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EFFECT OF HEAVY METALS CONTAMINATION ON THE BACTERIAL LOAD IN LOCAL AND IMPORTED POULTRY PRODUCTS

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Abstract
The focus of this study was to assessment the concentration of heavy metals lead (Pb), cadmium (Cd), Zinc (Zn) and copper (Cu) contents and contamination of bacteria in local and imported poultry in Baghdad city. The heavy metals detection by atomic absorption spectrophotometery (AAS). Bacterial contamination was detected by using plate count method and vitek 2 system. In most poultry samples the largest concentration of toxic heavy metals (Pb, Cd), were recorded higher than the acceptable limits set by FAO/WHO, EC, CN, this shows that foodstuffs were not safe from the toxins of these heavy metals. (Zn) concentration above the detectable levels that was anxiously to public health. The concentration of (Cu), in all samples was little or within the permissible value, these indicate the samples were safe of Cu poisoning. Contamination of bacteria (*E. Coli*, Enterobacter Sp., Salmonella Sp., *E. Coli O157:H7*, *Staph. aureus*, Pseudomonas Sp., Klebsiella Sp., Proteus sp. and *Yersinia enterocolitica*) was present in all meat samples with varying degrees. Statistical analysis by ANOVA revealed significant difference ($p< 0.05$) of metals in poultry samples. Hence, it requires many tests to be carried out on meat products before purchasing them from the market.
Keywords: heavy metals, bacterial load, Poultry, AAS,vitek.

抽象的

本研究的重点是评估巴格达市本地和进口家禽中重金属铅 **(Pb)**、镉 **(Cd)**、锌 **(Zn) 和**铜 **(Cu) 的**浓度以及细菌污染。原子吸收分光光度法（**AAS**）检测重金属。使用平板计数法和**vitek 2 系**统检测细菌污染。在大多数家禽样本中，有毒重金属（**Pb**、**Cd**）**的最大**浓度记录高于 **FAO/WHO**、**EC**、**CN** 设定的可接受限度，这表明食品对这些重金属的毒素并不安全。 **(Zn)** 浓度高于可检测水平，这对公共健康至关重要。 （**Cu**）**的**浓度，在所有样品中都很少或在允许值内，这表明样品是安全的**Cu中毒**。细菌（大肠杆菌、肠杆菌属、沙门氏**菌属**、**大**肠杆菌

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O157:H7、金黄色葡萄球菌、假单胞菌属、克雷伯菌属、变形杆菌属和小肠结肠炎耶尔森氏菌）污染存在于所有肉类样品中，并具有不同的度。方差分析的统计分析揭示了家禽样品中金属的显着差异（p < 0.05）。因此，在从市场上购买肉类产品之前，需要对它们进行许多测试。

关键词：重金属，细菌负荷，家禽，AAS，vitek。

Introduction

Heavy metals are divided into two main groups important elements like Cu, Zn, Fe, Mg and Co and non- important toxic elements like Pb, Cd, Ni and Cr (1). Presence of non- important elements in the body causes major chemical and neurological changes (2). The presence of important elements in low concentrations in the body for the continuity of living organs and their survival, it's called **trace metal** that found naturally in soil, water, rocks and fossils, and considers essential food nutrients in feeding poultry and livestock (1, 3). In any case, heavy metals wether regarding to be important or possibility toxic have an reverse effect on human and animal health if they are found in high concentrations above the body's very low needs(3,4).

Living organisms are exposed to heavy metals contamination due to their presence in a polluted ecosystem (3). One of the most important and largest sources of pollution is the combustion of fossil fuels, the use of disinfectants and sterilizers, the throwing of industrial waste, the use of fertilizers and pesticides (2). There are reasons related to the excessive consumption of minerals by chicken, including natural supply of water have rising concentrations of iron and magnesium, furthermore, toxic metals. Cobalt is naturally present in water, soil, rocks, air, animal, plant and also be liberated from burning coal and furnace. Zinc pollutes the ambient as a result of burning waste and refining of ores and mining, in addition copper pollution occurs through fossil fuels combustion,waste, wood production and phosphate fertilizers (3). Heavy metals contamination is linked to car pollution, especially lead, that reaches to the environment due to daily combustion of petrol. Lead considered a toxic metal that is strongly linked to enzymes and works to disrupt its functions and effects on the blood and nerves (1, 5). Another reason increased the chance of food contamination with various environmental pollutants, especially toxic heavy metals, is technological manipulation and amelioration of food products (4). Other methods of contamination with toxic heavy metals is the use of poultry feed producers shaving dust from the tanning industries as a protein source (6). Trace metals have an impact on water quality, agricultural products and human health, while industrial development has main role in pollution with toxic heavy metals (1). The percentage of heavy metals in food and the amount of drinking water is about 80% for (cd), 40% for (Pb) and 8% for (Hg) (5). In general, heavy metals are not susceptible to degradation and decomposition and have a longer shelf life with the possibility of their accumulation in different body organs leading to deleterious side effects. Therefore, the ingestion of these pollutants by animals led to the deposition of these residues in the meat. This defilement with minerals is a series of risks due to their toxins, biological aggregation and

