# Role of Trace & Toxic Elements in the Development of Anaemia in School going population of Hyderabad

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**Summary:** The aim of this research work was to measure the concentration of essential trace elements, including serum copper, iron and zinc in children suffering from anemia, and also to investigate the effect of heavy metal like lead on causing anemia. The study has been performed on school going children living in Hyderabad, which is a mini industrialized city. The study was carried out in anemic children studying in different schools of Hyderabad having different age groups. 10 ml venous blood samples were obtained after an informed consent form was signed.The samples were used to analyzed the trace elements (Fe,Cu, Zn) and toxic metal Lead (Pb). Trace elements include zinc and copper were found elevated in anemic children than healthy one, Copper is involved in many vital mechanisms in the body, energy production, connective tissue formation, and Fe metabolism, whereas copper found low with relation to the iron in anemic children because copper helps in the absorption of iron. Zinc is an essential micronutrient demanded by living being because of its significant position in-cooperation with structural constituent of proteins and as a cofactor in enzyme catalysis, there is difference between zinc and iron, they have been found to inhibit each other's absorption due to their competitive absorption pathways. Increased zinc levels found in children with low iron content, whereas control group have normal results of these elements which may be due to their dietary management. The results revealed the environmental pollution and the associated health risks on exposure to lead. Pb concentrations whereas the current research stated a considerable relationship of mild and severe anemia with 10-42.2µg/dl Pb concentrations, the variation in results may be due to a small sample size in the current study, Drinking water from corrosion of plumbing systems through the use of lead sellers and other lead containing materials in connecting household plumbing to public water supplies. Ground and surface water are also contaminated by lead consuming industry and agricultural activities. The concentrations of Pb greater than or equal to (≥)10 µg/dl in children related with an increased threat of mild and severe anemia, diminishing iron absorption . High Lead levels were related with lower concentrations of iron, ferritin and copper, in this study it was found that high levels of lead were found mostly in boys. Lead levels have also found in control group which was below 5 ug/dl, according to WHO ≥ 10 ug/dl is considered as high.

**Keywords**; Micronutrients, Anemia, Trace elements, Children, Lead.

## Introduction

Trace elements are vital micronutrients playing role by narrow, susceptible, and antioxidant function resultant from as a crucial part or else co-feature of major metabolic enzymes [1, 2]. Several enzymes have to be stimulated by metallic ions that counts 25% to submit their vital metabolic responsibilities in the living mechanism [3, 4]. Zinc is needed in the composition and responsibility of greater than three hundred enzymes of different biological structures inside the body. The role of zinc is reflected through the many features and activity in which it plays a systematic part e.g. cellular differentiation, nucleic acid metabolism, replication, insulin secretion and glucose metabolism are dependent on zinc availability [5]. Copper is an essential micronutrient and possess numerous principal functional specifications in the immune process and its feedback, and on assessing shows magnificent value in acute as well as chronic infections [5, 6]. The deficiency of iron is the main cause in developing anemia, the mechanism of absorption found unique in managing iron stock in the body. The proliferation of body mass during childhood development rushes transitory stimulus iron demands [7]. A number of different factors influence iron absorption including dietary plant

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phytates and tannins [8]. Some toxic substances specifically Lead intervene the iron absorption by entering the mechanism blocking iron through aggressive inhibition interferes with heam biosynthesis resulting in multifaceted attacks which has extremely terrible outcomes in children [9]. The human body exposed to Pb from environment where it is utilized by different industrial products e.g. car batteries, gasoline, toys, pipes, paint, cosmetics and traditional medicine [10]. Lead has been reported to be fatal to public since ancient period [11]. Above the 10 µg/dl concentration of Lead in blood describe as cut off by the Centre for Disease Control and Prevention (CDC) [12]. Public can be effected by lead in any time of their life period. Though, adolescent, pregnant girls and firm personnel are found higher at threat than others [13]. Due to the growth of central nervous system as well as playing habits young children be probable to exist additional responsive to lead than matures [12,13]. Child physiology has serious threats from Lead exposure counting learning difficulty, fall in IQ growth, problems regarding behavioral, such as inattention and violence, hearing loss and impaired growth [14]. In addition, anemia, male infertility, kidney malfunction, like situations were found amongst adolescents at higher levels about 50ug/dl to 100ug/dl [13]. In most developing countries a 20% of all children have greater value than fFthe mentioned cut off (10ug/dl) that estimated by Fewtrell and Clark [15]. The short-revenue family circle in Pakistan usually make use of distemper, as it is economical, owing to moist climate in nearly all metropolitan city areas, which origins painted walls to initiate stuffed, fading and flaking then immature kids living in such an setting are probably to be bare to lead. Moreover, deprived sanitation in city areas causes to water leaking that includes a supplementary issue toward this condition. Several editorials have been available as of Pakistan [16, 17]. A fresh investigation in Karachi demonstrated that 80.5% of study subject matters had Pb concentrations greater than >10 ug/dl [18].

