidney) were collected, washed with distilled water to remove the blood and dried with filter paper (Double ring) then stored in polyethylene bags. Samples were identified and frozen at -20°C till the time of analysis of heavy metals concentrations. Tissue samples either muscles or organs were digested according to the method applied by Ibeto and C.O.B.O., (Ibeto and C.O.B.O., 2010). Each digested sample was transferred to a pre-washed sample bottle for analysis. The sample analyses were done using Atomic Absorption Spectrophotometer (AAS) (Flame technique). Model (SensAA: GBC scientific EQUIPMENT Spectrophotometer) of animal Health Research Institute, Dokki, Giza, Egypt for estimation of lead, cadmium, iron and macro elements (Na, Ca, and Mg) levels in the digested samples (water, feed and tissue).

Statistical Analysis:

Data were expressed as mean standard error (SE) and analyzed using one-way analysis of variance (ANOVA) procedure and the statistical significance of correlation coefficients among different traits were carried by Duncan's test according to statistical analysis system (SAS,1988). As a post-hoc test using IBM SPSS statistics 22.0 software package and the chart was created by Microsoft Excel 2010 software. The 0.05 level of probability was used as the criterion for significance.

RESULTS AND DISCUSSION

Heavy metals pollution, along with environmental pollution, is increasing every day. It contaminates foodstuffs (Sarkar *et al.*, 2017) and causes disorders (Baydan *et al.*, 2017). Moreover, in the last few years, The frequency of renal failure and liver cirrhosis in a human was markedly increased which could be related to heavy metal pollution in Egypt (Kamel and El Minshawy, 2010).

Lead (Pb) in drinking water of different broiler farms in Ismailia was not detected in all farms except farm (A) in Fayed area which is in the mean with (0.05 ppm). This recorded value of lead was within the permissible limit stated by E.O.S, (2010) (0.05

mg/kg) and above the permissible limit of WHO, (2011) (0.01mg/kg) which could be attributed to farms location beside the roads and heavy traffic. These findings are nearly similar to those (Abd El-Wahab, 2019); who reported that Pb levels in water samples were in the mean of 0.002 ± 0.0004 , 0.003 ± 0.0004 , 0.002 ± 0.0003 , 0.025 ± 0.005 and 0.046 ± 0.01 ppm, respectively as shown in Table (2).

Table 2: Concentrations of heavy metals and macro elements (ppm) in drinking water of different farms, Ismailia province, Egypt.

Farm	Lead	Cadmium	Iron	Sodium	Calcium	Magnesium
A	0.05 ± 0.001^a	ND	ND	51.91 ± 8.7^d	21.6 ± 4.3^e	17.8 ± 4.3^c
B	ND	ND	0.06 ± 0.002^b	38.04 ± 4.6^e	37.3 ± 8.7^d	19.8 ± 5.6^c
C	ND	ND	0.06 ± 0.001^b	$449.60 \pm 25.3^{a*}$	205.6 ± 17.4^b	44.5 ± 11.4^a
D	ND	ND	0.07 ± 0.002^b	$222.57 \pm 19.7^{b*}$	$213.4 \pm 21.4^{a*}$	46.2 ± 9.6^a
E	ND	ND	0.11 ± 0.05^a	149.00 ± 23.7^c	54 ± 15.3^c	31.5 ± 8.7^b
WHO guidelines	0.01	0.003	0.3	200	200	125
Egypt	0.05	0.005	0.3	200	200	150

- Data was expressed as mean \pm SE.

- ND: Not Detected.

- (*): Significant difference ($P < 0.05$) from permissible limit according to WHO (2011) and E.O.S (1995).

-A: Fayed, B: El Tall El Kebir, C: Abu Khalifa, D: Taie and E: Sarabium.

-The permissible limit according to WHO, (2011).

-The permissible limit E.O.S, (1995).