biomagnification in the food chain (4). Chicken meat contain principal source of essential nutrients, comprise protein (amino acids), essential fatty acids such as omega- 3, vitamin E, minerals such as selenium, iron and zinc(5). The industry of poultry is the largest agriculture-based industries and the fastest growing in demand for chicken products as sources of protein and widely consumed all over the world, so their safety can't be negligent. Food safety is a major public problem and the exposure of food products, especially poultry meat to the dangers of heavy metals pollution is a widespread and deep concern issues for both food safety and human health, due to the toxicity in nature of these minerals, even in low concentrations (4, 5). One of the outcomes of ambient contamination is the entry of pollution into the food chain, so food is the main way for most minerals to enter the body (2). Meat is considered the best ecosystem for the grow and flourish of numerous pathogenic germs such as: *Enterobacteriaceae, Lactobacillus sp., Leuconostoc sp*., *Carnobacterium sp., Pseudomonas sp*., and *Bacillus sp.* that are distinguished as a foodborne pathogen that propagation foodborne disease (1).

Total coliform and *E. coli* employ as an indicator of the existence of pathogenic microorganisms in many of industry food (7). These germs are in charge of spoiling of refrigerated meat and its products. Incorrect processing and absence of warning about food safety facilitated from the transmission of most organisms to the consumers, Therefore, the correct management of animal breeding and manipulation of meat is call for the sake of health safety and preservation of the quality of meat and its products for a long time (1).

Materials and methods

Sample collection

Sixty samples of the local and imported raw chicken meat, the whole chicken for each sample collected from markets in Baghdad then sent with cool box to the Microbiology laboratory for isolation and identification of bacteria, also for detection of heavy metal lead (Pb), cadmium (Cd), Zinc (Zn) and copper (Cu).

Isolation and identification of bacterial contamination

In polyethylene sac (500) gram of raw chicken meat from chest and thigh then mixed well in the stomacher, each meat sample was inoculated into peptone broth with (25gr/225ml) and incubated at (37°C) for (24-48) hrs. After incubation, about 0.1ml of the inoculated broth were sub-cultured onto plates of Nutrient agar, Blood agar, Eosin methylene blue (EMB), MacConkey agar, Salmonella Shigella Agar (SS) agar, Sorbitol-MacConkey agar with cefixime telluride, and CIN agar. Biochemical is performed using vitek 2 system.

Heavy Metals Analysis

10 gram of meat was kept in oven at 80°C for 48 hrs. or until dried. The heavy metals Analysis was performed by adding concentrations HNO3 into the dried meat. (0.5 gr. of dried meat with 5mL of concentrations HNO3) were taken into the digestion flask. Then heated at 80-90 °C for 10 mint by placing on hot plat for digestion then raised to100 , More acid was added up to 3-5mL until clear solution was obtained. The samples were cooled at room temperature and filtered through filter paper and the volume was raised up to 25 ml with the help of non-ionize water. The blank sample was also prepared. Atomic Absorption Spectrophotometer was used for the detection of heavy metals presence in the meat samples (FAAS, Shimadzu AA-7000) (8).

Statistical Analysis: Statistical Analysis System- SAS (2012) program was used to detect the effect of difference factors in study parameters. Least significant difference –LSD test (Analysis of Variation-ANOVA) was used to significant compare between means in this study (9).

Results

The results of this study showed heavy metals concentrations in local and imported chicken meat samples were tested in table (1). The average counts of Pb concentrations were 0.45 ppm and 0.28 ppm in local and imported meat samples, respectively (fig.1). Mean concentrations of Cd were 0.073 ppm in local samples and 0.060 ppm in imported meat samples (fig.2).

