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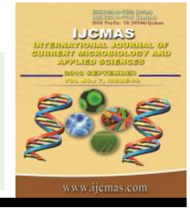

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A Study on the Levels of Heavy Metals in Poultry Eggs in Chittoor District of Andhra Pradesh, India

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A B S T R A C T

Chicken eggs are one of the main sources of protein but if contaminated by toxic heavy metals due to industrial waste, geochemical structures and agricultural activities is a serious problem for environmental and human health. The aim of our study is focused on evaluating the trace elements level in eggs that collected from the retail outlets in and around Chittoor district, Andhra Pradesh. The concentrations of As, Cr, Cu, Cd, Mn, Ni, Pb and Fe were measured using Inductively Coupled Plasma - Optical Emission Spectrometry. The result of our study showed that none of the samples had trace elements like Arsenic, Cadmium and Lead. The concentration of other trace elements like Chromium, Copper, Manganese, Nickel and Iron were ranged 0.04-0.15, 0.87-5.66, 0.22-0.66, 0.01-0.08, 1.29-8.54. Fe, Cu is found in high levels while, Cr, Mn, Ni burden occurred in less levels in all eggs. It seems that the regular national monitoring of egg producing chain specially the quality of chicken feed should be taken into account seriously in order to safeguard public health.

Introduction

The poultry industry is one of the largest sectors of agriculture throughout the world and the intensive poultry farming being increased the supply of economical, palatable and healthy food protein for growing urban populations. Despite substantial interest in the trace element content of eggs by poultry breeders, nutritionists and environmental scientists, available data about trace elements levels in eggs are scarce. Chicken eggs are one of the main sources of protein but if contaminated by toxic heavy metals due to industrial waste, geochemical structures and agricultural activities is a serious problem for environmental and human health Singh *et al.*, (2007).

Apart from these, chickens are also exposed to heavy metals by feed intake. Poultry could take up heavy metal from different sources, especially via nutrition. Therefore, metal residues may concentrate in their meat, and eggs Nisianakis *et al.,* (2009), Chowdhury *et al.,* (2011); Abdulkhaliq *et al.,* (2012) and consequently, the metals are passed to humans through chicken eggs. Since hen's eggs are considered as one of nature's highly-nutritious and economical food items in human daily diet, especially that of the children, it is of high account for human health, Surai and Sparks (2001); ALAshmawy (2013). Metallic elements are found in all living organisms where they play a variety of roles, as structural, components of control mechanisms (e.g. in nerves and muscles) and enzyme activator. Some metals are essential as copper (Cu), zinc (Zn), calcium (Ca), iron (Fe) and magnesium (Mg) those play a definitive role in the intrinsic mechanisms regulating vital biological processes. Whereas others are non-essential metals and even toxic in trace amounts, especially lead (Pb), cadmium (Cd), mercury (Hg) and arsenic (As), Dundar and Deryaoglu (2005).

Heavy metal contamination is a serious threat because of their toxicity, bio-magnification and bioaccumulation in food chain. The deficiency of elements leads to impairment of vital biological process but when they are present in excess, they become toxic. Copper is an essential trace element, normal constituent of animal tissues and fluids, crucial in haemoglobin synthesis and other enzymes functions. Toxic level of Cu may lead to Wilson's disease (excessive accumulation of Cu in liver, brain, kidney and cornea) and Menkes's disease, Tapero *et al.,* (2003). Zinc plays an important role in the maintaining of structure and function of large number of macromolecules and for over 300 enzymatic reactions, Prasad (1995) and also plays a role in immune function, protein synthesis, wound healing, DNA synthesis and cell division; consequently it supports normal growth and development during pregnancy, childhood and adolescence. Lead ingested by chicken through contaminated feed is deposited in bones and soft tissue. Development of abnormalities, deficits in intelligence quotient and neurotoxicity effects in infants, incidence constipation, colic, and anaemia are the main consequences of chronic exposure to Pb, Hariri *et al.,* (2015).

