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Estimation of chromium, copper and lead in milk by inductively coupled plasma-optical emission spectrometry in Tirupati, Andhra Pradesh

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Abstract
The aim of our study is focused on evaluating the trace elements level in milk that collected from the retail outlets in and around Chittoor district, Andhra Pradesh. The concentrations of Copper, chromium and Lead were measured using Inductively Coupled Plasma - Optical Emission Spectrometry. The study showed that the concentration of trace elements like chromium, Copper and Lead in 25 milk samples using using Inductively Coupled Plasma Optical Emission Spectrometry method (ICP-OES) were ranged 0-0.002, 0-0.004 and 0.15-0.68 respectively (Table 1) (Fig.1). Cu is found in high levels while Cr, Pb burden occurred in less levels in all milk samples. The averages of the concentration of the heavy metals in egg samples Cr, Pb, Cu were 0.00032, 0.00108 and 0.3632 respectively. Attention should be given to heavy metals as once they are present in concentrations greater than the acceptable daily intake, it may be leads to public health risk.

Keywords: Food safety, heavy metals, milk

Introduction
Milk is considered as an important foodstuff in our daily life which acts as excellent source of Calcium (Ca) which extensively helps in the growth and development of children and also helps to reduce osteoporosis in old people hence considered to be an essential source of nutrition. In recent years due to the existence of trace elements and heavy metals in dairy products and milk become one of the most threatening aspects has been recorded in various countries and regions. Till now about 38 micro and trace elements found in raw milk from different regions around the world have been reported (Dobrzañski *et al*. 2005) [9].

Micro elements such as copper (Cu), Iron, selenium (Se), and Zinc (Zn) are known to be essential for human growth. However, heavy metals such as Arsenic (As), Cadmium (Cd), Mercury (Hg), Lead (Pb) have health impacts on human wellbeing if it is more than maximum permissible level. The maximum permissible level of Pb in milk recommended by Codex Alimentarius Commission (2015) [7] and European Union Commission (EC) no. 1881 European Union (2006) [11] is 0.02mg/ml. According to Indian regulations (FSSAI, 2011) [12], the maximum permissible limits for Pb in milk are 0.02 and 0.1mg/ml. Heavy metal content in raw cow milk may vary depending on several factors i.e. lactation period of cows, health conditions, seasonal variations, climatic conditions, annual feed composition and environmental contamination (Yahaya *et al*. 2010) [22]. Apart from that conditions carried out for processing of milk may also have effective influence on the contents and retains of minerals in total composition of milk (Salah *et al*. 2013) [18]. Prolong or over exposure to heavy metals will leads to abdominal pain, hepatotoxicity, neurotoxicity, vomiting, decreasing of intelligence quotient (IQ) level, Alzheimer's disease, behavioural disorders, tissue injury, irritation of lungs, cancer etc. could be generated.

Complete elimination or prevention of chemical contaminants cannot be achieved from milk because the lipophilic contaminants will find its way into the persistent fat compounds from where heavy metals cannot be removed readily (Girma *et al*. 2014) [13]. Besides heavy metals are non-biodegradable in nature and become accumulated in the food chains via bio-transformation, bio-accumulation and biomagnifications (Aslam *et al*. 2011) [4]. Moreover, with this increasing scenario in milk production, it is assumed that the consumer population of the country would face significant health threat in the long run from consuming contaminated milk and milk products.

Thus the daily intake rate of heavy metal hazard quotients (HQ) and carcinogenic risk (CR) might be considered as exponentially increasing trend with the increasing rate of milk production. 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) [21]. 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) [14]. 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.

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) [20]. 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 (Cr, Cu and Pb) in milk to safeguard the public health in Chittor 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.

Methodology

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* Cr, Cu and Pb using Inductively Coupled Plasma Optical Emission Spectrometry method (ICP-OES).Milk samples were collected from places in and around chittor district of Andhra Pradesh. The milk samples (n=25) 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) [5] was adopted in this experiment for the preparation of the sample to determine heavy metals like Chromium, Copper and lead. 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 Cr, Cu and Pb 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 Cr, Cu and Pb from the milk samples the standard methods adopted by Boss and Fredeen, 1997 [6]. As per the procedure of these scientists Inductively Coupled Plasma - Optical Emission Spectrometry (ICP-OES Model) was used for the determination of Cr Cu and Pb from the milk 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 study showed that the concentration of trace elements like chromium, Copper and Lead in 25 milk samples using using Inductively Coupled Plasma Optical Emission Spectrometry method (ICP-OES) were ranged 0-0.002, 0-0.004 and 0.15-0.68 respectively (Table 1) (Fig.1). Cu is found in high levels while Cr, Pb burden occurred in less levels in all milk samples. The averages of the concentration of the heavy metals in egg samples Cr, Pb, Cu were 0.00032, 0.00108 and 0.3632 respectively.

