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Exposure of Toxic Trace Metals in Human Blood of Rural Population from District Okara, Pakistan; A Case Study

Rukhsar Saleem[1], Aimal Khan[2], Muhammad Afnan Khattak[2], Aliza Niazi[3], Lina Gouacem[3], Majid Ayaz[2*],

1. Environmental Toxicology laboratory, College of Earth and Environmental Sciences, University of the Punjab, Lahore, Pakistan

2. Department of Medical Laboratory Science and Biotechnology, Asia University, No. 500, Lioufeng Road, Taichung 41354, Taiwan.

3. Faculty of Natural Sciences, Institute of Earth Science, University of Selisia in Katowice, Poland.

Co-responding Author

Majid Ayaz,

Department of Medical Laboratory Science and Biotechnology, Asia University, No. 500, Lioufeng Road, Taichung 41354, Taiwan.

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KEYWORDS

Complete blood count, heavy metals, rural population, age groups, gender, correlation.

ABSTRACT:

Introduction: This study investigates the presence of heavy metals, specifically cadmium (Cd), lead (Pb), and chromium (Cr), in blood samples from a rural population in district Okara, and their correlation with Complete Blood Count (CBC) parameters.

Objectives: The primary objective is to measure the concentrations of Cd, Pb, and Cr and evaluate their relationship with CBC parameters, including hemoglobin, total leukocyte count (TLC), and red blood cell (RBC) count.

Methods: A total of 30 human blood samples were collected. Heavy metal concentrations were determined using atomic absorption spectroscopy (AAS). Cadmium was detected in 21 samples, lead in all, and chromium in 29, while copper (Cu) was not detected. Statistical analysis was conducted to explore correlations with CBC parameters.

Results: The highest mean concentrations were 0.22 mg/L for Cd, 1.596 mg/L for Pb, and 4.264 mg/L for Cr. Pb and Cr were higher in males, while Cd was higher in females. Strong positive correlations were found between heavy metals and CBC parameters, particularly lead (R^2= 0.9319 for hemoglobin, R^2= 0.9308 for TLC, R^2= 0.9308 for RBC).

Conclusions: Heavy metal levels were elevated in older individuals and females, impacting CBC parameters and indicating potential health risks from food and industrial exposure

1. Introduction:

Metals are substance have high electrical conductivity, luster and malleability. Metals form cations by losing their electron. The density of the heavy metal is more than 5 g /cm^3. Naturally, metals are founded in the earth‵s crust and their composition are dissimilar in different places. Their effect on living organism and environment is unsympathetic (Jaishankar *et al.,* 2014). Some metals show adverse effect or toxic even at low level of exposure like Pb, arsenic (As), cd, and Hg. Some metals are very necessary for host of physiological and metabolic functions, such as iron (Fe), copper (Cu), zinc (Zn), and selenium (Se). Insufficient amount of these traits may possible lead to raise consumption of toxic metals. Such ass, low blood calcium (Ca), Fe, and zinc are at accumulation possibility of high blood Pb levels (Silver *et al.,* 2018). Heavy metals on the public health concern very injuries resulting from exposure to harmful substances for a long term, because of heavy metal persistence in the environment. Even at low level heavy metals is toxic. Leads (Pb), cadmium (Cd), manganese (Mg) are natural occurring metals, which are also classified as endocrine disruptors (EDCs). The EDCs change the function of

endocrine system that causes unfavorable health effects (Hussain *et al.*, 2019).

The heavy metals are released in the environment by two type of sources that are natural and anthropogenic. The most considerable natural heavy metal sources are geological weathering and rock erosion. The concentration and composition of heavy metal is depending upon the condition of the environment and type of the rock. The other sources of heavy metals are weathering of mineral, forest fires, biogenic sources and particle release by vegetation. Instead natural, mostly heavy metal occurs due to the manmade activities. Mining, smelting activities, contaminated bios lids, fertilizer, insecticides, fungicides, and herbicides, industrial waste water, emission from automobile and landfills. Some individuals are firstly exposed to these contaminates in the workplace, for most people diet is the main sequence of exposure of these venomous elements. Industry, atmosphere, soil, water, foods and human are a cyclic order of heavy metal exposure (Morgan, 2012).