Cadmium is a toxic to virtually every system in the animal body. It is almost absent in the human body at birth but accumulates with age. However food is the primary source of cadmium exposure and its adverse health effects occur in the form of kidney damage but possibly also bone effects and fracture, Trampel *et al.,* (2003). It is also listed as a human carcinogen in Group 1 by the IARC. Decreased rate of glomerular filtration, significant proteinuria, and increased frequency of kidney stone formation are the chronic effects of oral exposure to this metal. Chromium is an essential element for human beings, especially since it acts on the organism, maintaining normal glucose tolerance. Chromium (III), found in most food and nutrient supplements, is an essential nutrient with very low toxicity, whereas Cr (VI) compound have been shown to be potent occupational carcinogens. Stainless steel vessels seem to be the main source of this element's contamination.

Iron is an essential trace element whose biological importance arises from its involvement in vital metabolic function by being cytochromes, Iron deficiency is the most prevalent single nutritional deficiency in the world and is the main cause of anaemia in infants, children, adolescents and woman of child bearing age. Manganese is usually occurring with iron and it one of the most abundant metal in the earth's crust. Manganese perform significant part in different metabolic process in human, animals, microorganism and plants the deficiency of manganese is very rare that is its presence everywhere and is found in many food.

The human health risk assessment requires identification, collection, and integration of information on hazardous chemicals, their exposure to humans, and also the relationship between exposure, dose, and adverse health effects Sobhanardakani (2017). Eggs are generally consumed by members of different income classes; thus this product's contamination can cause problems to consumers.

Hence immediate action required by the health regulatory authorities and the researchers in order to control the hazard due to heavy metals hazard which is being highly sensitive in posing risk to public health. There is a serious need of local database or risk assessment studies in local animals and foodstuffs to evaluate the potential risk or threat to humans from heavy metals because the Asian countries have different environmental and topographical conditions under which a large number livestock and poultry are growing.

Therefore the present study was designed to evaluate the levels of selected heavy metals (As, Cr, Cu, Cd, Mn, Ni, Pb and Fe) in egg to safeguard the public health in Chittoor district, Andhra Pradesh. This study will be useful in determining the potential risks from the toxic effects of heavy metals and to make recommendations for future implementations by the local health regulatory authorities.

Materials and Methods

The present study was carried out at the Department of Veterinary Public Health and Epidemiology, College of Veterinary Science, Tirupati, Sri Venkateswara Veterinary University to estimate the level of heavy metal residues *viz* As, Cr, Cu, Cd, Mn, Ni, Pb, Feusing Inductively Coupled Plasma Optical Emission Spectrometry method (ICP-OES). Hen's egg samples (commercially produced, home produced and organic eggs) were collected from different shops, homes and supermarkets in and around chittoor district of Andhra Pradesh. The egg samples (n=26) collected aseptically and carried to the laboratory in sterile polythene bags. The samples were maintained at 4ºC until processing.

Sample preparation

The procedure mentioned by Belton P.S. (2006) was adopted in this experiment for the preparation of the sample to determine heavy metals like Arsenic, Chromium, Copper, Cadmium, Manganese, Nickel, lead and Iron. The collected egg samples were cleaned and washed with demineralized water. Each egg were cut in the air cell end using pointed forceps and dissecting scissors was sterilized and rinsed with distilled water for each egg.

The content of each sample were placed in a chemically clean glass jar and weighed then blended. Samples were dried at 75°C until constant weight was obtained. The dried egg samples were subjected to digestion for further analysis by using wet digestion procedure. As per this method two grams of the sample was placed in a digestion tube and pre-digested in 10 ml concentrated HNO_3 at 135^0C until the liquor was clear.