Abdulkhaliq *et al*. 2013 have found the mean concentrations of metals (µg/g) in milk and dairy samples analyzed ranged between 0.022-0.057 for Cd, ND-0.93 for Pb, 0.62-0.85 for Cu and 3.2-12.91 for Fe and it indicated high concentrations of Pb and Cd especially in powder milk samples. The lowest concentrations of metals were found in white cheese followed by liquid milk. Enb *et al*. 2009 [10] from Egypt reported the concentrations of Metals, i.e. iron (Fe), copper (Cu),manganese (Mn) and zinc (Zn) were detected in buffalo's milk at 0.88, 0.201, 0.072 and4.35 mg/kg milk and were higher than in cow's milk (0.572, 0.131, 0.047 and 2.828 mg/kg, respectively).Also cow's milk contained chromium (Cr), nickel (Ni), cobalt (Co) and tin (Sn), at lower levels.

Similar study conducted by Nirgude 2015 in buffalo milk of Boisar-Tarapur area of Palghar district using Inductively Coupled Plasma Atomic Emission Spectroscopy had significantly lower concentration of Cu and Mn ($P<0.1$) Zn and Mn ($P<0.1$), similarly significantly higher concentration of Pb and Fe ($P<0.001$) and Fe and Al ($P<0.01$). Most of the elements show positive correlation coefficient with each other except Nickel. It shows negative correlation coefficient with all measured elements except Barium and Silicon. Strontium was found and detected in milk sample.

Abdl- khalik, 2012 estimated the residues of some heavy metals of toxicological concernin milk. One hundred and fifty samples from dairy farm, market raw milk andvendor's milk were collected from Zagazig City and were examined for presence of lead (Pb), cadmium (Cd), mercury (Hg) copper (Cu) and Zinc (Zn)by using atomic absorption spectrometry. The mean concentrations were 0.716, 2.001, 2.11 for Pb; 0.871, 3.39, 4.11 for Cd; 0.911, 5.79, 6.37 for Hg; 2.81, 7.21,7.72 for zinc and 2.111, 4.44, 4.45 (ng/L) for copper in farms, markets and vendors milk samples, respectively. The percentages of significant dairy farms, market milk and vendor samples exceeded the maximum permissible limits were30, 44 and 52% for lead, 26, 100 and 100% for cadmium, 12, 22 and 28% for mercury, 22, 26 and 32% for zinc, 50, 90 and 96% for copper.

Roy *et al*, 2009 [17] collected total of 65 cow and 126 buffalo milk samples were collected from different districts of Haryana covering industrial and non-industrial areas of two zones as per agro-climatic conditions of the state. Lead (Pb) content of cow and buffalo milk averaged 0.035±0.009, 0.048±0.009 and 0.100±0.007, 0.090±0.013 ppm in zone 1 and 2, respectively.

A recent study by Ahmed *et al*. 2017 in order to determine the seven elements was performed by Perkin Elmer Atomic Absorption (AA) spectrophotometer and highlighted the importance of seven heavy metals residual concentration including Cd, Cr, Cu, Fe, Mn, Ni and Zn in milk of Camel, Cattle, Buffalo, Sheep and Goat from various areas of Khyber Pakhtunkhwa (KPK), Pakistan. It revealed that milk of camel comprising of high levels of Zn (5.150 ± 0.021 mg/kg), Mn (0.094 ± 0.003 mg/kg) and Fe (1.580 ± 0.530 mg/kg) with a definite correlation. In the milk of buffalo, high concentration of noxious heavy metals including Cu (0.223 ± 0.010 mg/kg) and Cd (0.117 ± 0.086 mg/kg) were found whereas in goat milk, high Ni (1.152 ± 0.045 mg/kg) and Cr (1.152 ± 0.045 mg/kg) was observed and detected. The analysis showed that camel and buffalo have similar high concentration of heavy metals. Overall results showed that milk of cattle shows higher concentration of Zn, Mn and Fe along with Buffalo.

By using Inductively coupled plasma- optical emission spectrometry (ICP-OES), a study conducted by Dhanalakshmi *et al*. 2013 [8] in goat milk to find out the concentration of heavy metal residues which was collected from three different areas (industrial, semi- industrial and non-industrial) of Hosur (Tamil Nadu) and reported that heavy metals concentration were higher in industrial area, than from the semi-industrial and nonindustrial areas but well within the prescribed permissible limit. Among the five heavy metals analyzed in the milk samples, cadmium was higher than the other elements viz., lead, mercury, nickel and arsenic. From the study, it was concluded that, the concentration of heavy metals in goats milk were generally low but differed (P< 0.01) significantly.

