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Status of Serum Trace Elements among Preschool Children in Rural Bhubaneswar, Odisha, India

Priyadarsi Girija Sankar Sethy[1], Gandham Bulliyya[1], Tapash Ranjan Rautray[2], Shuchismita Behera[1] and Shantanu Kumar Kar[1]

[1]Regional Medical Research Centre, Indian Council of Medical Research, Chandrasekharpur, Bhubaneswar, Odisha, India
[2]Centre of Excellence in Theoretical and Math Sciences, SOA University, Bhubaneswar, Odisha, India

Correspondence should be addressed to Gandham Bulliyya, gbrmrcicmr@gmail.com

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Abstract One in every three malnourished children in the world is from India leading to high child morbidity and mortality. Out of these, preschool children constitute the most vulnerable segment of any community. The present study aimed at assessing the levels of trace elements in preschool children aged 2-5 years in rural Gram Panchayats (GP) of Bhubaneswar block, Odisha, India. A cross-sectional study was undertaken covering 176 children (boys 100 and girls 76) from 8 GPs selected on random basis. Blood was collected and lyophilized serum subjected to trace elements by proton induced X-ray emission (PIXE) technique. Overall, the mean levels of manganese (Mn), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), selenium (Se), bromine (Br) and lead (Pb) were 19.2±3.39, 0.86±0.52, 3.2±0.9, 4.04±0.87, 15.26±2.27, 1.03±0.95, 59.95±6.55 and 0.29±0.66 ppm respectively. The concentrations of Mn, Ni, Cu and Br were increased significantly with age, while levels of Co, Zn, Se, and Pb showed random variations. The mean Co, Ni, Cu, Se, Pb and Br levels were found to be higher among boys than girls however, only Cu and Br found to be significantly high. There were significant correlations observed between some of elements (Mn-Br, Co-Ni, Ni-Cu, Ni-Br, Cu-Zn and Cu-Br) and Se levels negatively associated with Pb. The mean concentrations obtained for most trace elements with an exception of Mn were within the ranges reported by earlier studies.
Keywords *Preschool Children; Trace Elements; PIXE; Odisha; India*

1. Introduction

Preschool children constitute the most vulnerable segment of any community. Their nutritional status is a sensitive indicator of community health and nutrition [1]. More than half (54%) of all deaths before age five years in India are related to malnutrition [2]. The overall scenario in the nutritional profile of preschool children in Odisha is much inferior to other states in India [3]. A community based studies show that the magnitude of under nutrition in preschool children is still a leading problem in Odisha

[4]. Over the last three decades, increasing attention has been directed towards specific deficiencies of trace elements. Although these elements are required in small amounts, organisms need these substances for their health and all forms of life [5]. Trace element determination in blood serum has become important to investigate the vital role in human metabolism as well as to obtain information regarding the health status of individuals [6]. Several studies [7-17] were undertaken in measuring trace elements in the serum/plasma using various techniques like instrumental neutron activity analysis (INAA), flame atomic absorption spectrometry (FAAS), Electro-thermal atomic absorption spectrometry (ETAAS) and Proton Induced X-ray Emission (PIXE) in different age groups but no such studies were reported for the most vulnerable preschool children in India. The current study aims at evaluating the levels of serum trace elements among the preschool children in Odisha by PIXE method, which can quantify trace element down to parts per million (ppm) or sub-ppm levels [18]. In this study some essential and toxic (Pb) elements were estimated among preschool children. The levels of the trace elements were presented and the results are compared with the data reported in the literature.

2. Materials and Methods

A cross-sectional study was undertaken in rural block of Bhubaneswar covering 8, out of 19 GP selected on random basis. The study sample included 176 children aged 2-5 years after getting informed written consent from caregivers and mothers. Age of children was recorded in months from the birth records available with mothers or Anganwadi Centers. Venous blood samples were collected from each child and allowed 30-40 minutes for spontaneous clotting and then the serum was separated by centrifugation at 3000 rpm for 10 minutes at room temperature. Sera were stored at -20°C in metal free vials until analysis. The serum samples were lyophilized to make powder form using freeze-dried vacuum concentrator (Labconco make no. 010112731E). Aliquots of 250 mg of dried serum was weighed and mixed with high purity graphite powder in 1:1 ratio by mass. The pellets were made by applying 6 Dalton pressure in a hydraulic press [19].