Heavy metals have adverse health effects on human, wild life and environment. The heavy metal cause adverse effect to the aquatic life through effluent, sewage, garbage leaching and smelting process by mixing of heavy metals in aquatic system. The phosphate and nitrogen are nutrients that are polluted by toxic algae and detrimental for aquatic animals. The tadpole and frog biodiversity can be diminishing by chemical contamination. Marine organism's development is badly impacted by the oil spill. It causes many diseases and also affects the reproduction of marine organisms. It can also cause gastrointestinal irritation and damage to liver, kidney and nervous system (Govind & Madhuri, 2014). Metal imbalances have negative impact on human development. Heavy metal is persistent to human body and have been adverse health effect on wide range of system including intestinal tract, central nervous system, reproductive, skeletal, mucus tissues, kidney and liver, cardiovascular, respiratory system (Silver *et al.*, 2018). Human exposure to heavy metal prospective risk factor for developing cognitive impairment. Cognitive impairment is shortage in thinking, learning and memory, visuospatial skill (Iqbal, *et al.*, 2018) and cause Multiple sclerosis (MS) is crippling disease of the nervous system (Dehghanifiroozabadi *et al.*, 2019). Long term effect of heavy metal has been shown to expand the liability to human diseases for example; heart disease, mutagenicity, diabetes, teratogenicity cancer and suicide (Yousaf *et al.*, 2013).Pregnant women and children are mostly affected by the heavy metals and also those people who work in polluted area. The four metals that are part of our studies are lead, chromium, cadmium and copper. Lead is a very toxic metal that cause considerable environmental contamination and health problems all over the world (Jaishankar *et al.*, 2014). The primary source of lead exposure are coal burning, transportation emission, mining, smoking, paints and pesticides and for nonsmoker source of lead exposure are food, polluted air, drinking water etc (Stojsavljevic *et al.*, 2019).Lead poisoning may cause many types of diseases including: cancer, cognitive impairment, Alzheimer disease, neurological disorder, higher blood pressure, kidney disorder and heart disease (Dehghanifiroozabadi *et al.*, 2019). The permissible limit of metal in the body is 0.1 mg/1 (Iqbal *et al.*, 2018). According to ATSDR ranking, seventh most poisonous heavy metal is cadmium. (Jaishankar *et al.*, 2014) The main sources of cadmium exposure are fertilizer, welding, Cd and Ni batteries, stabilizer, pesticides, cigarette smoke and nuclear fission plant (Jeong *et al.*, 2017).

The diseases caused by cadmium are bone damage, cancer, kidney damage; disturbed phosphorus and calcium metabolism; kidney stone and motor nerve disease. Permissible limit of cadmium is 0.06mg/1(Iqbal *et al.*, 2018).In human being copper is very important for the formation of enzyme but, if the amount of copper exceed in human body it cause severe damages in human body such as: hepatic and renal damage, mucosal irritation and corrosion, irritation, capillary damage and of C.N.S followed by depression (Sharma & Agrawal, 2005). The sources of copper metals is similar to the lead that is mining, metal piping, chemical industry, pesticides production, and copper is enter in human body through the source of meat and fish. Permissible limit of metal in the body is 0.1mg/ 1(Iqbal *et al.*, 2018).

In the environment chromium occur in several oxidation states ranging Cr_2^+ to Cr_6^+. The sources of chromium are burning of oil and coal, pigment oxidant, chromium steam, oil well drilling, petroleum, catalyst and metal plating tanneries (Jaishankar *et al.,* 2014). The diseases cause by chromium is gastrointestinal irritation, liver narcosis, nephrites and mucous membrane ulcer of nasal. The permissible limit of chromium is 0.05mg/l (Sharma & Agrawal, 2005).

Determination of trace amount of heavy metal in Pakistan is actually still a challenging in a field because of the appearance of high concentration of interfering matrix component in most environmental and biological sample. The key step of detection of heavy metal is capturing of ions from complex matrix (Farzin *et al.,* 2017).Previous studies have suggested that the sufficient dietary intake can reduce the toxicity of heavy metals. Particular nutrients and food avert accumulation of heavy metal in blood. In Pakistan, people are affected by heavy metals mostly from environmental and dietary sources. Related studies show in over the Pakistan drinking water has high level of heavy metals. Especially the main source of metal contamination in water is due to the pharmaceutical fertilizer and textile industries. Polluted water is a major source of human exposure to heavy metal in Pakistan (Iqbal *et al.,* 2018).