The higher concentrations of (Pb) in feed samples were in a mean of 0.71, 0.47, 0.47 and 0.22 ppm in farms B, A, C and D. The recorded limits were within permissible limits stated by EC, (2003); however (Pb) was not detected in farm (E). The high levels of lead in feedstuffs might be due to the exposure of feeders to a higher level of pollution from the surrounding environment such as sand from the floor, dropping and dry feed particles (hay), or due to different agricultural activities (Metawea, 2012). In addition to anthropogenic sources of lead pollution in the environment especially fossils fuels (Okoye *et al.*, 2011). Our results are similar to those obtained by Bukar and Sa'id, (2014), who analyzed poultry feed samples for Pb residues and results showed different concentrations of Pb in a range from 0.27- 0.80 mg/kg as shown in Table (3).

Table 3: Concentrations of heavy metals (ppm) in feed of different farms, Ismailia province, Egypt.

Farms	Lead	Cadmium	Iron	Sodium	Calcium	Magnesium
A	0.47 ± 0.01^b	0.44 ± 0.01^b	$150.2 \pm 0.37^{a*}$	797.9 ± 3.65^c	551 ± 3.65^c	442.6 ± 0.73^b
B	0.71 ± 0.01^a	$0.64 \pm 0.01^{a*}$	$138.4 \pm 0.62^{b*}$	844.7 ± 1.83^b	643 ± 3.65^a	444.6 ± 1.46^b
C	0.47 ± 0.01^b	$0.45 \pm 0.01^{b*}$	$110.9 \pm 0.31^{d*}$	932.5 ± 3.65^a	579 ± 1.46^b	442.5 ± 2.19^b
D	0.22 ± 0.01^d	ND	$109.2 \pm 0.15^{e*}$	852 ± 3.65^b	576 ± 3.65^b	452.7 ± 0.73^a
E	ND	ND	$123.2 \pm 0.81^{c*}$	800.7 ± 3.65^c	452.7 ± 3.65^d	445.8 ± 1.83^b
Permissible limits	^E 5 mg/kg	^E 0.5 mg/kg	^F 45-80 mg/kg	-	-	-

- Data was expressed as mean \pm SE.

- (E): The permissible according to EC (2003), (F): the permissible as stipulated by (FAO/WHO, 2000).

-ND: Not Detected.

- (*): Significant difference ($P < 0.05$) from the permissible limit.

-A: Fayed, B: El Tall El Kebir, C: Abu Khalifa, D: Taie and E: Sarabium

In this study, the concentrations of (Pb) determined in broiler tissues revealed that levels detected in all farms except farm (E) in which broiler tissue samples (breast,

thigh, gizzard, kidney and liver) were in a mean of 0.08 ± 0.011 , 0.134 ± 0.02 , 0.16 ± 0.02 , 0.29 ± 0.04 and 0.39 ± 0.06 ppm, respectively. Looking at these results, Pb levels were within permissible limits of E.O.S, (2010) in meat set as 0.1 mg/kg as shown in Table (4). The obtained results are the same as or slightly higher than those (Abbas, 2017) who, analyzed chicken meat for lead residues. The results revealed that the highest Pb concentrations were recorded in the liver (0.5 mg/kg) followed by the gizzard (0.329 mg/kg), then the muscle of the thigh (0.244 mg/kg) and then the breast muscle (0.207 mg/kg). The results were statistically significant ($p \leq 0.001$) in examined kidney and liver organ samples that were collected from farms A and B (which used tap water) and farms C, D and E (which used well water).

The results pointed the accumulation of lead in the liver and kidney is probably an indicator of its presence in water and feed. (Ahmed, 2002) High levels of metals in poultry products are mainly due to contamination of feed and water sources (Andrée *et al.*, 2010; Ersoy *et al.*, 2015). The presence of lead residues may be attributed to feeding contamination, climatic factors such as wind, use of agrochemicals and very importantly drinking water. Furthermore, lead could be emitted from the car exhaust to contaminate the environment or from water pipes (ogabiela *et al.*, 2011).

Table 4: Concentrations of lead (ppm) in broiler tissues (breast, thigh, gizzard, kidney and liver) from five different farms at Ismailia province, Egypt.