The trace element composition was determined using the PIXE analytical methodology at the Institute of Physics, Bhubaneswar. PIXE is simultaneous, reliable, rapid, multi-elemental, sensitive and non-destructive in nature to analyse the trace elements [20]. The proton beam with energy of 3 MeV and diameter 3 mm obtained from the 3 MV tandem pelletron accelerators was used to irradiate the samples in vacuum (10^{-6} Torr) inside a PIXE chamber. The targets (pellets) were held at 45° to the beam direction on a target holder that was mounted on an insulated stand surrounded by a cylindrical electron suppressor held at negative potential with respect to the target. The sample targets were bombarded with 3 MeV proton beams and the beam current was in the range of 25–30 nA. A Silicon (lithium) detector (active area 30 mm^2) was used with a resolution of 170 eV at 5.9 keV, with beryllium window placed at 90° to the beam direction to detect the characteristic X-rays emitted from the targets. Spectra were recorded using a multichannel analyser calibrated with ^{241}Am X-ray source [21]. No X-ray absorbers were used between the detector and target during data collection. The PIXE spectral analyses were performed using GUPIX-2004 software (University of Guelph, Guelph, Ontario, Canada). This provides a non-linear least squares fitting of the spectrum, together with subsequent conversion of the fitted X-ray peak intensities into elemental concentrations, using the fundamental parameter method for quantitative analysis. The serum concentrations of the trace elements such as Mn, Co, Ni, Cu, Zn, Se, Br and Pb were measured and expressed as ppm. For the checking of the reliability of the technique, a certified reference material NIST Bovine liver (1577b) was used as an international standard and comparison (Table 1). The measured values and the certified values are in good agreement and thus the experimental procedure adopted is reliable in analyzing the serum samples.

Table 1: *Concentration of Trace Elements (ppm) in Certified Reference Materials (Bovine Liver, NIST-1577b) Measured by Proton Induced X-Ray Emission Method*

Trace Elements	Certified Value	Measured Value	Percentage of Recovery
Manganese	10.5±1.7	10.3±1.2	98.10
Copper	160±8.0	157±10	98.13
Zinc	127±16.0	130±11	102.36
Selenium	0.73±0.06	0.71±0.05	97.26
Bromine	9.7	10.1±1.1	104.12
Lead	0.123±0.004	0.128±0.003	99.22

Statistical analysis was performed using SPSS program 11. The data was presented in mean±standard deviation and t-test was used for comparison between groups. Significance was considered when p value was less than 0.05. Pearson's correlation tests were performed for establishing associations between parameters.

3. Results

The characteristics of the study population are provided in Table 2. The study sample included 100 male and 76 female children aged 2-5 years from different socioeconomic and ethnic groups which comprised 93 general (GEN), 49 scheduled caste (SC) and 34 scheduled tribe (ST) children.

Table 2: *Characteristics of Study Population in Bhubaneswar Rural Block, Orissa, India*

Gram Panchayat	No. of Children	Sex		Community		
		Boys	Girls	SC	ST	GEN
Andharua	20	12	8	5	4	11
Chandaka	24	13	11	7	4	13
Daruthenga	26	14	12	8	3	15
Kantabada	18	11	7	4	10	4
Dadha	20	12	8	4	2	14
Raghunathpur	21	13	8	6	4	11
Kalyanapur	23	12	11	8	2	13
Kalarahang	24	13	11	7	5	12
Total	176	100	76	49	34	93