## Experimental

### *Study Design*

This study is planned as cross-sectional on the causes of anemia in Hyderabad and carried out on 200 students living in Hyderabad and studying in different schools having age group 10 – 16 years, divided into four groups in which 40 boys of aged 10 – 13 years and 40 with aged 14 – 16 years, 40 girls of aged 10 – 13 years and 40 having aged 14 – 16 years, out of those 160 children was taken as samples and 40 normal control subjects. The study was took place in schools situated in different areas of city of Hyderabad. The blood samples were taken after the ethical approval by the school committee from random subjects to monitor the prevalence of anemia in students; students were excluded with any physical disability those having chronic illness like hemolytic anemia appear on analysis of complete blood count, the control group has included those students having good physical health also take part in playing sports and were monitored by their sports board. The aim of the research was thoroughly briefed to the parents of the students. A 10 ml blood samples were taken from each student for the analysis different parameters performed at Asian Institute of Medical and Health Sciences (AIMS) a life line trust, Hyderabad, and Hi-tech laboratory, university of Sindh.

### *Research Area*

The study has been performed on school children living in Hyderabad, which is a mini industrialized city previously known as Neroon Kot with two industrial zones (Hyderabad and kotri industrial zone) and multiple unarranged mini industries beyond the city producing a huge amount of chemical waste with no proper disposal of it. It is the 5$^{th}$ major metropolitan city in the country with nearly around 90 million populations.

### *Sample size*

Adjusting for a non-response rate of 2%, with a confidence interval of 95%, the estimated sample size for the study using the formula N=z2pq/d2 was 200 study participants. Demographic data variables were collected through interview of study participants using a well-structured questionnaire

### *Inclusion Criteria*

The children those studying in respective schools were selected by an organized random sampling, having age between 10 to 16 years from both genders with must have one guardian present in respective city.

### *Exclusion Criteria*

An exclusion criterion of subjects was found that those children of above 16 years or below 10 years having hemolytic chronic anemia as well as persons suffering from persistent sickness related with anemia.

The control group subjects were chosen amongst those who attending the outdoor clinic for assessing physical strength.

*Data Collection*

Parents of students were acknowledged over the objective of the research and their approval was received. Details associated to age group, sex, place of residence, and details regarding source of drinking water, literacy rate of parents along with occupation, and also socio-economic category, mostly data was obtained from the mothers of students those were under study. According to the WHO report, anemia has been defined as hemoglobin level less than <11 g/dl, the considered students were separated into anemic and healthy groups [19].

*Blood Sampling*

A 10 ml venous blood samples were obtained from those students who were agreed to participate in the research followed a strict standardized skin cleaning protocol which is essential for avoiding surface contamination; the tubes were appropriately labeled with unique pathology numbers assigned each participant on the study.

*Sample Preparation*

The samples were prepared for the analysis of trace elements (Cu, Zn) and toxic one Lead (Pb) as reported by (Folin O and AVu HS, 1919) [20], Diluted 1 ml serum with 8 ml of water and a half volume (0.5ml) each of sodium tungstate (100g $NaWO_3.2H_2O$/l) and 0.33 mol/l $H_2SO_4$ (20 ml of concentrated acid was diluted to 1 liter, standardized against a known NaOH solution, and adjusted if necessary) to make 1:10 dilution of serum. Then the sample was filtered through whatman filter paper No. 42. The acid liberated the whole of the tungstic acid from an equal volume of the tungstate, and practically all that was taken down by the protein precipitate leaving sufficient excess acid to give a filtrate with a pH between about 3.5 and 4.9 [21]. The serum samples were aliquoted into eppendorf® tubes and stored in a Sanyo Scientific® refrigerator (Sanyo Electronic Company-Ltd. Japan), at -30C for the assessment of lead, copper and zinc by the atomic absorption spectrophotometer (Perkin Elmer HGA 700-Germany), also for the estimation of serum iron on Hitachi 902 auto analyzer using Roche reagents kits. Atomic absorption spectrometer (Varian specter AA-20) with standard burner head and air acetylene flame was used to investigate the metal ions sodium, potassium, calcium ,magnesium, iron, copper, zinc and cadmium. Procedure for estimation was performed in triplicate with integration time 3 seconds and delay time 3 seconds. Na, K, Ca, and Mg after proper dilution were investigated. Component (250ml) contain HNO3 (1ml) was heated gently was at 90-950C, concentrated up to 15-20 ml. and the finally adjusted volume up to 25ml. Components of Fe, Cu, Zn, and Cd from the solution.