Malhat *et al*. 2012 [15] investigated the residues levels of five metals (Cadmium, Copper, Lead, Iron and Zinc) in cow milk collected from different sites in El-Qaliubiya governorate, Egypt and found that the highest average concentration are those of iron (16.38 lg/g) followed by zinc(10.75 lg/g) and lead (4.404 lg/g), while the lowest mean concentration are 2.836 and 0.288 lg/g for copper and cadmium, respectively.

A similar type of study conducted by Shahriar *et al*.2014 and estimated the content of copper in most of all raw milk and milk products were in the range from 0.02 mg/kg to 0.25 mg/kg. The highest level of copper was found 0.244 mg/kg in milk products. The concentration of lead in milk and milk products were in the range from 0.007 mg/kg to 0.02 mg/kg. The highest concentration of lead was found 0.019 mg/kg in raw milk.

Conclusion

However, the number of analyzed heavy metals and sample size were limited in our study and further studies are necessary to evaluate the contents of ''essential'' and ''toxic'' heavy metals on a greater number of milk samples from various region of Andhra Pradesh and to confirm the absence of possible toxicological risks. Special attention should be given to heavy metals as once they are present in concentrations greater than the acceptable daily intake, it may be difficult to reduce them to an acceptable level during processing. It is suggested that control and monitoring of water and feed for livestock and application of appropriate containers in transit of raw milks may be helpful for production of healthier milk.

Table 1: Showing the concentration of Chromium, Lead and Copper in Milk by Inductively Coupled Plasma Optical emission Spectrometry method (ICP-OES)

Wavelength	267.716	220.353	327.393
Sample No	**Chromium (ppm)**	**Lead (ppm)**	**Copper (ppm)**
1	0	0	0.42
2	0	0.002	0.35
3	0	0.003	0.22
4	0	0.001	0.27
5	0	0.001	0.39
6	0.001	0.001	0.57
7	0	0	0.41
8	0.002	0	0.21
9	0	0.002	0.44
10	0	0.003	0.32
11	0	0	0.21
12	0	0.004	0.27
13	0	0.003	0.15
14	0	0.002	0.25
15	0	0	0.19
16	0	0	0.24
17	0	0	0.18
18	0.001	0	0.38
19	0	0	0.47
20	0	0	0.52
21	0.002	0	0.33
22	0	0.002	0.48
23	0	0	0.57
24	0	0.003	0.68
25	0.002	0	0.56

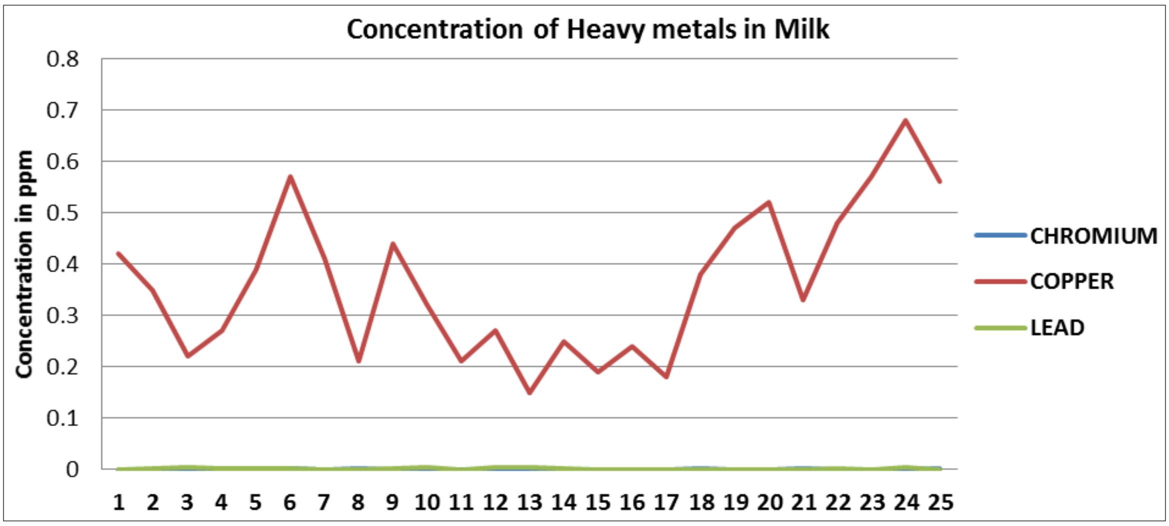

Fig 1: Concentration of heavy metals in Milk (ppm) using Inductively Coupled Plasma Optical emission Spectrometry method (ICP-OES).