SC-Schedule Caste, ST- Schedule Tribe, GEN-General Caste

The mean serum concentration of trace elements in children by age and sex are presented in Table 3. The mean Mn, Co, Ni, Cu, Zn, Se, Br and Pb levels were 19.23±3.39, 0.86±0.52, 3.20±0.90, 4.04±0.87, 15.26±2.27, 1.03±0.95, 59.95±6.55 and 0.29±0.66 ppm respectively. The level of Mn was high and the selenium deficiency was marked in the population. Levels of Pb in the serum were at very low concentrations indicating that the study population is free from the influence of Pb contamination in excess of the normal level. Mean concentrations of Mn, Ni, Cu and Br were increased with age, however, elements like Co, Zn, Se and Pb showed random variations. The concentrations of Co, Ni, Cu, Se, Br and Pb were found to be higher for boys as compared to girls, while Mn and Zn level tend to be in reverse trend. Sex variation was significant observed for Cu ($p<0.05$) and Br ($p<0.01$). The mean values of Mn, Se and Br showed increasing trend with respect to age.

Table 3: *Mean (±SD) Concentrations of Serum Trace Elements (ppm) by Age and Sex*

Age years	Sex	N	Manganese	Cobalt	Nickel	Copper	Zinc	Selenium	Bromine	Lead
2	T	26	15.85±3.21	1.01±0.50	2.75±0.55	3.50±0.38	15.37±1.29	0.94±1.28	55.14±5.64	0.09±0.13
	B	14	14.36±3.41	1.26±0.24	2.82±0.35	3.52±0.12	14.87±1.27	1.11±1.56	56.10±6.59	0.10±0.22
	G	12	17.59±1.90**	0.61±0.41**	2.67±0.73	3.47±0.56	15.95±1.10*	0.74±0.87	54.02±4.29	0.08±0.02
3	T	41	19.83±3.05	0.64±0.64	3.23±0.59	4.04±0.82	14.51±2.02	1.02±0.89	59.13±5.44	0.42±0.74
	B	20	19.82±3.20	0.73±0.64	3.14±0.63	4.15±0.85	13.95±2.17	0.88±0.81	60.09±5.99	0.44±0.71
	G	21	19.85±2.97	0.56±0.63	3.31±0.55	3.94±0.80	15.04±1.74	1.16±0.97	58.22±4.83	0.40±0.79
4	T	57	19.40±3.54	0.84±0.45	3.20±1.04	3.99±0.96	15.43±2.79	1.01±0.85	61.10±5.25	0.30±0.68
	B	34	19.58±4.12	0.76±0.44	3.30±1.28	4.15±0.97	16.07±2.21	0.98±0.74	63.25±5.00	0.25±0.56
	G	23	19.14±2.51	0.97±0.47	3.05±0.48	3.74±0.90	14.93±2.02	1.04±1.01	57.92±3.88**	0.39±0.84
5	T	52	20.25±2.47	0.96±0.45	3.41±1.03	4.36±0.87	15.43±2.79	1.09±0.91	61.72±7.83	0.27±0.72
	B	32	20.04±2.61	1.02±0.49	3.52±1.24	4.44±0.96	15.35±3.13	1.25±1.06	62.41±8.73	0.35±0.87
	G	20	20.58±2.24	0.85±0.38	3.23±0.54	4.24±0.71	15.56±2.20	0.83±0.53	60.63±6.18	0.14±0.36
Pooled	T	176	19.23±3.39	0.86±0.52	3.20±0.90	4.04±0.87	15.26±2.27	1.03±0.95	59.95±6.55	0.29±0.66
	B	100	19.05±3.86	0.92±0.52	3.27±1.08	4.15±0.91	15.25±2.53	1.07±1.00	61.35±7.11	0.30±0.68
	G	76	19.47±2.64	0.77±0.52	3.11±0.59	3.88±0.81*	15.29±1.89	0.97±0.87	58.10±5.23**	0.27±0.65

B: Boys, G: Girls, T: Total, * p < 0.05, ** p < 0.01

From the correlation matrix (Table 4), it was observed that the concentrations of elements Mn, Ni, Cu and Br increased with the age. Among the inter elemental relationships, significant correlations observed between elements Mn-Br, Co-Ni, Ni-Cu, Ni-Br, Cu-Zn and Cu-Br. There was a significant negative correlation observed between levels of Pb-Se.