*Environmental Assessment*

To analyze the quality of drinking water, samples of water to be used for the drinking purpose were collected from both hand pump as well as tap water sources from different areas of the city for the assessment of lead (Pb) level using the atomic absorption spectrophotometer (Graphite Furnace, Perkin Elmer HGA 700-Germany). The detection limit for water analysis was 10 ug/L.

**Results and Discussion**

This research was conducted on 200 students with age group ranging from 10 years to 16 years were divided in to five groups, 10-13 years girls and boys as well as 14-16 years girls and boys, the fifth group was control subjects were selected from healthy children having age 10-16 years both girls and boys, all groups have 40 number of subjects, According to the Lead concentration, ranging between 3 to 25 µg /dl, about 51.7% (n = 76) of students had lead concentrations ≥10 µg/dl (high lead levels) and 48.2% (n = 71) had lead concentration <10 µg/dl (low lead levels), The socio-economic factors were deliberated between the high and low lead concentrations (Table-1). Though, higher lead concentrations were amongst students 14-16 years and those utilizing ground water, however no statistical variation was noticed between those having high Pb concentrations, Pb levels were found increased in boys than girls and those who have low socio-economical standard. In addition, increased values were also in students of low education level (secondary level). Fig 1 shows the Average mean value of Fe, Cu and Zn for 14-16 years boys, Comparing the results of Fe, Cu, Zn and Pb of group 10-13 years boys with control shown in Fig 2 we found clear decrease in Fe, and Cu, and significance increase found in anemic people($p<0.001$) than control group (Table-3). Fig 3 shows the mean value of Fe, Cu and Zn for the girls with age group 10-13 years, while Fig 4 illustrates that Comparing the results of Fe, Cu, Zn and Pb of group 14-16 years girls with control we found clear decrease in Fe ($p<0.001$) and significance increase of Pb found in

anemic people (p 0.001) than control subject (Table-4). While all age group compression of Fe, Cu, Zn and Pb depicted in Table-2 and 5 respectively.

Fig 5 shows the Correlation between Fe and Zn for 14-16 year's girls Whereas Fig 6 demonstrate the Correlation between ferritin and Fe for 10-13 years boys.

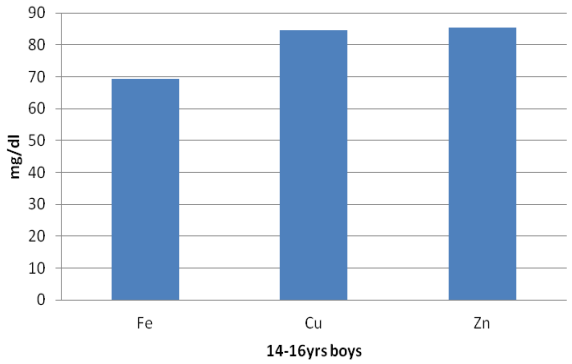


Fig. 1: Average mean value of Fe, Cu and Zn for 14-16 years boys.

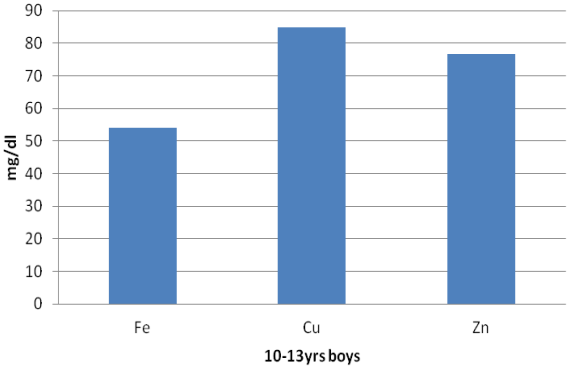


Fig. 2: Average mean value of Fe, Cu and Zn for 10-13 years boys.