The analysis of heavy metal exposure in the environment can be estimate by two main techniques that is based on the different profile data, therefore allow the confirmation and verification of data. One of them includes determining the heavy metals concentration and other one evaluate the exposure of heavy metals (Morgan, 2012).The objectives of study are to obtain the basic and practical knowledge about heavy metal pollution in blood sample from Okara district. The basic objectives of study are:

1. To investigate the level of heavy metals in blood samples of rural population of district Okara. 2. To measure the association of heavy metals with CBC parameters of human blood.

2. Materials and methods:

Sample collection and preparation:

The study includes 30 clinically healthy normal people (women/men Ratio = 22/8). The selected blood contributor consisted only of adults, age vary from 20 to 61 years. The blood samples were collected within a two months' period (December 2018 – January 2019). All examined subjects were voluntary blood donors and were selected by medical professionals. The samples were collected from Doctor's lab Okara. The gathered data include the information including age, gender, place of residence, body weight, height, occupation and personal life style were obtained through interview. CBC test were conducted because of the effect of the heavy metal on their parameters. Blood sample 3ml were collected in EDTA K3 vacationer tube (bio tube, inc.) the whole blood sample mixed well and transferred into the storage box until unless next process proceeds.

Sample storage:

Blood samples were stored at room temperature in EDTA K3 vacationer tube and away from direct contact of sunlight.

Glassware, reagents and instruments

Different reagent H2O2, HNO3 and distilled water were used during the experiment. Glassware utilized during experimentation was insulin syringes (1ml), beakers, conical flask, and 100 ml round bottle conical flask, conical funnel, and stirrer. For filtration, Whatman filter paper 42 was used. Hot plate was used for heating sample.PH meter was used for measuring pH value of digested sample. Plastic bottles were used for storage of digested solution of samples. AAS is used for the estimation of heavy metals in human blood.

Digestion of samples

For Acid digestion take 0.5ml whole blood sample of all patient were exactly taken from accurately labeled test tubes through insulin syringe and place in a small beaker and mixture of concentrated HNO3, H2O2 (2:1, v/v) was recent prepared and 3ml of this mixture add by measuring cylinder. Use stirrer after adding acid solution. After that, place the beaker in hot plate and mix it well with stirrer and heat for 1 minute until show fumes that are light yellow in color. After cooling, the

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digested sample were filtered through filter paper and brought to a needed volume with distilled water about 50 ml and then shift this solution in a beaker and use pH meter for measuring PH value of sample solution. Shift this solution in bottles and labeled them.

Measurement of metals level

Atomic Absorption Spectroscopy (AAS), (Model: A Analyst 800, PerkinElmer) present in college of earth and environmental sciences was used. Blank solution was run to calibrate the instrument and pre prepared standard solutions were used for determination of heavy metals in digested sample solution. The heavy metals (lead, chromium, and cadmium, copper) were analyzed in digested sample. All samples were analyzed for each metal detection by AAS. Respective metal lamp was used for estimation of heavy metals. On the basis of recorded absorbance in software the heavy metal concentration was calculated (Rehman and Husnain, 2012).

Statistical analysis

Statistical analysis was done with EXCEL 2007. The EXCEL software help for evaluation or concentration range of heavy metals in human blood by using for all parameters and also used to identify the normality of distribution of values. This parameter represented the comparison across different age groups, which are expressed as mean and standard deviation, also used the SPSS software to analyse the significant and no significant value by using ANOVA (t test), independent test and post hoc (LSD) test. If the p is less than 0.05 the result is significant or greater than 0.05 result is no significant. Corral function is used for correlation between the heavy metals and CBC parameters. The highest mean value of cadmium is 0.22 and least is 0.019, highest value of lead is 1.596 and least is 0.089 and the highest value of chromium is 4.264 and least value is 0.097. The average mean concentration of chromium, lead and cadmium is 1.73, 0.89, 0.1 respectively, which shows in table 4.2.