Table 4: *Correlation Matrix for the Trace Element Contents in Children*

Indicator	Age	Manganese	Cobalt	Nickel	Copper	Zinc	Selenium	Bromine	Lead
Age	1.000								
Manganese	0.347**	1.000							
Cobalt	0.061	-0.128	1.000						
Nickel	0.203**	-0.141	0.241**	1.000					
Copper	0.280**	0.042	0.138	0.439**	1.000				
Zinc	0.083	0.093	-0.019	-0.111	0.159*	1.000			
Selenium	0.047	0.146	-0.072	0.000	-0.049	-0.020	1.000		
Bromine	0.316**	0.224**	-0.085	0.316**	0.156*	0.057	0.107	1.000	
Lead	0.037	0.108	-0.040	-0.051	-0.001	0.066	-0.202**	-0.016	1.000

*Correlation is significant at the 0.05 level
**Correlation is significant at the 0.01 level

Table 5 presents the values (mean±SD) of trace elements in this study population as against earlier reports. In the present study it was found that the levels of Mn and Co were relatively high, and levels of Ni, Cu, Zn, Se and Pb were closer to earlier studies.

Table 5: *Concentrations of Serum Trace Elements (ppm) in Preschool Children, Orissa, India*

Trace Elements	Mean ± SD (95% CI)	Earlier Studies on Preschool Children
Manganese	19.23±3.39 (5.82-32.98)	0.08±0.147, 0.32± 0.18 [18, 24]
Cobalt	0.86±0.52 (0.01-1.87)	0.05±0.08, 0.24±0.25, 0.61±0.33 [24, 14, 18]
Nickel	3.20±0.90 (1.80-9.75)	0.06±0.06, 2.6-7.5, 11.7±6.5 [18, 25, 14]
Copper	4.04±0.87 (2.28-6.53)	1.63±0.37, 3.61±0.10 [27, 31]
Zinc	15.26±2.27 (7.30-20.70)	7.4 ±0.25, 15.15±0.19, 16.8±0.08 [14, 31, 8]
Selenium	1.02±0.95 (0.0-4.7)	0.45-1.21, 0.90 [28, 29]
Bromine	59.95±6.55 (42.60-79.20)	59.1-63.0, 100-187 [7, 31]
Lead	0.29±0.66 (0.0-3.10)	0.08±0.01, 0.21 ± 0.105, 0.20±0.10 [13, 15, 17]

4. Discussion

Trace elements are involved in many biological processes supporting life. The concentrations of trace elements in the body are influenced by a number of factors such as gender, age and dietary intakes. Deficiencies in trace elements are usually diagnosed with common symptoms such as malaise, loss of appetite, anemia, infection, skin lesions, and low-grade neuropathy [5]. Intoxication by trace elements is common in situation like flue, fever, nausea, vomiting, diarrhea, anemia, and affect central nervous system causing neuropathy [22]. On the other hand, excess intake of these trace elements leads to diseases and toxicity, for which a fine balance is essential for maintaining health.

In our study Mn concentration was found to be 19.23±3.39 ppm and the levels increased with age. A significantly high content of Mn detected among children in the present study, which is not the case in earlier reports [18]. In a study conducted on neonates, Mn levels in blood at full-term and 6 months were found to be 0.32 and 0.21 ppm [14]. As compare to earlier studies and reference values, Mn levels in this population seem very high. Food is the main source of Mn for general population, with air and water contributing about 1% of the daily intake. Children often eat soil that contains Mn, however, there is little information on how well Mn in soil can be taken up from the stomach into the body if children eat it. Most soils contain Mn values range from 40–900 ppm. Further, Mn can be absorbed in higher-than-usual amounts if the diet is low in iron. Anaemia is the most common among children in Odisha [3]. It is an established fact that low ferritin levels are associated with increase in Mn absorption [23].

The mean Co level in the study was 0.86±0.52 ppm. Sex wise the level of Co is higher in case of boys than girls. Marriott et al., [14] reported serum cobalt levels among Turkish children as 0.24±0.15 µg/l (ppm). In this study, Co levels were relatively higher as compared with earlier studies [18, 24].