Farms	Breast muscle	Thigh muscle	Gizzard	Kidney	Liver
A	0.11±0.03a	0.20±0.02a*	0.27±0.02a	0.53±0.08a*	0.69±0.08a*
B	0.11±0.01a	0.17±0.01a	0.16±0.03b	0.52±0.02a*	0.73±0.02a*
C	0.11±0.02a	0.22±0.04a*	0.24±0.04a	0.26±0.02b	0.39±0.00b
D	0.07±0.03a	0.06±0.04b	0.11±0.01b	0.13±0.03c	0.15±0.04c
E	ND	ND	ND	ND	ND
Permissible limits	0.1 mg/kg	0.1 mg/kg	0.5 mg/kg	0.5 mg/kg	0.5 mg/kg

-Data was expressed as mean ± S.E.

-Different superscripts (a,b,c,d) between rows indicates significant difference at $P \leq 0.001$

-The permissible limit according to E.O.S, (2010)

- (*): Significant difference from permissible limit according to E.O.S.

-A: Fayed, B: El Tall El Kebir, C: Abu Khalifa, D: Taie and E: Sarabium.

(Cd) levels were not detected in the drinking water of different broiler farms in Ismailia province as shown in Table (2). In other studies, lower levels of cadmium were reported in the Slovak Republic by Rapant *et al.*, (2017) in groundwater samples by a mean value of 0.0010 mg.L-1 and Sobhan Ardakani *et al.*, (2015) In Razan Plain (Iran), detected that cadmium levels in groundwater resources during spring and summer seasons 0.21 ± 0.04 ppb in spring and 0.30 ± 0.08 ppb in summer, (0.00021 ± 0.00004 ppm) and (0.0003 ± 0.00008 ppm). In addition to El Assal and Abdel-Meguid (2017), who reported the least cadmium levels in the River Nile water at four sites in Great Cairo. However, the concentration of cadmium determined in broiler feed of different broiler farms was only detected in farms A, B and C with means 0.44, 0.64 and 0.45 ppm. These values were within permissible limits except farm (B) in which is higher than permissible limits stated by EC (2003) (0.5mg/kg) Table (3). The recorded results were in accordance with this reported by Abdou *et al.*, (2016) in El-Fayoum province, Egypt. According to current knowledge, cadmium is not added or used as feed additives for animal growth. Usually, Cd is found in mineral supplements as phosphates, Zn oxides, and Zn sulphate as an impurity, thus Cd can enter into the production process through these feed ingredients, which might result in severe dietary contamination (Zhang *et al.*,

2012). The high levels of cadmium in poultry products emanate mainly from contamination of feeds (Rahman *et al.*, 2014).

The concentrations of cadmium determined in broiler tissues revealed that cadmium levels were not detected in all farms except farm (B) as shown in Table (5). In which broiler tissue samples (breast, thigh, gizzard, kidney and liver) were in a mean of 0.02 ± 0.01 , 0.03 ± 0.01 , 0.04 ± 0.01 , 0.12 ± 0.00 and 0.28 ± 0.01 ppm, respectively. The obtained cadmium levels in broiler meat samples were within the permissible limits of E.O.S, (2010) ($0.05 \mu\text{g/g}$). The kidney and liver tissue samples had significantly ($p \leq 0.001$) higher concentrations of Cd (0.12 ± 0.00 and 0.28 ± 0.01 ppm, respectively), compared to the muscle tissue samples (0.02 ± 0.01). Cadmium occurs throughout the body with the highest concentrations in the liver, kidney, spleen and bone. Exposure to cadmium causes kidney and liver damage, skin irritation and also causes ulceration (Thirulogachandar *et al.*, 2014) 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).

Table 5: Concentrations of Cadmium in broiler tissues (breast, thigh, gizzard, kidney and liver) from five different farms (A, B, C, D and E) at Ismailia province, Egypt.

Farms	Breast muscle	Thigh muscle	Gizzard	Kidney	Liver
A	ND	ND	ND	ND	ND
B	0.02 ± 0.01 a	0.03 ± 0.01 a	0.04 ± 0.01 a	0.12 ± 0.00 a	0.28 ± 0.01 a
C	ND	ND	ND	ND	ND
D	ND	ND	ND	ND	ND
E	ND	ND	ND	ND	ND
Permissible limits	$0.05 \mu\text{g/g}$	$0.05 \mu\text{g/g}$	$1 \mu\text{g/g}$	$1 \mu\text{g/g}$	$1 \mu\text{g/g}$

-Data was expressed as mean \pm S.E.

