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TRACE ELEMENTAL ANALYSIS OF HAIR FOR TOXICITY USING PARTIAL INDUCED X-RAY EMISSION (PIXE) TESTING FOR FORENSIC SCIENCE APPLICATIONS

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ABSTRACT

Background: Trace metals and elements are the vital component of the body but their excessive amount can be the hallmark for survival. Workmanship of industries like pharmaceutical, paint, and textile are more prone to accumulate them in their bodies and cause toxicity. Hair analysis of workers could be useful for criminal investigations. PIXE is technique which plays vital role in detection of elements and metals with fine accuracy to even less than 1 ppm (particles per million) and provides all present metallic profiles simultaneously.

Method: PIXE is a novel technique used for identification of metals and advantageous over other methods due to its efficacy on the range of metals, the amount of the sample and the quickness. Samples of 31 people (related from three industries pharmaceutical, paint and textile) including 5 normal were analyzed for detection of trace elements from hair.

Results: Trace metals (Se, Cr, Fe, Cu, Zn, and Ni) identified from workers of all three industries pharmaceutical, paint and textile with varying concentrations and with observable difference from control group (people not working in industry). Results are encouraging as it could have vital applications in forensic science as hair from crime scene could lead to significant information about the standard of living of person and ultimately to access him.

Conclusion: Outcome of present study will help us to detection the toxicity of trace metals in workers expressing in terms of diseases and for identification purpose, it is a powerful marker in criminal investigation.

Keywords: Forensic, metal, toxicity, hair, trace element

INTRODUCTION

Trace metals are the important part of body in biochemical processes. Human hair is one of most distinctive feature in human body and a healthy person sheds hair regularly. The US environmental protection agency suggests that human hair could be considered an important indicator for worldwide biological monitoring of toxic trace elements [1]. Hair is a unique for metal analysis because of its structural stability the advantage over this course is the alteration in metallic content is restricted [2]. Metal deposition in the body due to their excess amount expresses in form of diseases. The mining's workers accumulate mercury and gold ultimately their children have high value of these metals in hair than any other region [3]. Such high value of specific metal in hair is the unique identity of specific region where person resides and helpful in determining lifestyle of person and his identity as well. Long-term deposition of metals and their particular environmental intake and recognition for person's specific area from metallic hair profile for forensic aspect can be a marker for identification for particular area, work place or industry. Industrial workers working in process area in pharmaceutical, paint, and textile industry are more prone to accumulate metals.

Particle Induced X-ray Emission (PIXE) is persistent technique with accuracy and sensitivity, which has been used to detect the metals in many fields such as biology, medicine, zoology, archeology, botany, environmental science and forensic science [4]. PIXE`s accuracy and sensitivity is evident from the fact that it gives all metallic profile of periodic table to even one atom of the metal. In many cases from decades, PIXE is being used to detect the metals from bones, gunshot wounds and from hair. This method is quick and requires few mili grams of a sample, which is the vital issue in the forensic science for identification [5]. At crime scene, samples mostly present in very minute amount even to a single hair for carrying out the detection and identification. While using other techniques, sample is required in high quantity and in stable form which could not be guaranteed in case of crime scenes. The simultaneous determination of all metals with precision is also very important.. The detection limits achieved with PIXE analysis are dependent upon a number of well-understood parameters, such as atomic number, matrix effects, solid angle of the detector, detector X-ray sensitivity (which is X-ray energy dependent), irradiation time and beam intensity. In general, the detection limits vary with the square root of the irradiation time or beam current. Although this method is, to some extent, similar to X-ray fluorescence already used by some forensic scientists, it has higher sensitivity and generally requires much smaller sample making it suitable for forensic science applications.

MATERIALS AND METHODS

Sample collection and storage

Hair samples of thirty one (31) male industrial donors working in pharmaceutical, textile and paint industry along with 5 persons (not working in any industry) as reference were collected with informed written and oral consent. All industrial donors were educated to wash their hair prior to sampling with shampoo to clean dust and apparent industrial contaminants following recommendations of international atomic energy agency (IAEA) [6]. The recommended one inch hair length closest to scalp with total weight 5 g from each donor were cut with stainless scissor and stored in sterile plastic bags in cool and dry atmosphere.

Inclusion criteria

The individuals having age from 21 – 55 years and actively working in industry for more than four years were studied in this research.

Exclusion criteria

The individuals are excluded from this research having age less than 20 years and working in industry less than 4 years.

Glassware\reagent

The glassware used for this processing and preparation of the samples and reference samples was washed with 5 % (w/v) detergent solution and rinsing with distilled water and then kept overnight for drying in oven at 60 °C before use.