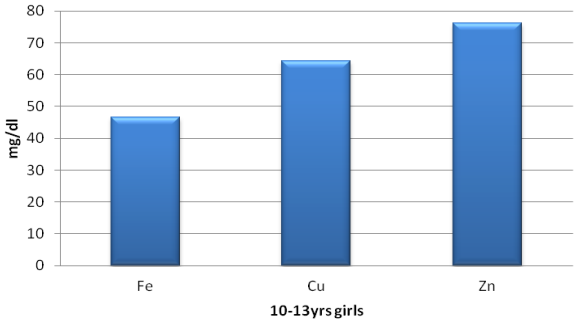


Fig. 3: Average means value of Fe, Cu and Zn for 10-13 years girls.

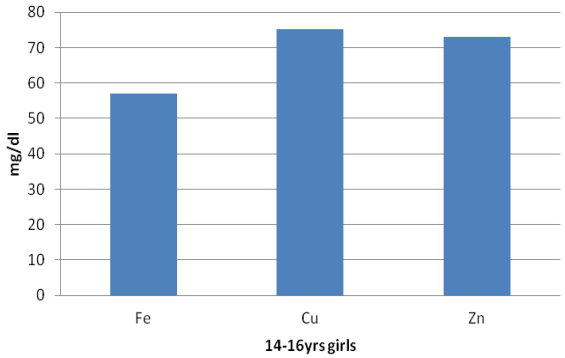


Fig. 4: Average mean value of Fe, Cu and Zn for 14-16 year's girls.

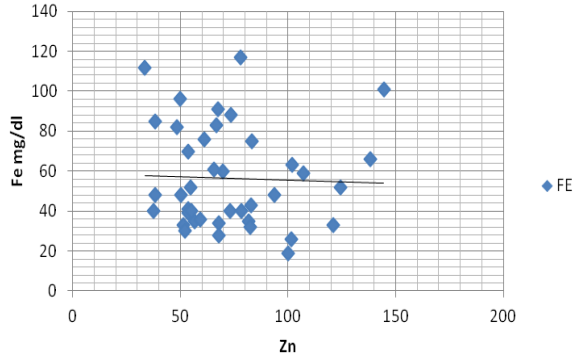


Fig. 5: Correlation between Fe and Zn for 14-16 year's girls.

Table-1: Distribution of individual characteristics in relation to blood lead levels.

| S# | Characters of study group | Low <10 ug/dl(71) No (%) | High >10 ug/dl(76) No (%) | Test Of Significant |
|---|---|---|---|---|
| 1) | Gender | | | |
| | Boys | 35(49.2%) | 40(52.6%) | $X^2$- 1.91, p- 0.3 |
| | Girls | 36(50.7%) | 36(47.3%) | |
| 2) | Age | | | |
| | 10-13 Years | 33(46.4%) | 35(46%) | $X^2$- 083, p- 0.5 |
| | 14-16 Years | 38(53.5%) | 41(53.9%) | |
| 3) | Education | | | |
| | Secondary | 40(56.3%) | 48(63.1%) | $X^2$- 0.21, p- 0.8 |
| | Higher | 31(43.6%) | 38(36.8%) | |
| 4) | Socioeconomical Level | | | |
| | Middle | 35(49.2%) | 25(32.8%) | $X^2$-0.14, p- 0.6 |
| | Low | 36(50.7%) | 51(67.1%) | |
| 5) | Source of Drinking Water | | | |
| | Tap Water | 51(71.8%) | 37(48.5%) | $X^2$-0.03, p- 1.0 |
| | Hand Pump Water | 20(28.1%) | 39(51.4%) | |

$X^2$= Chi-square test.

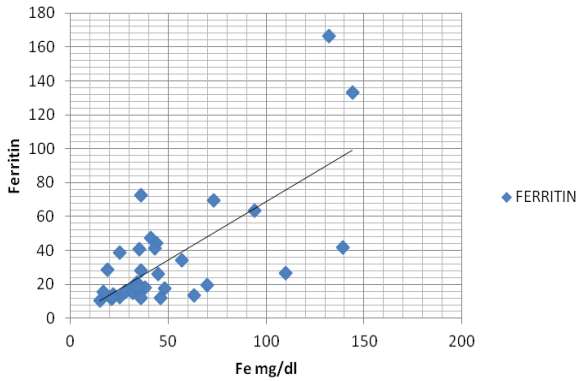


Fig. 6: Correlation between ferritin and Fe for 10-13 years boys.