-Different Superscripts (a,b,c,d) between rows indicate significant difference at $P \leq 0.001$

-The permissible limit according to E.O.S, (2010).

-A: Fayed, B: El Tall El Kebir, C: Abu Khalifa, D: Taie and E: Sarabium

Iron in drinking water of different broiler farms in Ismailia was detected in all farms except farm (A) in Fayed area, which in mean with 0.06, 0.06, 0.07 and 0.11 ppm in farms B, C, D and E Table (2). These recorded values of iron in water samples were within the permissible limit stated by E.O.S, (1995) (0.3 mg/l) and WHO, (2011) (0.3 mg/l). The higher levels of iron in broiler feed samples collected from different broiler farms were accumulated in this order: farms $A > B > E > C > D$. the given results revealed that iron levels detected were in a mean of 150.2, 138.4, 110.9, 109.2 and 123.2 ppm in farms A,B,C,D and E, respectively as shown in Table (3). And these recorded limits were exceeded the permissible limits of WHO, (1992 & 1995) (80 ppm) and FAO, ($45\text{-}80 \text{ mg/kg}$). The obtained results were in agreement with or slightly higher than those obtained by (Islam *et al.*, 2017) in Dhaka city, Bangladesh that ranged from 57.3 to 121.9 mg/kg. The high levels of iron in feed can be attributed to the farmers and companies that manufacture poultry feed adding high amounts of iron in order to avoid deficiencies as it leads to severe anemia and loss of pigmentation, also decreased growth rates (Ma *et al.*, 2014). A study stated that the addition of ferrous Sulphate in broiler ration leads to better growth, Feed Conversion Ratio and maximum per bird net profit (Talpur *et al.*, 2016).

The concentration of Iron in the gizzard, thigh muscle, liver and breast muscle of broiler showed that iron levels detected in all broiler tissues (breast, thigh, gizzard,

kidney and liver) were in a mean of (185.07 ± 3.77, 164.30 ± 9.56, 204.43 ± 20.13, 198.43 ± 7.21 and 231.03 ± 21.38 ppm, respectively in farm A), (139.40 ± 3.88, 141.07 ± 35.11, 247.83 ± 7.68, 237.30 ± 12.64 and 267.77 ± 14.32 ppm, respectively in farm B), (175.30 ± 4.79, 184.77 ± 10.88, 209.00 ± 15.76, 185.90 ± 7.47 and 201.57 ± 4.18 ppm, respectively in farm C), (104.39 ± 1.03, 108.30 ± 1.89, 111.31 ± 1.14, 110.20 ± 1.11 and 118.54 ± 2.56 ppm, respectively in farm D) and (241.16 ± 4.86, 209.40 ± 3.40, 239.92 ± 6.45, 235.17 ± 6.07 and 241.21 ± 3.96 ppm, respectively in farm E) as shown in Table (6), and they exceeded the permissible limit recorded by (Demirezen and Uruç, 2006). (30-150 mg/kg) in broiler tissue samples. It is worth mentioning that liver samples, which were collected from farm (B), seemed to be the organ, which accumulated the highest values (267.77 ± 14.32 ppm) of iron. While breast muscle organs that were collected from farm (D) showed the lowest value of iron (104.39 ± 1.03 ppm). Iron is the basic micronutrient that is standardized in poultry feeding. They accumulated in tissues and organs of birds in different concentrations which depends on many factors as dose, a form of these elements and other physiological factors (Smulikowska and Rutkowski, 2005). The high iron concentrations in tissues may be attributed to their high concentrations in feed. This cause is more reliable when we look at the obtained iron concentration in broiler farms feed. The previously mentioned reason was supported by (Rehman *et al.*, 2012) which showed that elements used in feed deposit in the body and cause harmful impacts.

Table 6: Concentration of iron (ppm) in broiler tissues (breast, thigh, gizzard, kidney and liver) from five different farms (A, B, C, D and E) at Ismailia province.