The concentration of Ni among the children was 3.20±0.90 ppm, whereas Iyengar and Woittez [25] reported a concentration ranged 2.6-7.5 ppm. Another study [14] reported the Ni level of 11.7±6.5 ppm among children. The level of Ni in case of boys is more than that of girls.

In case of Cu, the mean serum concentration was 4.04±0.87 ppm and more in case of boys than the girls. The level of Cu increased with increase of age. The serum Cu level in preschool children was found to be 3.61±0.01 [26]. Serum Cu levels in malnourished and well children in Tanzania reported to be 16.5 and 21.2 mmol/l [7]. Co levels reported to be significantly higher in children with iron deficiency anaemic (189±49 µg/dl) than those of controls (163±37 µg/dl) [27]. In the rural community of Canagua, Merida State, Venezuela estimated the Cu among pre-school children aged 2-6 years to be 1.18±0.30mg/l, and the level decreased with age [10]. But in the present study, it is not same and the level of Cu is slightly higher in case of boys than girls.

The concentration of Zn in this study population was 15.26±2.27 ppm. Similar concentrations of Zn (15.15±0.19 ppm) documented for preschool children [26] from Nigeria and for malnourished children it was 12.5 ppm in Tanzania [7]. Serum Zn levels are inversely associated with severity of protein energy malnutrition in children using weight-for-age of Harvard Standards. Zn levels fallen consistently with malnutrition grades -I, -II, -III respectively with 8.27, 6.77, 5.3 ppm as compared to 10.9 ppm in the well-nourished group [16]. Levels of serum Zn found to be low in children with iron deficiency anemia (IDA) group as compared to control group [15, 27]. Zn levels of neonates at full-term and 6 months reported to be at 12.0±2.6 ppm and 13.8±2.5 ppm respectively [14]. The mean serum concentrations of zinc among children aged 2-6 years found to be 7.4±2.5 and the levels increase with age [10].

In the present study, mean Se concentration was 1.03±0.99 ppm ranging from 0.03 to 4.7 ppm. A study by Arnaud et al., [28] on serum Se concentrations among newborns found to be varying 0.45-

1.21 ppm. Muntau et al., [29] reported a statistically significant age dependency: increase in Se levels from 1 to 5 years (0.64-0.90 ppm). Se concentrations found in children is within the normal ranges. The concentration of Se in case of boys is little higher than girls. It is observed that, Se could not be detected (negligible amount) in 31.68% of samples, which indicating the deficiency of Se in the population. It may be due to low soil Se levels and dietary intakes.

The concentration of Br among child population was 59.95±6.55 ppm, with significantly higher levels in boys than in girls. The levels of Br increased with age. Serum Br level among malnourished children (59.1 µmol/l) was found to be lower than the healthy control groups (63.0 µmol/l) in Tanzania [7]. In a study conducted on Taiwan preschool children from revealed significant differences between Atayal and Bunun aboriginal groups [30].

Of the total samples, 20.6% (21.3 % boys and 18.9 % girls) children had detected lead in their serum at very low concentrations indicating that the study population is free from the influence of lead contamination in excess of the normal level. The Pb level in this study was 0.29±0.66 ppm. Pb levels are shown to influence negatively with iron status of population groups. Anemic children from Lucknow, India showed a significant association of elevated Pb levels to an extent of more than 1 µg/dl [13] and those children having iron deficiency anemia shown to be have lower Pb levels than that of their normal (0.13±0.04 vs 0.08±0.01 ppm) [15]. Moreover, a negative linear correlation of Pb with Zn and iron ($P < 0.01$), hence deficiency of Zn and iron reflecting Pb toxicity levels in children [17]. Similar findings were observed in our study showing inverse association between Pb and Se.

5. Conclusion

The concentrations of Mn and Co were relatively high, and levels of Ni, Cu, Zn, Se and Pb were closer to earlier studies which may be due to living environment, preference of dietary intakes of the given population, quality of diet and soil contents of these elements. The results provide baseline information of trace elements for the vulnerable section of child population, which needs further study in assessing its deficiency and intoxicant effects independently and in combinations.

References

[1] Sachdev, H.P.S. *Assessing Child Malnutrition: Some Basic Issues*. Food and Nutrition Bulletin India. 1995. 16 (4) 1-5.