Table-2: Shows mean values of trace & toxic elements of Group 10-13 years' girls

| Parameter | Cases | Controls | P- value |
|---|---|---|---|
| | Mean + SD | Mean + SD | |
| Fe (mg/dl) | 46.5±18.2 | 91.6±28.4 | 0.0008 |
| Cu(mg/dl) | 64.2±24.3 | 108.2±41.8 | 0.012 |
| Zn(mg/dl) | 76.08±30.8 | 95.8±31.3 | 0.072 |
| Pb(µg/dl) | 12.1±3.96 | 3.45±2.83 | 0.0001 |

For the preservation of life Fe, Cu, and Zn are essentially needed. Lead is a heavy metal and it can be harmful for human health. For this reason, serum levels of these elements were determined in students. Also, because of heavy water and air pollution in Hyderabad, Pb levels in serum of our subjects were determined. The present study revealed the environmental pollution and the associated health risks on exposure to lead. The skeleton is an important endogenous source of labile lead as the bones and teeth contain more than 95%of the total lead in the body [22].

Although worldwide economic and scientific growth, more than a quarter of the world's residents remains anemic. Similar study was done in India by Nitin B Jain in 2005 in which he reported that 40% Childs were anemic with Pb level 10-19.2 ug /dl [19]. Andrew Hall, in his research found that anemia in school Childs, boys aged 12-14 years were more anemic than girls 40% and 50% anemia was among 7-11 years Childs, In his research he depicted some causes which are infections caused by worms, micronutrients deficiency specially iron [23] .

We observed in this research, that the serum iron level was lower with mild anemia than other subjects group and on comparing with control group shows significance (Table-2), Fig 7shows the Correlation between ferritin and Iron for the group 10-13 years girls whereas Fig 8 illustrate the Correlation between ferritin and Fe for 14-16 year's girls in another study in Thailand reveals that the ratio of anemia among school children was as high as 31% [24]. About half of this burden is a result of iron deficiency anemia. Intake of sufficient iron in diet develops physical and mental performance, work productivity, and well-being, impaired scholastic performance and even long-term child outcomes. Among children, iron may improve cognitive, psychomotor, and physical development [25]. However, iron alone or with combination reduce only 40-60% of the anemia even large proportion of anemia is unresponsive to supplementation. In our results it was observed that deficiency of micronutrients often coexist and the interactions between them are important, so it was observed that anemia in result may be due to the deficiency of other micronutrients [24].

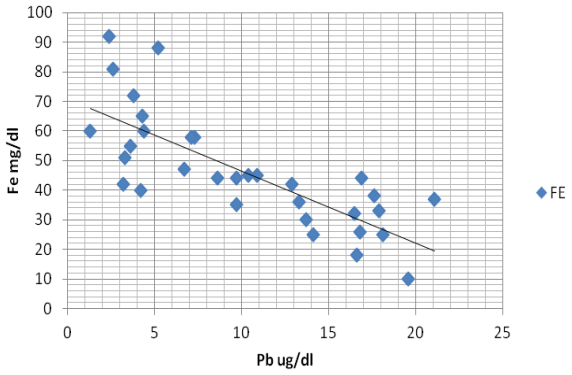


Fig. 7: Correlation between ferritin and Fe for 10-13 years girls.

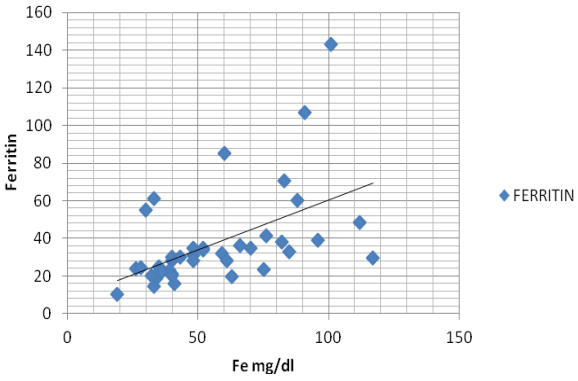


Fig. 8: Correlation between ferritin and Fe for 14-16 year's girls.