Farms	Breast muscle	Thigh muscle	Gizzard	Kidney	Liver
A	185.07±3.77 ^b	164.30±9.56 ^{ab}	204.43±20.13 ^b	198.43±7.21 ^b	231.03±21.38 ^{bc}
B	139.40±3.88 ^c	141.07±35.11 ^{bc}	247.83±7.68 ^{a*}	237.30±12.64 ^{a*}	267.77±14.32 ^{a*}
C	175.30±4.79 ^b	184.77±10.88 ^{ab*}	209.00±15.76 ^{b*}	185.90±7.47 ^{b*}	201.57±4.18 ^{b*}
D	104.39±1.03 ^d	108.30±1.89 ^d	111.31±1.14 ^c	110.20±1.11 ^c	118.54±2.56 ^d
E	241.16±4.86 ^a	209.40±3.40 ^{a*}	239.92±6.45 ^{ab*}	235.17±6.07 ^{a*}	241.21±3.96 ^{ab*}
Permissible limits	30-150 mg/kg	30-150 mg/kg	30-150 mg/kg	30-150 mg/kg	30-150 mg/kg

-Data was expressed as mean ± S.E.

-Different Superscripts (a,b,c,d) between rows indicate significant difference at P ≤0.001

-The permissible limit according to Demirezen (2006).

-(*): Significant difference from permissible limit according to Demirezen and Uruç,(2006).

-A: Fayed, B: El Tall El Kebir, C: Abu Khalifa, D: Taie and E: Sarabium

Concerning macro elements, the tap water sodium levels were 51.91 and 38.04 ppm in farms A and B, respectively. The sodium levels in well water were 449.60, 222.57 and 149 ppm in farms C, D and E, respectively. The sodium levels in the well water of farms C and D were higher than the permissible limits stated by WHO guidelines and the Egyptian organization for standardization (E.O.S). The high concentrations of sodium in broiler drinking water attributed to the source of water in which farm A and B water sources were tap water while farms C, D and E used well water, which is higher in total dissolved salts.

The calcium levels in tap water were 21.6 and 37.3 in farms A and B, respectively. However, the calcium levels in well water were 205.6, 213.4 and 54 ppm in farms C, D and E respectively. The levels of calcium in well water of farm C (Abu Khalifa Area) and D (Taie Area) were higher than permissible limits stated by WHO and E.O.S. Concerning magnesium concentrations, their levels were 17.8 and 19.8 ppm in farms A and B, respectively. Moreover, magnesium levels in well water of C, D and E were 44.5, 46.2 and 31.5ppm, respectively as shown in Table (2). The high level of

calcium in farms C and D may be attributed to the source of water, as the used water was derived from a well.

The sodium levels in broiler feed were 797.9, 844.7, 852 and 800.7 ppm in farms A, B, D and E respectively while farm C has shown the highest sodium concentration of all farms which was 932.5 ppm. The high sodium levels in poultry feed may be attributed to different sources of feed and the addition of premixes to help in the growth of chickens as Watkins *et al.*, (2005) mentioned that Levels of Na and Cl in drinking water and in the diet significantly affected the live performance of broilers in which Water sources of Na and Cl can be used to provide part or all of a chick's needs for these minerals, and adjustments in dietary levels of Na and Cl should be made based on levels of these minerals in the drinking water. This aids in reducing litter moisture for situations in which water supplies are high in saline content. The calcium levels were 551, 643, 579, 576 and 452.7 ppm in farms A, B, C, D and E, respectively. However, WHO and E.O.S have not standardized a permissible limit for calcium. The magnesium levels were 442.6, 444.6, 442.5, 452.7 and 445.8 ppm in farms A, B, C, D and E. However, WHO and E.O.S have not standardized a permissible limit for magnesium as shown in Table (3).