[2] Arnold, F., Parasuraman, S., Arokiasamy, P., and Kothari, M., 2009: *Nutrition in India*. National Family Health Survey (NFHS-3), India, 2005-06. Mumbai: International Institute for Population Sciences; Calverton, Maryland, USA: ICF Macro.

[3] Bulliyya, G. *Secular Deterioration in Nutritional Status of Young Children: An Alarming Menace for the State of Orissa*. Man in India. 2003. 83 (1-2) 49-71.

[4] Mahapatra, A., Geddam, J.J.B., Marai, N., Murmu, B., Mallick, G., Acharya, A.S., Bulliyya G., and Satyanarayana, K. *Nutritional Status of Preschool Children in the Drought Affected Kalahandi District of Orissa*. Indian Journal of Medical Research. 2000. 38 (11) 90-94.

[5] World Health Organization, 1996: *Trace Elements in Human Nutrition and Health*. Geneva: World Health Organization. 1-229.

[6] Mitiko, S., Omar, J., Nairo, M.S., Marina, B.A.V., and Wilson, J.F. *Trace Element Contents in Serum of Healthy Elderly Population of Metropolitan Sao Paulo area in Brazil.* Journal of Trace Element in Medicine and Biology. 2007. 21 S1; 70-73.

[7] Chuwa, L.M., Mwiruki, G., Bilal, M.G., Mnubhi, E.K., and Swai, A.B. *Serum Iron, Zinc, Copper and Bromine in Malnourished Children in Dares Salaam, Tanzania*. East African Medical Journal. 1996. 73 (5) S21-S23.

[8] Singla, P.N., Chand, P., Kumar, A., and Kachhawaha, J.S. *Serum, Zinc and Copper Levels in Children with Protein Energy Malnutrition.* Indian Journal of Pediatrics. 1996. 63 (2) 199-203.

[9] Rukgauer, M., Klein, J., and Kruse-Jarres, J.D. *Reference values for the Trace Elements Copper, Manganese, Selenium and Zinc in the Serum/Plasma of Children, Adolescents and Adults.* Journal of Trace Element in Medicine and Biology. 1997. 11 (2) 92-98.

[10] Brunetto, M.R., Alarcon, O.M., Davila, E., Contreras, Y., Gallignani M., Rondon, C., Burguera, J.L., Burguera, M., and Angarita, C. *Serum Trace Elements and Fat-Soluble Vitamins A and E in Healthy Pre-School Children from a Venezuelan Rural Community.* Journal of Trace Element in Medicine and Biology. 1999. 13 (1-2) 40-50.

[11] Chien-Yi Chen. *Chromium, Iron, Selenium and Zinc Levels in Serum from Preschool Children in Central Taiwan.* Biological Trace Element Research. 2004. 100 (2) 169-184.

[12] Hassan. S.E., Abdelrazik, N.M., Abd El-Aziz, A., and R., EL-Iraqi. *Assessment of the Relation between Trace Elements and Antioxidant Status in Children with Protein Energy Malnutrition.* The Internet Journal of Pediatrics and Neonatology. 2004. 4 (1) 1-12.

[13] Ahamed, M., Singh, S., Behari, J.R., and Siddiqui, M.K. *Interaction of Lead with Some Essential Trace Metals in the Blood of Anemic Children from Lucknow, India.* Clinica Chimica Acta. 2007. 377 (1-2) 92-97.

[14] Marriott, L.D., Foote, K.D., Kimber, A.C., Delves, H.T., and Morgan, J.B. *Zinc, Copper, Selenium and Manganese Blood Levels in Preterm Infants.* Archives of Disease in Childhood - Fetal and Neonatal Edition. 2007. 92 (6) F494-F497.

[15] Turgut, S., Polat, A., Inan, M., Turgut, G., Emmungil, G., Bican, M., Karakus, T.Y., and Genc, O. *Interaction between Anemia and Blood Levels of Iron, Zinc, Copper, Cadmium and Lead in Children.* Indian Journal of Pediatrics. 2007. 74 (9) 827-830.