Thereafter, 10 ml of HNO_3, 1 ml of $HClO_4$ and 2 ml of H_2O_2 was added and temperature was maintained at 135^0C for 1 hour until the liquor becomes colourless. The product of the digestion was allowed to evaporate slowly to near dryness. The dried product after digestion cooled and dissolved in 1M $HNO_{3.}$ The digest was subsequently filtered through Whatman filter paper No. 1 and diluted to 25 ml with 1M $HNO_{3.}$ The digested liver samples were presented for Inductively Coupled Plasma Optical emission Spectrometry method (ICP-OES).

Determination of heavy metals

Standard curve for the analysis of heavy metals like As, Cr, Cu, Cd, Mn, Ni, Pb and Fe was prepared from stock solutions (standard concentrations of 1000mg / ml) of analytes. To cover the optimum emission working range (0.01 to 5.00 mg / ml) further serial dilutions were prepared. Usually freshly stored standard curves in the system software, where available and the same were used. Blank solutions were also being prepared accordingly.

For the determination of As, Cr, Cu, Cd, Mn, Ni, Pb and Fe from the egg samples the standard methods adopted by Boss and Fredeen (1997). As per the procedure of these scientists Inductively Coupled Plasma - Optical Emission Spectrometry (ICP-OES Model) was used for the determination of As, Cr, Cu, Cd, Mn, Ni, Pb and Fe from the egg samples. During this experiment the samples were analyzed under the instrumental operating conditions like RF power 1.0 kW, outer argon flow 12.0 L/min, intermediate and inner argon flow 1.0 L/ min and the nebulizer uptake rate (ml / min) was 1.0. Samples were run in replicate and integrated computer results of the determination will be recorded.

Results and Discussion

The result of our study showed that the concentration of other trace elements like chromium, Copper, Manganese, Nickel and Iron were ranged 0.04-0.15, 0.87-5.66, 0.22-0.66, 0.01-0.08 and 1.29-8.54 respectively (Table 1) (Fig. 1 and 2). Fe, Cu is found in high levels while, Cr, Mn, Ni burden occurred in less levels in all birds eggs. The average of the concentration of the heavy metals in egg samples Cr, Mn, Ni, Fe and Cu were 0.06, 0.40, 0.50, 4.63 and 1.98 respectively. The result of our study showed that none of the samples had trace elements like arsenic, chromium, cadmium and lead. The mean and standard deviation of the concentration of heavy metals are listed in (Table 2).

A study was conducted by Zafar Khan*et al.*, (2016) in Peshawar found the liver contains significantly higher concentration of lead (Pb), Cadmium (Cd), Chromium (Cr), Iron (Fe), Manganese (Mn) and Zinc (Zn) as compared to thigh and breast muscle and the mean concentrations of toxic heavy metals; Pb, Cd and Cr in albumen samples were 0.13, 0.06 and 0.09 (ppm) respectively. Concentrations of Pb, Cd and Cr in egg albumen of Dir Lower were 0.13, 0.05 and 0.05and in Malakand were 0.12, 0.05 and 0.07 ppm, respectively.

The mean concentrations of essential elements Fe, Mn and Zn in egg yolk of Peshawar were 1.27, 0.31 and 2.05 while that of Dir Lower were 1.05, 0.19 and 1.97±0.04 ppm, respectively. The mean levels of Fe, Mn and Zn in egg yolk from Malakand were 1.13, 0.20 and 2.00±0.06 ppm, respectively.

Another study by Demirulus (2013) revealed the average concentrations of heavy metals were found as follows: Zn: 35.6 ppm and 42.2 ppm in yolk, 4.3 ppm and 7.4 ppm in albumen; Cu: 2.7 ppm and 10.5 ppm in yolk, 7.0 ppm and 1.5 ppm in albumen; Cd: 0.34 ppm and 1.24 ppm in yolk, 0.31 ppm and 1.25 ppm in albumen; Mn: 1.9 ppm and 6.8 ppm in yolk, 2.0 ppm and 4.5 ppm in albumen; Ni: 1.7 ppm and 3.1 ppm in yolk, 2.8 ppm and 3.7 ppm in albumen respectively and several studies conducted by different authors like Farhani *et al.,* (2015) found the mean concentrations of heavy metals in egg-white as follows: 0.119 for Al, 0.785 for As, 0.750 for Pb, 0.249 for Cd, 0.270 for Hg and 0.186mg/kg for Sb and similarly Abbasi *et al.*, (2015)showed the mean concentrations of cadmium, lead, arsenic, nickel, copper, iron and zinc in collected chicken eggs were 0.01, 0.074, 0.03, 0.014, 1.46, 34.37 and 12.55 mg/kg, respectively.