Our results revealed that the sodium residues in breast muscles of farms A and C showed highly significant ($P \leq 0.001$) levels than farms B, D and E. while farm B displayed significantly ($P \leq 0.001$) higher levels than farms D and E. there was no significant difference observed between farms D and E. The sodium residues of thigh muscles in farm A displayed highly significant ($P \leq 0.001$) levels than the other farms (B, C, D and E). While farms B and C showed significantly ($P \leq 0.001$) higher levels than farms D and E. there was no significant difference detected between farms B and C. In gizzard, the sodium concentrations revealed significantly ($P \leq 0.001$) higher levels in farm A than in the rest farms (A, C, D and E). while farm D showed significant ($P \leq 0.001$) higher levels than farms A, C and E. there was no significant difference observed between A and C. finally, farm D showed the least significant level than the other farms. In kidney samples, the sodium residues demonstrated significantly ($P \leq 0.001$) higher levels in farm C than farms A, B, D and E. while, farms A and B showed significantly ($P \leq 0.001$) higher levels than farms D and E. there was no significant difference observed neither between farms A and B nor between farms D and E. In liver samples, the sodium residues revealed significantly ($P \leq 0.001$) higher levels in farm A than farms B, C, D and E. while, farm D showed significantly ($P \leq 0.001$) higher levels than farms A, C and E. There was no significant difference observed between farms A and C as shown in Table (7). In comparing with this study, Shamshad *et al.*, (2017) In Lahore city (Pakistan), recorded low sodium concentrations in leg tissues, liver and heart muscles were mean as 6.727 ± 1.54 , 8.827 ± 1.61 and 7.902 ± 0.67 mg/kg, respectively.

While the obtained results were higher than those reported by Njinga *et al.*, (2016) In Metropolitan city (South Africa), reported that the mean sodium concentrations in chicken liver samples ranged as 59.229 -157.284 ppm., Majewska *et al.*, (2016) In Poland, reported that the mean sodium concentrations in chicken liver samples were 811.00 ± 10.43 mg/kg. and Seong *et al.*, (2015) In Korea, mentioned that the mean values (mg/kg) in the liver, and gizzard were $1.034.00 \pm 39.95$ and 947.71 ± 36.32 mg/kg, respectively.

On the other hand, the results demonstrated that the calcium residue in breast muscles of farm B exhibited significantly ($P < 0.001$) higher levels than other farms whereas a non-significant difference was observed between farms A and C levels. Also, Farms D and E exhibited non-significant alteration between them with significant ($P < 0.001$) least values than other groups.

Thigh muscles calcium residue was significantly ($P < 0.001$) higher than other groups. Calcium residue in thigh muscles of groups B and C exhibited non-significant variations between each other. Farms A and E exhibited a significant ($P < 0.001$) reduction in thigh calcium residue than other farms with no significant difference observed between both groups.

The gizzard calcium residue showed a significant elevation in farm B than other farms. Farm C exhibited significantly ($P < 0.001$) higher gizzard Calcium residue than farms A, D and E. There was a non-significant difference observed between farms A and D. group E exhibited the most significant ($P < 0.001$) reduction in the former value than other farms. The calcium residue in the Kidney of farm D revealed significantly ($P < 0.001$) higher levels than other farms whereas a non-significant difference was observed between farms A, C and E levels. Also, Farm B exhibited significant ($P < 0.001$) least values than other groups.

Farm A exhibited significantly ($P < 0.001$) higher liver calcium residue than other farms. Farm B showed significantly ($P < 0.001$) higher liver calcium residues than farms C, D and E. There was a non-significant difference observed between Farms C and D. group E exhibited the most significant ($P < 0.001$) reduction in the former value than other groups as shown in Table (7).