Sample preparation

To make pellet, each sample was charred at 180C for 1 hour then converted into powdered form by using standards and stainless graduated steel hand – press pellet marker (Perkin – Elmer) in Aluminium carbide mortar [7]. The pellet of hair samples and standard were prepared from each sample, and then the pellet and standard were mounted on 35 nm slide frame with adhesive tape and preserved until analysis.

Particle Induce x-ray Emission (PIXE)

Particle Induce X-ray Emission (PIXE) system developed in 1970 by Sven Johansson of Lund University, Sweden, PIXE is more suitable analytical technique than other techniques involving the measurement of the optical radiations. This technique belongs to Ion Beam Analysis, in which the specimen is used to analyze the target for a beam of accelerated particles.

PIXE analysis

The pelletized samples were irradiated with 3.0 MeV proton beam from the 5 MV Pelletron Tandem accelerator installed at Experimental Physics Lab, National Centre for Physics, Islamabad. The calibration and standardization of the newly installed PIXE chamber for both thick (TiV/Fe alloy) and thin (SRM 2783) targets were carried out using NIST standard reference materials. The diameter of the collimated proton beam was 2 mm. A 100 µm thick Mylar, "funny" filter was used during the measurements and this reduced the count-rate to ensure a dead time of less than 10% at beam currents of 2–5 nA. Observation of the samples after irradiation showed no apparent damage. The emitted X-rays were detected using a 30 mm^2 Si (Li) detector with an energy resolution of 138 eV (FWHM) at 5.9 keV of Mn. The PIXE data was analyzed using the computer code GUPIXWIN [8].

RESULTS AND DISCUSSION

The exposure of different metals from different origins can enlighten us the geographical location of the person, various diseases, employment etc. From environmental exposure, metals can deposit in human hair [9]. The bioavailability of detected metals from hair can be used to assess the relationship among metals in soil sample from various locations [10]. Six metals (Selenium, Chromium, Iron, Copper, Zinc, and Nickel) were detected from hair of industrial donors working in different industries. The arithmetic mean of selenium and zinc were 334.6 ppm and 3.30 ppm which were significant differences from all studied subjects in pharmaceutical industry which having P – value $p < 0.05$, in compare to the chromium, iron, copper and nickel having not much more differences between the pharmaceutical employees, the arithmetic mean for chromium, iron, copper and nickel were 1.33,1.79,2.22 ppm and 1.44 ppm showed non – significant differences because all the means were in normal ranges and the P – value were p > 0.01. The highest concentration of chromium, iron, copper and nickel were 2.30, 3.30, 5.20 and 3.10 µg/g and the lowest concentration of these metals were 0.45, 0.00, 0.87 and 0.50 µg/g respectively which show the discrimination between the individuals and exposure of metals to the employee working in different industries. The highest concentration of a metal was found to be Selenium (334.6 µg/g) found and the lowest concentration of this metal was 29.70 µg/g. The selenium level was found in highest quantity in pharmaceutical industry as compare to the other industries. The concentration of zinc in pharmaceutical industry was also found noticeably (highest 5.70 µg/g and lowest 0.00 µg/g) compared to paint industry. Selenium usually is found in much higher concentration in hair of people working in pharmaceutical industry due to the use of this metal in pharmaceutical industry processing.

The analysis of average metal contents and basic statistical parameter were calculated for donors of different industries. In textile industry, the arithmetic mean for the chromium, zinc, Nickel and Iron were 6.72 ppm, 3.76ppm, 8.49 ppm and 8.93 ppm show significant differences between data groups. The P – value were p > 0.05 which determines significant variation among the employees working in commercial textile industry. Selenium and Copper show the arithmetic mean 54.2 ppm and 0.6169 ppm found show non – significant differences like the chromium, zinc, nickel and iron. The P – value for these metals were P>0.00, exhibiting no difference in selenium and copper level in hair of textile workers. Hence, selenium and copper cannot be considered as the environmental contaminant in textile industry because the selenium and copper level were much more nearer to the control samples which were all in normal levels and show no variation in the results.

In workers of textile industry the concentration of Cr was ranged from highest 50.0 µg/g to lowest value 31 µg/g. While concentration of Iron was varied from 63.00 µg/g to lowest value 22 µg/g. Nickel was found in textile workers from 67.00 µg/g to 0.26µg/g and Zn was determined in highest concentration 5.7 µg/g to lowest concentration 0.00 µg/g in textile workers. Nickel has been reported to be found in higher concentration from workers of textile industry [11]. In paint industry , workers are usually engaged in variety of activities from refining raw material to mixing, processing and paint formation making them exposed to metals and toxic elements. The arithmetic means for iron and copper were calculated 10.88 ppm and 9.367ppm respectively both indicating significant difference with P value P >0.05. Also Zn shown significant difference with arithmetic means 1.31ppm and P value P >0.01. The Iron was detected in workers of paint industry ranging from 79.90 µg/g to 0.48 µg/g. The copper was determined in range of 67.90 µg/g to 0. 93 µg/g. The Zn was found to be lowest determined metal in workers from Paint industry with maximum quantity 2.90 µg/g to lowest quantity 0.64 µg/g.