3. Result and Discussion

Concentration profile of heavy metals in human blood

In human blood samples four heavy metals i.e. lead, chromium, cadmium, and copper were analyzed by using AAS method. Three metals deducted in the blood samples of people live in Okara district and copper is not present. The CBC tests were conducted in doctor lab in Okara.Table 1 shows the mean concentration of chromium, lead and cadmium is 1.73mg/L, 0.89mg/L, 0.1mg/L respectively. The concentration of chromium is clearly higher than lead and cadmium in human blood samples. Heavy metal mean concentration also shows in figure 1. The following patterns chromium > lead > cadmium and the metal copper is not detected during testing.

Table 1: Average concentration (mg/L) of heavy metals in human blood

Metals	**Cr (mg/L)**	**Pb (mg/L)**	**Cd (mg/L)**
Average mean	1.73	0.89	0.1
S D	1.226347	0.424964	0.091649
Higher concentration	4.264	1.596	0.22
Lowest concentration	0.097	0.089	0.022

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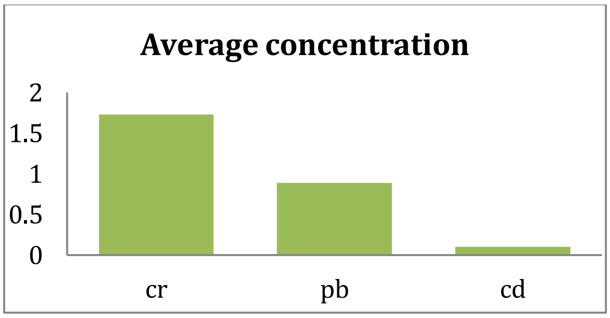

Figure 1: The mean concentration of metals in blood samples

The average concentration of heavy metal in female are chromium 1.776mg/L, lead 0.8411mg/L and cadmium 0.1183mg/L respectively shows in figure 2 that indicate concentration of chromium in female is higher as compared to other metals. Heavy metal concentration in female shows the following pattern chromium> lead > cadmium. The average concentration of heavy metals in males shows in figure 3 i.e. chromium 1.9001mg/L, lead 1.0315mg/L and cadmium 0.0725mg/L. Heavy metals concentration reveal the following pattern chromium > lead > cadmium. According to our research the result reveal that the concentration of chromium is higher in males than females and the level of cadmium is higher in female rather than males and the lead concentration in male in higher as compared to females. The comparison between the concentration of metals in male and female samples are shows in figure 4.