Table.1 Showing the concentration of heavy metals in egg samples (ppm) by Inductively Coupled Plasma - Optical Emission Spectrometry

SAMPLE NO	CHROMIUM	MANGANESE	NICKEL	IRON	COPPER
1	0	0.263	0.025	1.554	1.12
2	0.061	0.277	0.047	3.225	0.087
3	0.054	0.347	0.018	3.189	2.35
4	0.041	0.441	0.022	5.341	1.28
5	0.055	0.412	0.024	4.526	0.98
6	0.084	0.357	0.057	8.225	1.27
7	0.037	0.227	0.044	8.541	5.66
8	0.082	0.257	0.053	5.367	4.57
9	0.055	0.524	0.087	2.158	0.87
10	0.05	0.664	0.043	2.571	1.58
11	0.067	0.247	0.037	5.324	2.34
12	0.052	0.319	0.049	4.589	1.08
13	0.0957	0.665	0.057	3.664	2.22
14	0.102	0.348	0.055	8.547	1.97
15	0	0.525	0.029	5.664	1.55
16	0	0.354	0.068	7.658	1.49
17	0.124	0.429	0.071	5.367	3.24
18	0.075	0.712	0.049	3.35	1.59
19	0.088	0.274	0.051	5.124	1.27
20	0.114	0.366	0.043	6.66	1.8
21	0.081	0.297	0.071	4.246	1.6
22	0.057	0.269	0.059	3.278	0.98
23	0.08	0.557	0.047	1.295	1.41
24	0.089	0.442	0.076	2.506	2.07
25	0.153	0.382	0.081	2.387	4.55
26	0.097	0.476	0.057	6.214	2.61

Table.2 Showing the average, mean, standard deviation of heavy metals in egg samples

S.NO	HEAVY METALS IN EGGS	AVERAGE	MEAN	STANDARD DEVIATION
1	CHROMIUM	0.068	0.071±0.007	0.036
2	MANGANESE	0.401	0.401±0.026	0.137
3	NICKEL	0.050	0.050±0.003	0.018
4	IRON	4.630	4.637±0.414	2.115
5	COPPER	1.982	1.982±0.248	1.269

Fig.1 Showing the average concentration of manganese in eggs (ppm) by inductively coupled plasma - optical emission spectrometry