[16] Jain, A., Varma, M., Agrawal, B.K., and Jadhav, A.A. *Estimation of Serum Zinc and Alkaline Phosphatase in Malnourished Children.* Current Pediatric Research. 2007. 12 (1 & 2) 27-30.

[17] Yonghua Wu, Xu Yang, Jia Ge and Jie Zhang. *Blood Lead Level and its Relationship to Certain Essential Elements in the Children Aged 0 to 14 Years from Beijing, China.* Science of the Total Environment. 2011. 409 (16) 3016-3020.

[18] Esfahani, S.T., Hamidian, M.R., Madani, A., Ataei, N., Mohseni, P., Roudbari, M., Hadjizaeh, N., and Haddadi, M. *Serum Trace Elements in Children on Maintenance hemodialysis.* Acta Medica Iranica. 2007. 45 (5) 351-354.

[19] Rautray, T.R., Vijayan, V., Sudarshan, M., and Panigrahi, S. *Analysis of Blood and Tissue in Gallbladder Cancer.* Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms. 2009. 267 (17) 2878-2883.

[20] Rautray, T.R., Vijayan, V., and Panigrahi, S. *Analysis of Indian Pigment Gallstones*. Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms. 2007. 255 (2) 409-415.

[21] Rautray, T.R., Narayanan, R., Kwon, T.Y., and Kim, K.H. *Accelerator Based Synthesis of Hydroxyapatite by MeV Ion Implantation.* Thin Solid Films. 2010. 518 (12) 3160-3163.

[22] Chan, S., Gerson, B., and Subramaniam, S. *The Role of Copper, Molybdenum, Selenium, and Zinc in Nutrition and Health.* Clinics in Laboratory Medicine. 1998. 18 (4) 673-685.

[23] Finley, J.W. *Manganese Absorption and Retention by Young Women is Associated with Serum Ferritin Concentration.* American Journal of Clinical Nutrition. 1999. 70 (1) 37-43.

[24] Mehmet Emre Tascilar, Ilker Tolga Ozgen, Ayhan Abaci, Muhittin Serdar and Osman Aykut. *Trace Elements in Obese Turkish Children*. Biological Trace Element Research. 2011. 143 (1) 188-195.

[25] Iyengar, V., and Woittez, J. *Trace Elements in Human Clinical Specimens: Evaluation of Literature Date to Identify Reference Values*. Clinical Chemistry. 1988. 34 (3) 474-481.

[26] Ugwuja, E.I., Nwosu, K.O., Ugwu, N.C., and Okonji, M. *Serum Zinc and Copper Levels in Malnourished Pre-School Age Children in Jos, North Central Nigeria*. Pakistan Journal of Nutrition. 2007. 6 (4) 349-354.

[27] Ece, A., Uyanik, B.S., Iscan, A., Ertan, P., and Yigitoglu, M.R. *Increased Serum Copper and decreased Serum Zinc Levels in Children with Iron Deficiency Anemia*. Biological Trace Element Research. 1997. 59 (1-3) 31-39.

[28] Arnaud, J., Preziosi, P., Mashako, L., Galan, P., Nsibu, C., Favier, A., Kapongo, C., and Hercberg, S. *Serum Trace Elements in Zairian Mothers and their Newborns.* European Journal of Clinical Nutrition. 1994. 48 (5) 341-348.

[29] Muntau, A.C., Streiter, M., Kappler, M., Roschinger, W., Schmid, I., Rehnert, A., Schramel, P., and Adelbert, A. *Age-Related Reference Values for Serum Selenium Concentrations in Infants and Children*. Clinical Chemistry. 2002. 48 (3) 555-560.

[30] Chen, C., Lin, D., and Wei, Y. *Serum Sample Levels of Bromine, Iron, Scandium and Zinc in Preschool Children of Atalal and Bunun Aborigines Living in Central Taiwan*. Journal of Radioanalytical and Nuclear Chemistry. 2006. 268 (1) 83-90.

[31] Tietz, N.W., 1995: *Text Book of Clinical Guide to Laboratory Tests*. Philadelphia, PA: Saunders Company.