Moreover, magnesium residues in different broilers organs in five tested farms. The levels of magnesium residue in breast muscles showed significantly ($P < 0.001$) higher values in farm B than in other farms. Both farms A and C revealed a significant ($P < 0.001$) increase in breast muscles than farms D and E that exhibited the least significant ($P < 0.001$) Thigh magnesium levels. No significant difference was observed between Farms A and C as well as Farms D and E. The magnesium residue in thigh muscles exhibited higher levels in farm D than in other farms. Farms B and C demonstrated significantly ($P < 0.001$) higher thigh magnesium residue than farms A and E. no significant difference was observed between Farms B and C or A and E with the most significant reduction in farms A and C when compared with other farms. The gizzard magnesium levels exhibited significantly ($P \leq 0.001$) higher levels in farm D than farms A, B, C and E. whereas, farms C and E magnesium residues showed significantly ($P \leq 0.001$) higher levels than farms A and B. In addition, magnesium concentration showed significantly ($P \leq 0.001$) higher levels in farm B than farm A. there was no significant difference observed between farms C and E. In the kidney, the magnesium residues demonstrated significantly ($P \leq 0.001$) higher levels in Farm D than farms A, B, C and E. while, farm C showed magnesium values significantly ($P \leq 0.001$) higher than farms E, B and A. there was no significant difference between farms B and E. Magnesium concentrations in liver revealed significantly ($P \leq 0.001$) higher levels in farms C and D than farms A, B and D. moreover; there was no significant difference neither between farms C and D nor between farms A, B and D as shown in Table (7) The obtained results are nearly similar or slightly less than those recorded by (Chowdhury *et al.*, (2011), In Comilla, magnesium residues were with mean of 426.96 ± 18.23 and 401.86 ± 20.21 mg.kg⁻¹ in meat samples from local and farm cock, respectively. The Mg is considered to be one of the seven essential macro-minerals required as much as or greater than 100mg/day. The human body contains approximately 20-28 mg of Magnesium of which over 50% is stored in the system and the rest is found in muscle, soft tissues and bodily fluids (Krause, 2008). Despite known importance, however, no interpretation could be concluded in the current study.

Table 7: Concentrations of Sodium, Calcium and Magnesium (ppm) in broiler tissues (breast, thigh, gizzard, kidney and liver) from five different farms (A, B, C, D and E) at Ismailia province.

Farms	Element	Breast muscle	Thigh muscle	Gizzard	Kidney	Liver
A	Na	277.83±3.45 ^a	363.23±5.19 ^a	420.70±12.38 ^c	711.70±39.24 ^b	420.70±12.38 ^c
	Ca	38.70±0.73 ^b	36.27±0.93 ^c	31.73±2.22 ^c	44.03±4.43 ^b	65.17±2.70 ^a
	Mg	280.60±10.45 ^c	274.50±11.94 ^b	218.10±7.38 ^d	202.07±3.14 ^d	206.40±3.75 ^b
B	Na	217.93±4.47 ^b	315.90±1.59 ^b	536.03±6.04 ^a	722.20±10.11 ^b	536.03±6.04 ^a
	Ca	60.63±2.23 ^a	49.27±2.68 ^b	72.50±4.45 ^a	27.95±4.74 ^d	54.40±3.48 ^b
	Mg	316.33±4.35 ^b	307.73±17.77 ^a	237.60±5.94 ^c	217.13±5.96 ^c	211.07±5.71 ^b
C	Na	271.40±12.63 ^a	301.47±3.04 ^b	408.67±8.96 ^c	861.93±47.05 ^a	408.67±8.96 ^c
	Ca	43.50±2.81 ^b	53.93±6.86 ^b	39.70±2.67 ^b	42.50±2.52 ^b	31.33±1.96 ^c
	Mg	380.90±9.69 ^a	315.73±2.25 ^a	262.50±9.22 ^b	248.37±6.02 ^b	260.80±7.92 ^a
D	Na	180.00±10.60 ^c	279.00±9.19 ^c	450.03±10.66 ^b	450.03±7.37 ^c	450.03±10.66 ^b
	Ca	16.87±1.13 ^c	66.70±4.84 ^a	28.40±0.98 ^c	67.03±2.78 ^a	31.70±2.34 ^c
	Mg	384.57±7.33 ^a	332.83±3.16 ^a	282.30±10.19 ^a	264.57±5.92 ^a	268.70±6.21 ^a
E	Na	169.58±8.58 ^c	257.97±7.32 ^d	185.34±4.34 ^d	436.01±14.70 ^c	286.83±4.34 ^d
	Ca	21.89±8.76 ^c	29.80±2.38 ^c	16.77±0.72 ^d	33.02±4.24 ^b	19.05±3.30 ^d
	Mg	322.73±6.84 ^b	314.73±4.53 ^a	261.03±10.83 ^b	219.27±4.02 ^c	221.90±4.93 ^b

-Data was expressed as mean ± S.E.

-Different Superscripts (a,b,c,d) between rows indicate significant difference at P ≤0.001

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