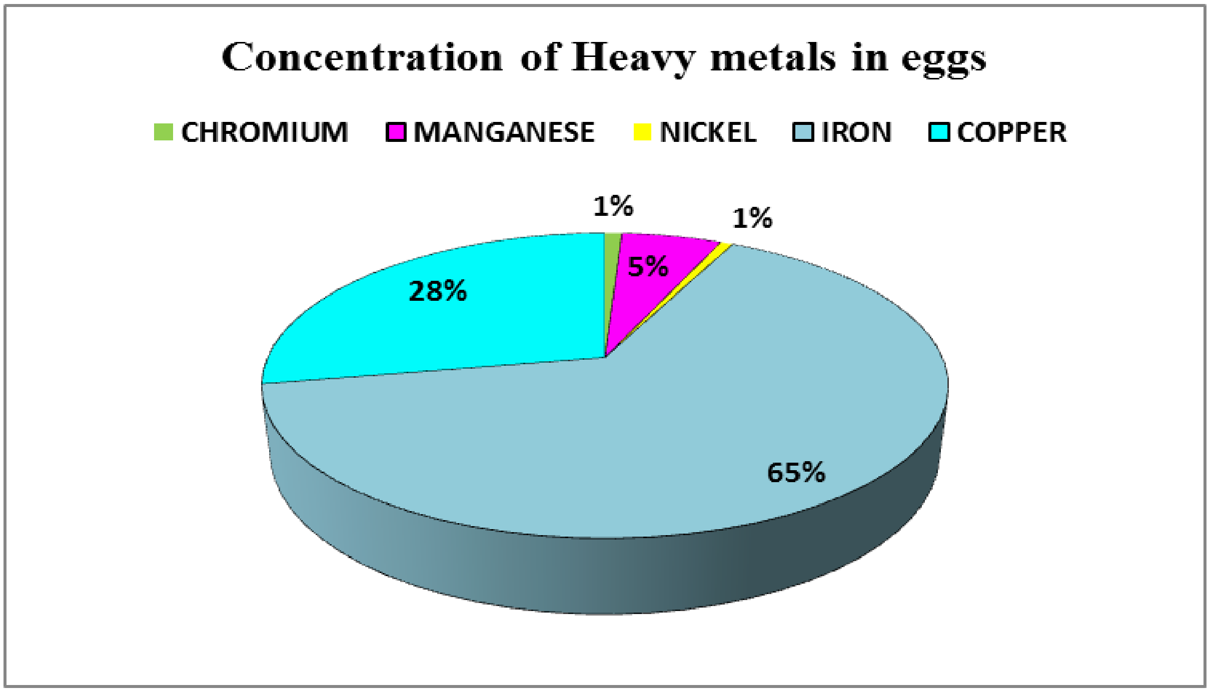
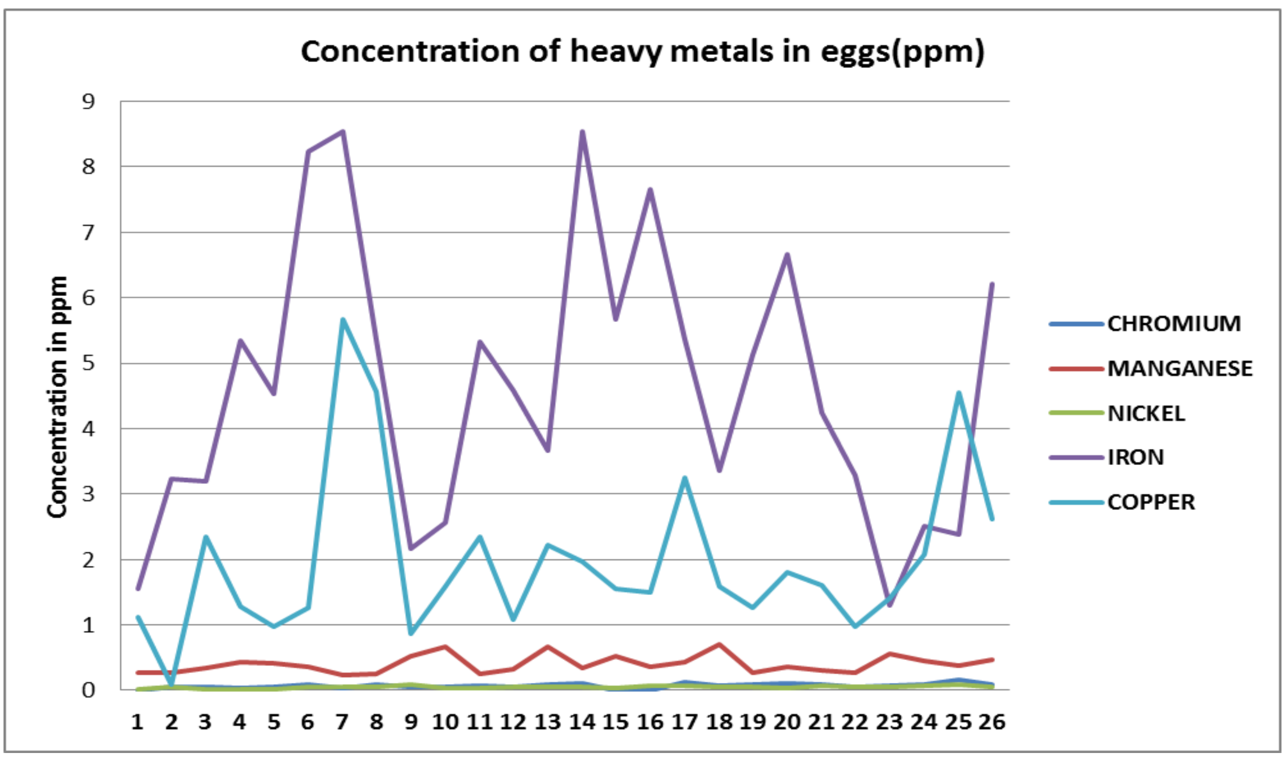