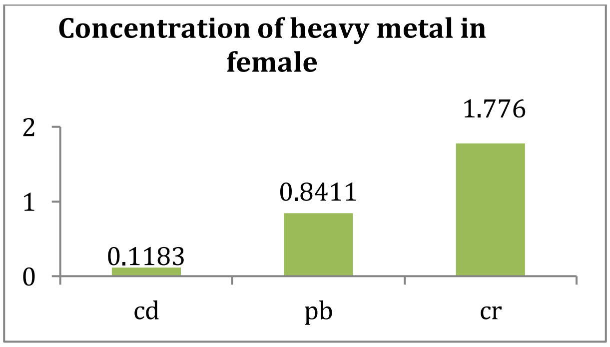

Figure 2: Average Concentration of metals in female blood samples

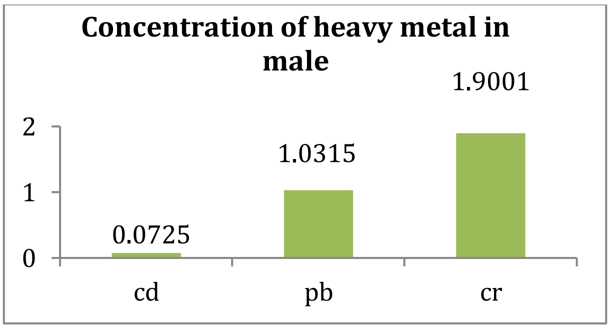

Figure 3: Average Concentration of metals in male blood samples

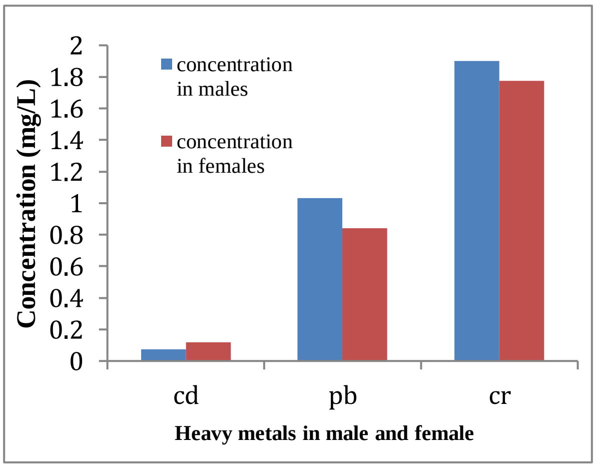

Figure 4: comparison between male and female samples

Toxic trace metals and human demographics:

The figure 5 summarized the concentration of chromium metal in different age group in which the graph shows that the chromium concentration is highest in age group between the 30-40 years. Chromium concentration shows the following pattern (30-40)> (40-50) > (20-30) > (50-60).

The concentrations of lead in different age group are varies:

Figure 6 show the concentration of lead is highest in 50-60 years' age group. Lead concentration shows the following pattern (50-60) > (20-30) > (30-40) > (40-50).

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The concentration of cadmium in different age group varies:

Figure 07. Show the concentration of cadmium is highest in 40-50 years' age group.

Cadmium concentration shows the following pattern (40-50) > (30-40) > (20-30) > (50-60).