References

1. Abdl-khalik A, Azza Ayoub GM, Raslan MT. Estimation of some heavy metals in cow's milk in zagazig city. Assiut vet. med. J. 2013; 59(138).
2. Abdulkhaliq A, Swaileh KM, Hussein RM, Matani M. Levels of metals (Cd, Pb, Cu and Fe) in cow's milk, dairy products and hen's eggs from the West Bank, Palestine. International Food Research Journal. 2012; 19(3):1089-1094.
3. Ahmad I, Zaman A, Samad N, Ayaz MM, Rukh S, Akbar A *et al*. Atomic Absorption Spectrophotometery Detection of Heavy Metals in Milk of Camel, Cattle, Buffalo and Goat from Various Areas of Khyber-Pakhtunkhwa (KPK), Pakistan. J Anal Bioanal Tech. 2017; 8:3.
4. Aslam B, Javed I, Hussain KF, Ur-Rahman Z. Uptake of Heavy Metal Residues from Sewerage Sludge in the Milk of Goat and Cattle during Summer Season. Pak. Vet J. 2011; 31(1):75-7.
5. Belton PS. Trace Element Analysis of Food and Diet RSC Food Analysis Monographs Royal society of chemistry, Thomos Graham House Science Park, Milton road Cambridge, CB4 0WF, UK, 2006.
6. Boss CB, Freeden KJ. Concepts, Instrumentation and Techniques in Inductively Coupled Plasma Optic Emission Spectrometry, 2nd edition, Perkin Elmer Corporation, USA, 1997.
7. Codex Alimentarius Commission. General standard for contaminants and toxins in food and feed (Codex Stan 193-1995): Codex Alimentarius Commission, 2015. http://www.fao.org/input/download/standards/17/CXS_193e_2015.pdf.
8. Dhanalakshmi B, Gawdaman G. Determination of heavy metals in goat milk through ICP-OES. Asian J Dairy Food Res. 2013; 32:186-90.
9. Dobrzañski Z, Kolacz R, Górecka H, Chojnacka K, Bartkowiak A. The content of microelements and trace elements in raw milk from cows in the Silesian region. Polish J Environ Stud. 2005; 14(5):685-9.
10. Enb A, Abou Donia MA, Abd-Rabou NS, Abou-Arab AAK, El-Senaity MH.Chemical Composition of Raw Milk andHeavy Metals Behavior during Processing of Milk Products. Global Veterinaria. 2009; 3(3):268-275.
11. European Union. Commission regulation (EC) no. 1881/2006 setting maximum levels for certain contaminants in foodstuffs. Official journal of European Union. 2006; 364:5-24.
12. Food Safety and Standards (Contaminants, Toxins and Residues) Regulations (FSSAI). Ministry of Health and Family Welfare. (Food Safety and Standards Authority of India), 2011. https://www.suratmunicipal.gov.in/Content/Documents/Downloads/FssAct/Fss Act1.pdf.
13. Girma K, Tilahun Z, Haimanot D. Review on Milk Safety with Emphasis on Its Public Health. World J Dairy Food Sci. 2014; 9(2):166-83.
14. Hariri E, Abboud MI, Demirdjian S, Korfali S, Mroueh M, Taleb RI. Carcinogenic and neurotoxic risks of acrylamide and heavy metals from potato and corn chips consumed by the Lebanese population. J Food Compos. Anal. 2015; 42:91-97.
15. Malhat F, Hagag M, Saberand A, Fayz AE. Contamination of Cow's Milk by Heavy Metal in Egypt. Bull Environ Contam Toxicol. 2012; 88:611-613.
16. Nirgude NT. Heavy Metals and Mineral Elements in Milk of Buffaloes at Boisar-Tarapur Industrial Area, Palghar District, Maharashtra, India. A.I.J.C.S.R. 2015; 2(1).
17. Roy Bharathidhasan DS, Veena Mani, Harjit Kaurand, Neelam Kewalramani.Heavy Metal Contents in Cow and Buffalo Milk Samples from Haryana. Indian J Anim. Nutr. 2009; 26(1):29-33.
18. Salah F, Ahmed AEA. Assessment of Toxic Heavy Metals in Some Dairy Products and the Effect of Storage on its Distribution. J Ameri Sci. 2012; 8(8):665-70.
19. Shahriar SMS, Akther S, Akter F, Morshed S, Alam MK, Saha I *et al*. Concentration of Copper and Lead in Market Milkand Milk Products of Bangladesh. International Letters of Chemistry, Physics and Astronomy, 27:56-63.
20. Sobhanardakani S. Tuna fish and common kilka: Health risk assessment of metal pollution through consumption of canned fish in Iran. J Consum Prot. Food Saf. 2017; 12(2):157-163.
21. Tapiero H, Tew KD. Trace elements in human physiology and pathology zinc and metallothioneins. Biomedicine and pharmacotherapy. 2003; 57(9):399-411.
22. Yahaya MI, Ezo GC, Musa YF, Muhamad SY. Analysis of heavy metals concentration in roadside soils in Yauri, Nigeria. Afr J Pure Appl Chem. 2010; 4(3):022-30.