Fig.2 Showing the average concentration of manganese in eggs (ppm) by inductively coupled plasma - optical emission spectrometry



Sobhanardakani (2017) reported the mean concentrations (mg/kg) of Pb, Cd, Cr, and Cu in the samples has been 0.29±0.16, 0.18±0.04, 0.31±0.03, and 2.81±1.56, respectively. Also, the mean contents of Cd and Cr have surpassed the maximum permissible levels (MPL), established by WHO/FAO.

The Study conducted on the important heavy metal (i.e.) lead by Trampel *et al.,* (2003) found the contamination of the lead in yolks varied from less than 20 to 400 ppb, and shells were found to contain up to 450 ppb lead though Albumen contained no detectable amount and also they have correlated lead content of the egg yolks with blood lead levels but the deposition of lead in the shells did not correlate well with blood lead levels and they revealed that mean tissue lead accumulation was highest in kidneys (1,360 ppb), with livers ranking second (500 ppb) and ovarian tissue third (320 ppb). Muscle contained the lowest level of lead (280 ppb) and his study about lead contamination of egg yolks and edible chicken tissues represents a potential public health hazard. Likewise Dey and Dwivedi (2010) detected the limits for Cd and Pb was 0.001pg/g and 0.02 pg/g, respectively and the Pb concentration in egg samples ranged between 0.142 and 0.936 pg/g (mean k standard deviation: 0.489 k 0.081 pg/g), and Cd concentrations ranged between 0.030 and 0.180 pg/g (0.072 f 0.004 pg/g). In his study, the majority of samples had Pb and Cd concentrations that exceeded 0.020pg/g and0.005 pg/g, respectively.

Spliethoffa *et al.,* (2014) detected lead was between 10 and 167 µg/kg and were significantly associated (p<0.005) with lead concentrations in soil. The association between soil and egg lead has been evaluated in his study leads to denote the transfer efficiency from soil-to-egg and suggesting that there may be important geographic differences in this transfer. They have developed models that suggested that lead concentrations in >50% of eggs from a henhouse would exceed store-bought egg concentrations (<7–13 µg/kg; 3% above detection limit) at soil lead concentrations >120 mg/kg, and that the concentration in one of six eggs from a henhouse would exceed a 100 µg/kg guidance value at soil lead concentrations >410 mg/kg. His models also suggested that the availability of dietary calcium supplements was another influential factor that reduced egg lead concentrations. Estimates of health risk from consuming eggs with the lead concentrations were measured generally were not significant.

In conclusion, with regards to presence of heavy metals in eggs, the necessity of vigorous regular national monitoring of eggs contamination as well as quality of safe animal feed as a main source of contamination should be emphasized**.** Feeds supplement added to hen's diet should be measured and calculated its residues in eggs to avoid undesirable increase in their amounts. Since knowledge of eggs' metal levels is becoming increasingly important and egg consumption is a bio indicator in addition to monitor environmental pollution.

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