The serum Zn levels were found decrease in 10-13 years girls and boys group (Table-2, 3) show weak negative correlation with Fe and on comparing with control group reveals non significance, Fig 9 shows the Correlations between Fe and Zn for 10-13

years girls while Fig 10 demonstrates the Correlation between Fe and Zn for 10-13 years boys. Inverse proportion is found in Zn and Fe absorption from the gastrointestinal tract [26.27]. Same theory was reported by Solomons and Jacob that high doses of inorganic iron (Fe) may decrease zinc absorption in the lumen [26].

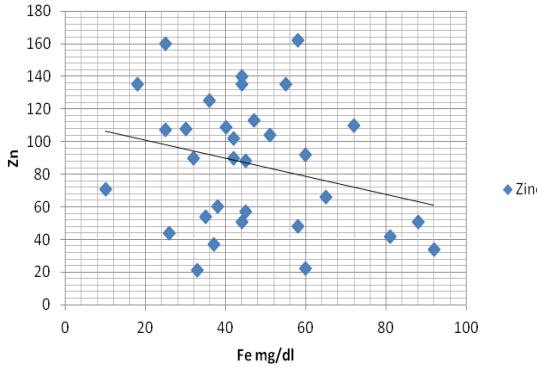


Fig. 9: Correlations between Fe and Zn for 10-13 years girls.

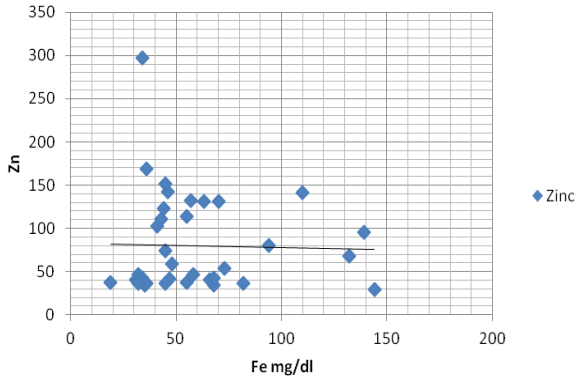


Fig. 10: Correlation between Fe and Zn for 10-13 years boys.

Table-3: Shows mean values of trace & toxic elements of Group 10-13 year's boys.

| Parameter | Cases | Controls | P- value |
|---|---|---|---|
| | Mean ± SD | Mean ± SD | |
| Fe (mg/dl) | 53.9±31 | 100.4±44.8 | 0.007 |
| Cu(mg/dl) | 84.9±28.6 | 125.2±34.5 | 0.003 |
| Zn(mg/dl) | 76.6±41.1 | 115.2±38.6 | 0.042 |
| Pb(µg/dl) | 15.8±3.6 | 4.08±2.9 | 0.0004 |

Cu level was found in mild decrease in all group specially in girls groups than control group (Table-2, 4) group 10-13 years girls show non significance while 14-16 years girls show significance with control group, Fig. 11 shows the Correlation between Fe and Cu for 10-13 years boys while Figs 12 demonstrate the Correlation between Fe and Cu for 14-16 year's girls. in this research Cu shows positive correlation with Fe while negative correlation with Pb, more over in another study done by James A Bush in USA, iron deficiency was reported in Childs because of prolong intake of cow's milk, cow's milk has low quantities of Cu to balance the metabolism [28].Cu has a major role in Fe metabolism, oxidation of ferrous to ferric state is carried out by the ferroxidase activity of major plasma Cu protein, ceruloplasmin. In that way, depletion of Cu could impair Fe absorption [28].

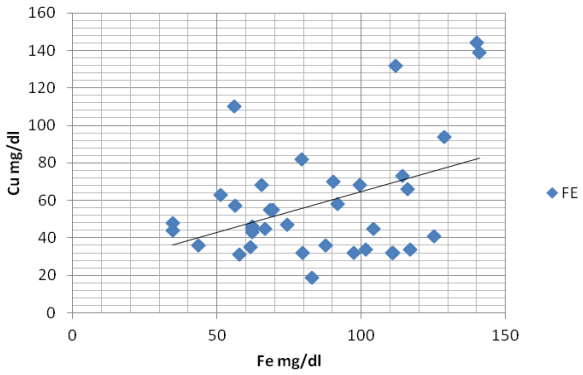


Fig. 11: Correlation between Fe and Cu for 10-13 years boys.