The average counts of Cu concentrations were 1.75 ppm and 1.40 ppmin local and imported meat samples, respectively (fig.3). Mean of total Zn count were 76.8 ppm in local samples and 69.5 ppm in imported meat samples (fig.4).

Table (1) average and standard error of the heavy metals concentration in local and imported chicken meat

Heavy Metal	Local chicken meat	Imported chicken meat	Permissible upper limits		
			FAO	EC	CN
Lead Pb	0.45 ± 0.022	0.28 ± 0.025	0.1	0.1	0.2
Cadmium Cd	0.073 ± 0.021	0.060 ± 0.018	0.05	0.05	0.1
Copper Cu	1.75 ± 1.0	1.40 ± 0.5	1	1	10
Zinc Zn	76.8 ± 15.0	69.5 ± 12.0	20	20	100

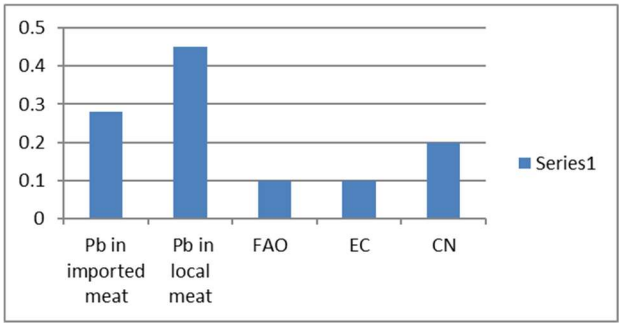

Fig. (1) The average of Pb in local and imported chicken meat samples

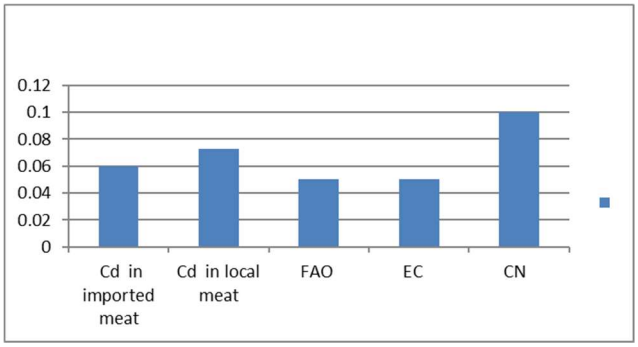

Fig. (2) The average of Cd in local and imported chicken meat samples

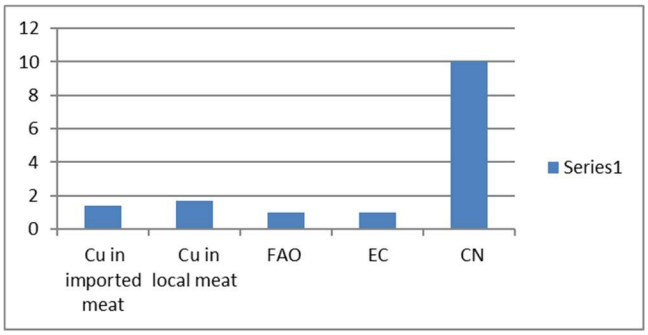

Fig. (3) The average of Cu in local and imported chicken meat samples

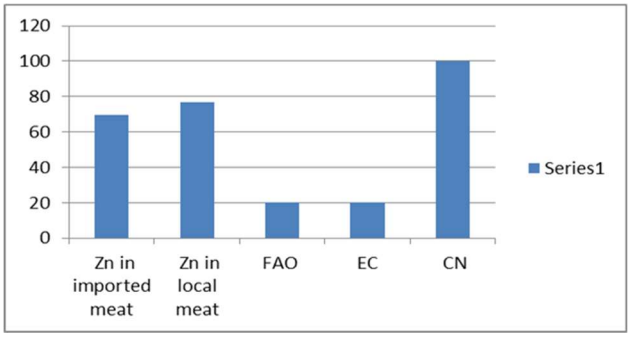

Fig. (4) The average of Zn in local and imported chicken meat samples

The results of this study showed the percentage of bacterial isolates in local and imported chicken meat samples were tested in Table (2). The results of bacterial isolation showed the presence of 65 isolates of local chicken meat samples where many samples contained mixed infection classified into 9 types includes *E. Coli* (75%), Enterobacter Sp. (25%), Salmonella Sp. and *E. ColiO157:H7* (15%), *Staph. aureus* (10%), Pseudomonas Sp. (7.5%), Klebsiella Sp., Proteus sp. and *Yersinia enterocolitica* (5%). Also, the results of the bacterial isolation of imported chicken meat samples showed the existence of 49 isolates divided into 9 types involve *E. coli* (20%), Enterobacter Sp. and *Staph. aureus* (30%), *E.coli O157:H7* and Salmonella Sp. (25%), Klebsiella Sp., Pseudomonas Sp. and *Yersinia enterocolitica* (10%), and Proteus sp. (5%).

Table (2) percentage of isolated bacteria from local and imported chicken meat samples

Isolated bacteria	Local chicken meat 40 sample		Imported chicken meat 20 sample	
E. Coli	30	75%	20	100%
Enterobacter Sp.	10	25%	6	30%
Salmonella Sp.	6	15%	5	25%
E. ColiO157:H7	6	15%	5	25%
Staph. aureus	4	10%	6	30%
Klebsiella Sp.	2	5%	2	10%
Proteus sp.	2	5%	1	5%
Pseudomonas Sp.	3	7.50%	2	10%
Yersinia enterocolitica	2	5%	2	10%

Discussion

Generally, heavy metals are important to maintain various functions in the bodies of living organisms with small quantities, they become deleterious if they exceed the standard levels, causing disorders of cell metabolism and thus poisoning (10). Many of serious chemicals materials intervention into the ambient widely that tainted water and aggregation in the soil in different united forms. Plants take their nutrients from the soil at the same time absorption of toxic chemicals and heavy metals that are consumed by animals and accumulate in their meat. Therefore, plants play a fundamental role in polluting the food chain (11).