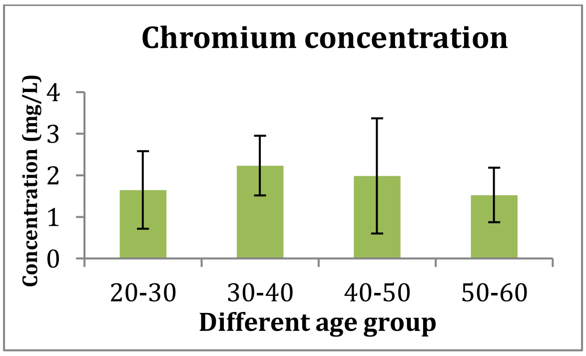

Figure 5: Concentration of chromium in different age groups

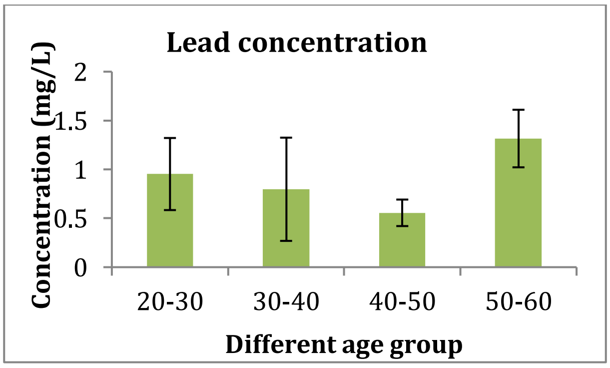

Figure 6: Concentration of lead in different age groups

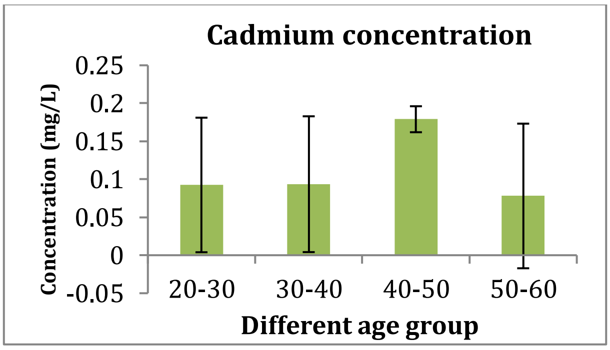

Figure 7: Concentration of cadmium in different age groups

Relation between CBC parameters with heavy metal

The relation between CBC parameters with heavy metal in all graph CBC parameters are on x axis and average mean of heavy metal (cadmium, chromium, lead) on y axis. And they gave the value of y and R^2. All parameters and heavy metals were shows positive relationship is if one factor increases other factor also increases. R^2 means how close each data point fit to the regression line and how well the regression line predicts actual values. The value of R^2 between 0 to 1.If 0.5< r <0.075 mean slight positive correlation, 0.75<r<0.99 mean strong positive correlation, r = 1 mean perfect positive correlation. The study reveals that the exposure of heavy metals were causes adverse health effect by changing the level of CBC parameters. A strongly positive correlation of chromium with CBC parameters shows in figure 8, 9 and 10 (hemoglobin R^2= 0.698, TLC R^2= 0.0807 and R.B.C R^2= 0.758), In cadmium (hemoglobin R^2= 0.766, TLCR^2 = 0.862, R.B.C R^2= 0.862) that shows in figure 11,12,13 and figure 14,15,16 reveals the highly positive correlation of lead (hemoglobin R^2= 0.9319, TLC R^2=0.9308, R.B.C R^2= 0.9308).

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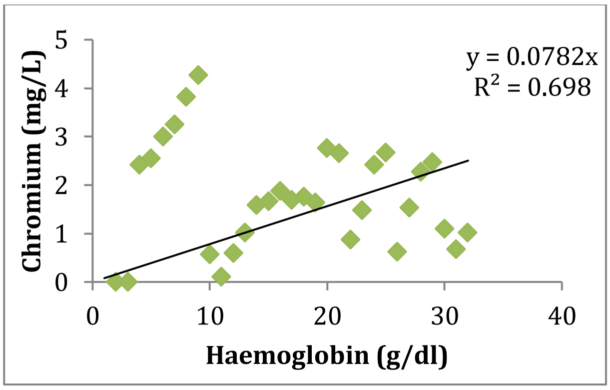