The highest iron concentration was found to be 79.90 µg/g and the lowest were 0.48 µg/g, copper were at highest at 67.90 µg/g and the lowest were 0.93 µg/g and zinc were 2.90 µg/g and the lowest value concentration were .64 µg/g, which indicate the best variation between all the employees working in paint industry [12]. The trace and toxic metals are transferred from the nail to human from the outer environment [13], the heavy and toxic metals are accumulated underneath the nails of the employees working in different industry during their duty. The hair is studied to know the relationship between minerals composition of human hair and illness like different infections, of the upper and lower respiratory tract, migraine, vertigo, asthma, dandruff [14].

The highly processed food causes the increase levels of some heavy and trace elements in hair [15]. The hair analysis can lead us to morphological characteristics and personal identification of person thus helping in forensic investigations. The hair is also the good source to detect the toxicity, drug and substance in abuse cases. The Forensic Sciences has largely simplified the identification of different heavy and trace metals accumulation in human hair. By employing forensic techniques to analyze human hair, we can check even six months old profile consisting elemental record giving rise to leading information about geographical origin and about lifestyle of person. The selenium was found in higher concentration in hair of people working in Pharmaceutical industry and lower concentration in textile and paint industry. Selenium in pharmaceutical industry is higher as (334.6 ± 39.8) as shown in Figure 1 while statistical parameters and comparison with control samples is entailed in Table 1.

Chromium contents were detected in higher concentration in hair of people working in textile industry as (6.72 ± 6.18) than people from other industries as it has been explained in figure 2. The comparison of concentration of Chromium with reference group and people from different industries has been demonstrated in table 2.

Paint and textile industry workers have higher iron than those of working in any other industry. It was found in workers of paint industry (10.8 ±9.86) and then workers of textile industry as (8.49± 7.78). Pharmaceutical workers are lesser exposed to iron as shown in figure 3. The comparison of concentration of Iron with reference group and people from different industries has been demonstrated in table 3.

Copper was found in higher concentration in workers from paint industry (9.36±8.36) than those of working in other industries as shown in figure 4. The comparison of concentration of Copper with reference group and people from different industries has been demonstrated in table 4.

Workers from textile and pharmaceutical industries were found to have exposed more to zinc than any other of element. Textile workers have higher content of zinc (3.76± 1.28) and pharmaceutical workers have (3.30±0.55) as shown in figure 5. The comparison of concentration of Zinc with reference group and people from different industries has been demonstrated in table 5.

Nickel was found in higher concentration in workers of textile industry (8.93±8.29) and then second highest concentration was found in people working in paint industry (0.76 ± 0.01) as it has been described in figure 6. The comparison of concentration of Nickel with reference group and people from different industries has been demonstrated in table 6.

Table 1: Concentration comparison of Selenium with control group and people from different industries. Pairwise comparison of industries with reference group is done by Bonferoni test. ** Significant at p<0.05 *significant at p<0.01 NS no significant p>0.05

Groups	Mean ± S.E	N (Samples)	Minimum µg/g	Maximum µg/g
References Group	129.2 ±19.8	5	96.0	187.0
Pharmaceutical Industry	334.6± 39.8	10	29.7	460.6
Textile Industry	54.2± 6.0	8	20.6	76.1
Paint industry	44.0± 4.5	8	33.7	74.8
P value	0.000^{**}	Total # 31		