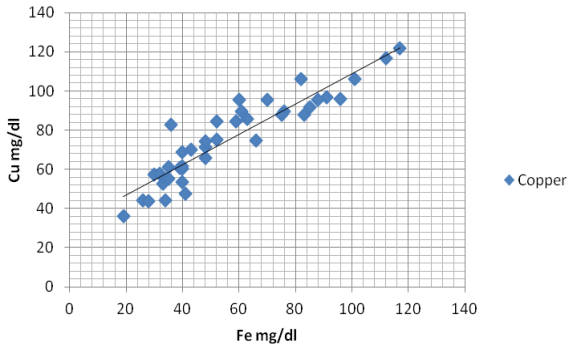


Fig. 12: Correlation between Fe and Cu for 14-16 year's girls.

Table-4: Shows mean values of trace & toxic elements of Group 14-16 year girls.

| Parameter | Cases | Controls | P- value |
|---|---|---|---|
| | Mean ± SD | Mean ± SD | |
| Fe (mg/dl) | 56.4+25.4 | 101.4+32.6 | 0.0001 |
| Cu(mg/dl) | 75.1+21.1 | 116.4+25.8 | 0.007 |
| Zn(mg/dl) | 72.9+26.9 | 105.6+30.2 | 0.019 |
| Pb(µg/dl) | 9.53+4.85 | 4.74+2.68 | 0.001 |

Table-5: Shows mean values of trace & toxic elements of Group 14-16 year boys.

| Parameter | Cases | Controls | P- value |
|---|---|---|---|
| | Mean ± SD | Mean ± SD | |
| Fe (mg/dl) | 69.2+23.9 | 101+27.2 | 0.01 |
| Cu(mg/dl) | 84.6+23.4 | 114.7+19.1 | 0.05 |
| Zn(mg/dl) | 85.3+39.02 | 93.1+31.5 | 0.61 |
| Pb(µg/dl) | 17.07+4.68 | 5.7+2.7 | 0.002 |

Of the children 51.7% had blood lead level≥10 µg/dl, similar to a study done by Amal A Hegazy [29]. Which accounted a considerable relationship of mild and severe anemia with 10-20µg/dl Pb concentrations whereas the current research stated a considerable relationship of mild and severe anemia with 10-42.2µg/dl Pb concentrations, the variation in results may be due to a small sample size in the current study, same high quantity of Pb was reported in study carried out in Egypt. Pb is a poisonous material that gathered in the body and could origins severe health harms; particularly for children [30]. Students live near small industries in congested city areas have more lead Pb concentrations in their blood up to 30-40 µg/dl and have mild as well as severe anemia as reported in 2010 in Egypt [29].The cutoff value of l0µg/dl defined by the Center for Disease Control and Prevention as a limit for elevated Pb concentrations principally is based on neurological toxicity [31]. Deficiency of certain trace elements such as iron enhances Pb absorption in the intestine [25]. While the Pb concentrations in blood of the students living in Hyderabad was found to be same level according to world standards, the concentration of Pb in blood of the boys 10-13 and 14-16 years groups is found to be significantly increased (Fig 13) as compared to control group and in the same groups strong negative correlations were found between Pb and Fe (Fig 14, 15). In comparison with girls groups, 10-13 years group has Pb conc. moderately high which was significance as compared to controls (Table no 3), while 14-16 years girls have decrease values of Pb with strong negative correlation between Fe and Pb (Fig 16, 17), the high blood lead levels among boys than girls may explain by more outdoor hobbies in boys.