Figure 8: Correlation of hemoglobin with chromium metal

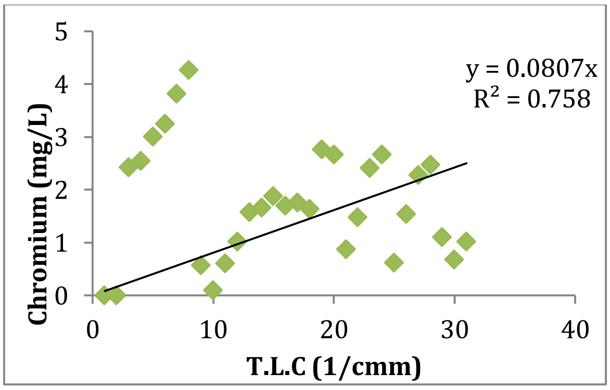

Figure 9: Correlation of TLC with chromium metal

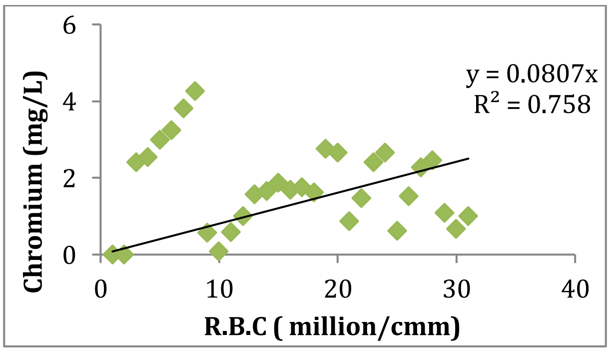

Figure 10: Correlation of RBC's with mean of chromium metal

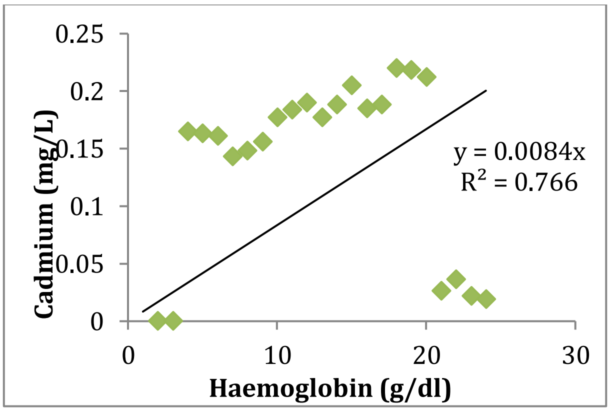

Figure 11: Correlation of hemoglobin with mean of cadmium metal

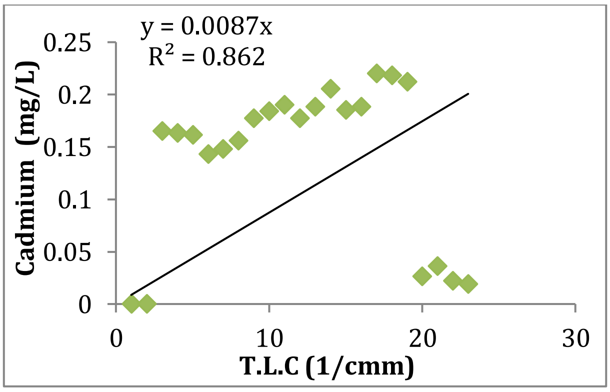

Figure 12: Correlation of TLC with mean of cadmium metal

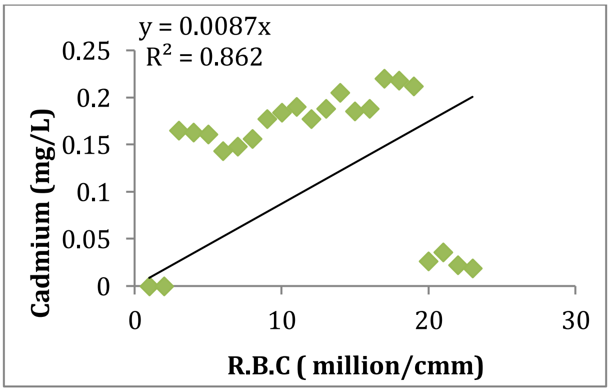

Figure 13: Correlation of RBC with mean of cadmium

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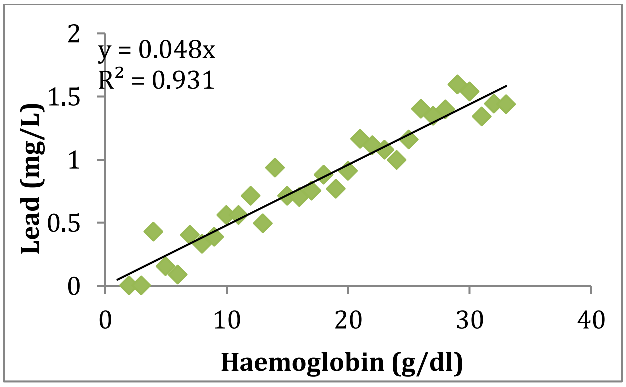

Figure 14: Correlation of hemoglobin with mean of Lead metal

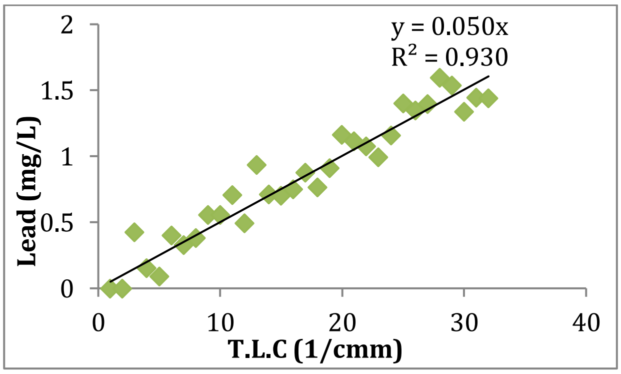

Figure 15: Correlation of TLC mean of Lead metal

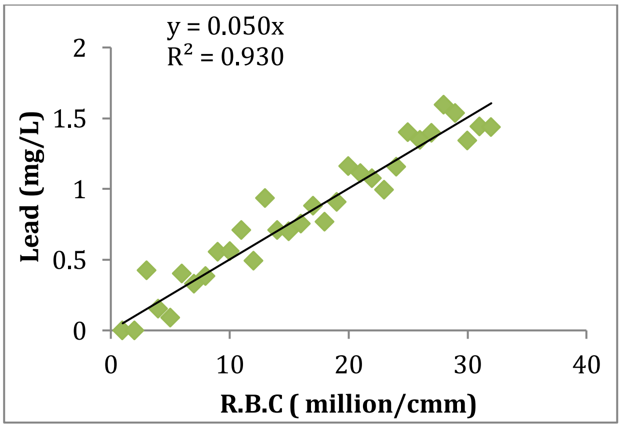

Figure 16: Correlation of RBC with mean of lead metal

Heavy metal increases with the passage of time because of the anthropogenic sources. In previous study the correlation study between the maternal blood and heavy metals shows the good correlation (0.58-0.81)except for manganese and iron (Raghunath *et al.*, 2000).