The results in Fig.(1) displays that local meat samples have highest **Pb** concentrations were (0.45 ppm) that exceeded the permissible limit

set by FAO/WHO, EC, CN (12,13,14), that harmful to human health, compared with imported meat samples (0.28 ppm), that correspond with (5). Statistical analysis of local and imported meat samples showed that **Cd** concentrations were significantly higher ($p< 0.05$) were (0.073 ppm), and (0.060 ppm) respectively, they exceeded the permissible limit set by FAO/WHO, EC, CN, that not safe to human health, that similar with results of (1,12), While disagree with (11) their findings the Cd content within acceptable value set by FAO/WHO and EC were (0.05 ppm). The concentrations of **Zn** were highest in local and imported meat samples approximately (76.8), (69.5) ppm respectively, they were above the standard value of Zn about (20 ppm) according to FAO and EC (12, 13, 14) while the value was less than permissible limits according to CN were (100 ppm), that similar with results of (10). The present study revealed that copper **Cu** content in local and imported meat samples were (1.75), (1.40) ppm respectively, that is close to the limit value set by FAO/WHO and EC were (1 ppm), and not exceeded the detectable levels according to CN were (10 ppm), so the food are reasonably safe from Cu toxicity while disagree with (10).

Conclusion:

Globally safety of food is a complex issue and one of the biggest challenges. Its needs precautionary and deterrent measures along the food chain from the field to the market. Tainted of chicken meat with heavy metals and microorganisms is facilitate to enter to human body and creates a series of risks. Results of the current study, showed a significant difference in the concentration of metals, where Pb, Cd and Zn recorded high concentration that exceeded the permissible limits, except Cu content with standard value, in addition the existence pathogenic bacteria in all samples. In order to maintain food safety and reduce pollution with metals, it's become compulsory for the feed producers to constantly noting and preservation the standard limits of heavy metals in poultry feeds. Feed manufacturers must conduct a continuous assessment of heavy metals in their products to persist them within safe limits. The rising concentration of Pb due to ambient pollution with Pb principally fossil fuels and car use this calls for the adoption of substitution revival energy sources like bioethanol and biodiesel. Also Preservation of meat and its products refrigerated from slaughter until reaches to the consumer to prevent the growth and spread of pathogenic microorganisms. We recommend the sources of contamination in local areas should be determined and reduced through direct surveillance, in addition activation the role of censorship on imported products and identifying heavy metals because of their impact on human and animal health.

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