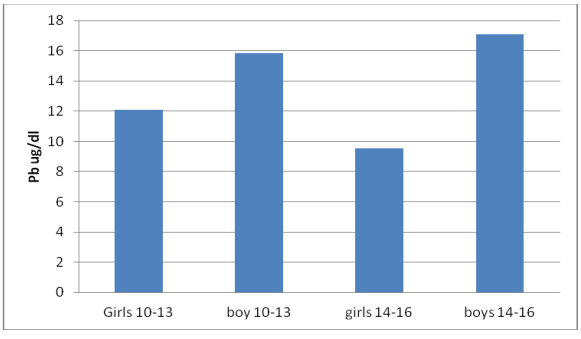


Fig. 13: Average values of lead Pb of all anemic groups

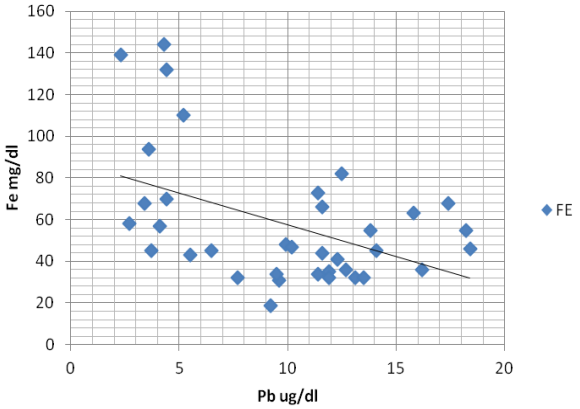


Fig. 14: Correlations between Fe and Pb for 10-13 years boys.

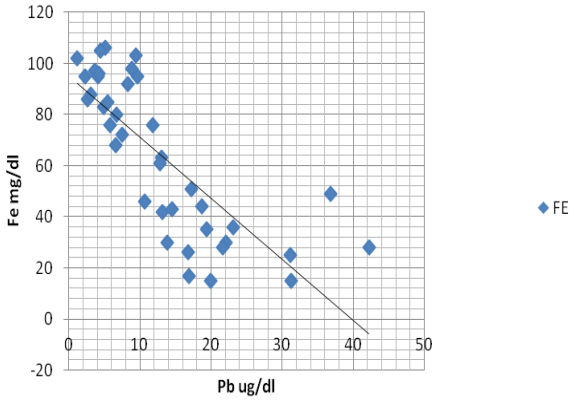


Fig. 15: Correlations between Fe and Pb for 14-16 years boys.

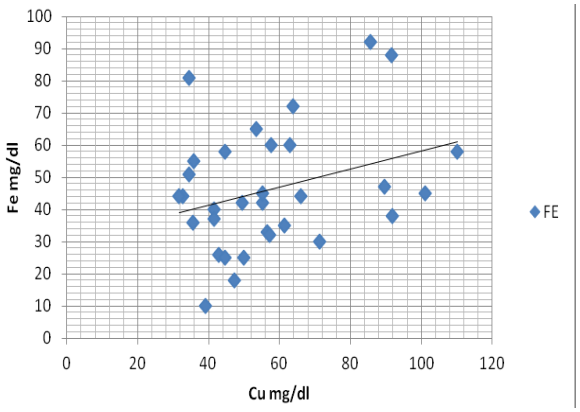


Fig. 16: Correlations between Fe and Cu for 10-13 years girls.

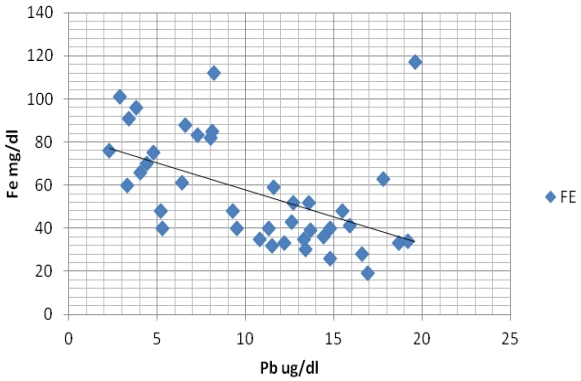


Fig. 17: Correlations between Fe and Pb for 14-16 year's girls.

In Pakistan, low-income families in their household commonly utilize water-soluble paint because of its cheaper price due to humid weather in these cities due to which bulging of paints start, deteriorating and peeling children lived in such environment are likely to be exposed to Pb. Another additional factor was the poor water piping in such cities results to water leaking from pipes results in increasing divesting condition [32]. For example; a study in Karachi showed that 80.5% of study subjects had blood lead levels above 10 ug/dl, which is defined as "elevated blood lead levels"[33].

## Conclusion

In this study all groups of children were assessed and found that school children living in Hyderabad have moderate anemia with elevated hemoglobin indices which shows low socio economic and hygienic level, the boys group were more anemic with low iron and ferritin content. Trace elements include zinc and copper were found elevated in anemic children than healthy one, Copper is involved in many vital mechanisms in the body, energy production, connective tissue formation, Fe metabolism, whereas copper found low with relation to the iron in anemic children because copper helps in the absorption of iron. . Increased zinc levels found in children with low iron content, whereas control group have normal results of these elements

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