Heavy metal population is increasingly a serious problem of public concern with the rapid population explosion, urbanization and industrialization in Okara.

DISCUSSION

The predominant source of exposure of metal lead for the non-smoker population is contaminated air, food, and drinking water. The long term exposures have adverse health impact. The blood is the best sample for measuring short-term exposure of lead to individuals (Stojsavljević *et al.*, 2019). Result reveals that the level of lead is dramatically less than chromium and cadmium in Okara. Our study shows that concentration of lead higher in male than female (figure 4). The higher Blood lead level values were also reported by Forte *et al.*,. (2011), Wilhelm *et al.*,. (2004) and others; our result is in agreement with these studies. Our result shows that people of older group was highly impacted by heavy metals in comparison with people of younger group (Figure 5). It was reported that lead increased with age as a result of storage in teeth and bones. In fact, bones hold about 90% of the body burden of this heavy metal that could be remaining about 30 years (Forte *et al.*, 2011). Our result is in agreement with the general ecotoxicology and environmental safety. (Stojsavljević *et al.*, 2019)

For the general population dietary intake is the main source of cadmium. Cadmium is highly toxic heavy metal. Risk of Renal disease and bone fracture has been associated with long term exposure of cadmium (Forte *et al.*, 2011). Our studies indicate the mean value of cadmium is 0.1mg/l. Our study is in agreement with general of Environmental international (Madrigal *et al.*, 2018) which results shows the mean blood cadmium was 0.11µg/l. Result from our research designated that the cadmium concentration is higher is female (age group: 40-50 years) than male. Our study shows the blood cadmium level in females is less than other studied, that is in disagreement with journal of environmental sciences that show cadmium the blood level 0.52µg/l (Li, Zhuang *et al.*, 2018). The higher Blood lead level values were also reported by

(Raghunath *et al.*, 2000) and others studies and we are in disagreement with these studies.

According to our study the average concentration of chromium is 1.73mg/l that shows the level of chromium is higher than other metals in Okara. Moreover, the Cr blood contents determined during the whole study period is higher reported by (Tahir *et al.*, 2017), our study is not in agreement with these studies. Chromium is mineral that the body can use in small doses for body metabolic functions, like digesting food. The chronic exposure causes serious diseases. The deficient chromium level can lead high cholesterol level. The level of chromium in female is 1.776mg/L and in male 1.9001mg/L (fig 4) clearly shows that the level of chromium is higher in male than female. The age groups 30-40 years have high level of chromium. The source of chromium include road dust contaminated by emission of chromium based tobacco, food stuff, cement dust, catalytic converters, smoke etc. Man at age group 30-40 years exposed to chromium at workplace during working in industries using chromium, especially the stainless steel welding, chromate production, chrome pigment industries and chrome plating. However, Zeng and Bhangare also reported higher concentration of chromium than lead and cadmium (Zeng *et al.*, 2016). Our study is in agreement with these studies.

Overall, results of our studies show that the Okara population have higher concentration of chromium than other metals, its mean residents that are near chromate production facilitates higher Cr exposure. There is also concern that residents whose homes have been built on landfills using slag from smelters and chromate producing facilities may be exposed to chromium by breath and dermal contact.

4. CONCLUSION

The study was managed to provide basic information about concentration of heavy metals in human blood. The result obtains from 30 male female samples) from Okara. According to our findings the concentration of heavy metals is higher in female blood sample than male, and higher level of heavy metals was estimated in old age group. Health risk complicated due to the venomous cause by the heavy metal after making their passage in to human metabolism. It is very important to calculate that how to reduce the level of heavy metal. Continuous heavy metal assessment should be done to protect the human and reduce such anthropogenic activities that promote heavy metal exposure.

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