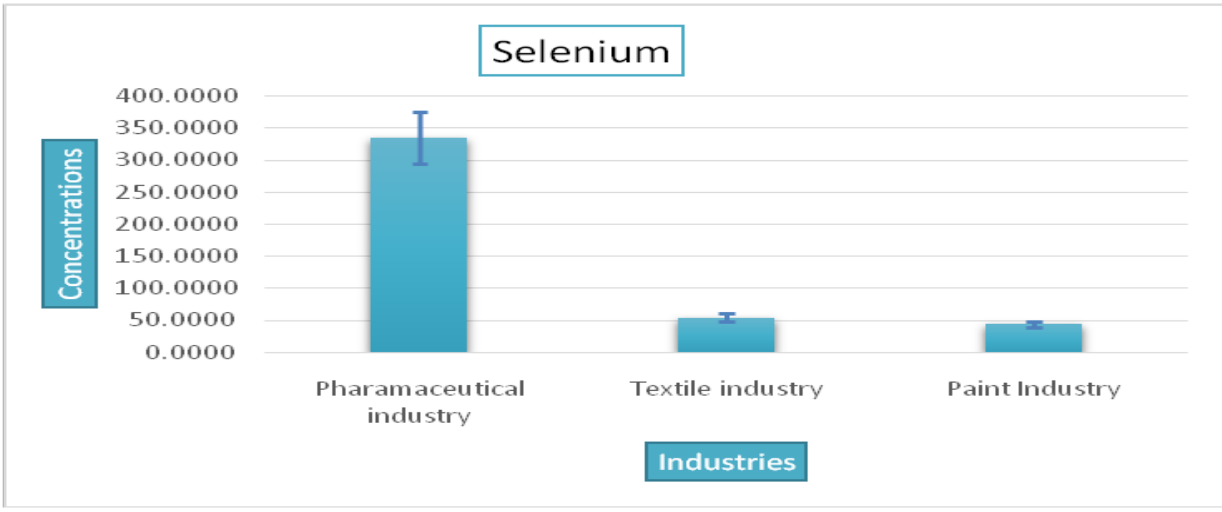

Figure 1: Concentration of Selenium found in workers from different industries

Table 2: Concentration comparison of Chromium with control group and people from different industries. Pairwise comparison of industries with reference group is done by Bonferoni test. ** Significant at p<0.05. *significant at p<0.01 .NS no significant p>0.05

Groups	Mean ± S.E	N (Samples)	Minimum µg/g	Maximum µg/g
References Group	1.32± 0.37	5	0.50	2.60
Pharmaceutical Industry	1.33±0.23	10	0.45	2.30
Textile Industry	6.72± 6.18	8	0.31	50.0
Paint industry	0.78± 0.031	8	0.65	0.91
P value	0.000**	Total # 31		

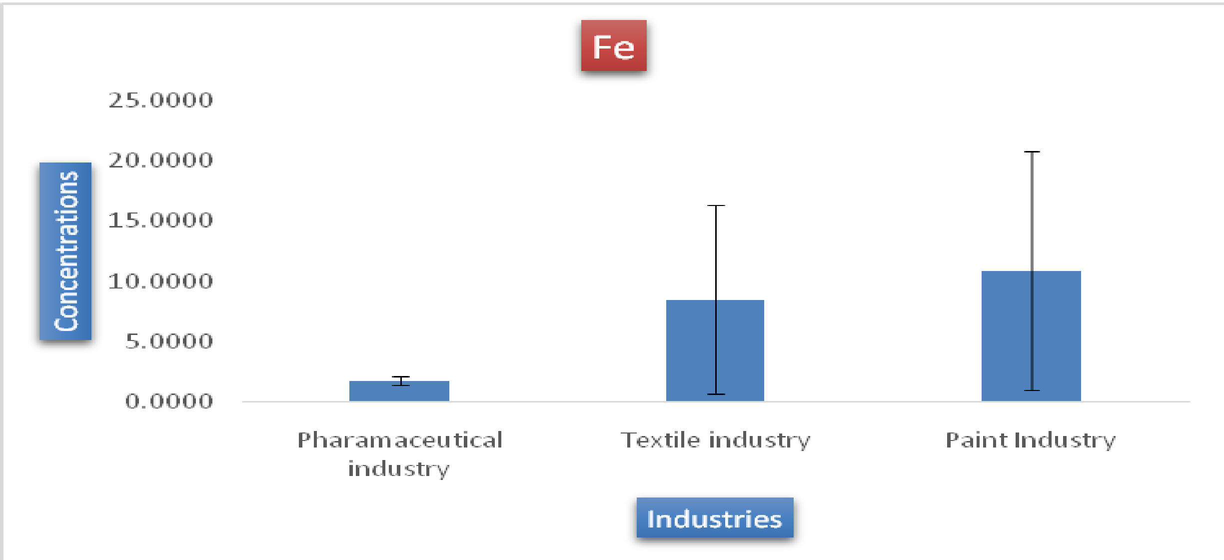

Figure 2: Concentration of Chromium found in workers from different industries

Table 3: Concentration comparison of Iron with control group and people from different industries. Pairwise comparison of industries with reference group is done by Bonferoni test. ** Significant at p<0.05 . *significant at p<0.01 . NS no significant p>0.05

Groups	Mean ± S.E	N (Samples)	Minimum µg/g	Maximum µg/g
References Group	2.68±0.28	5	2.10	3.70
Pharmaceutical Industry	1.79± 0.36	10	0.00	3.30
Textile Industry	8.49±7.78	8	0.22	63.00
Paint industry	10.8± 9.86	8	0.48	79.90
P value	0.000**	Total # 31		

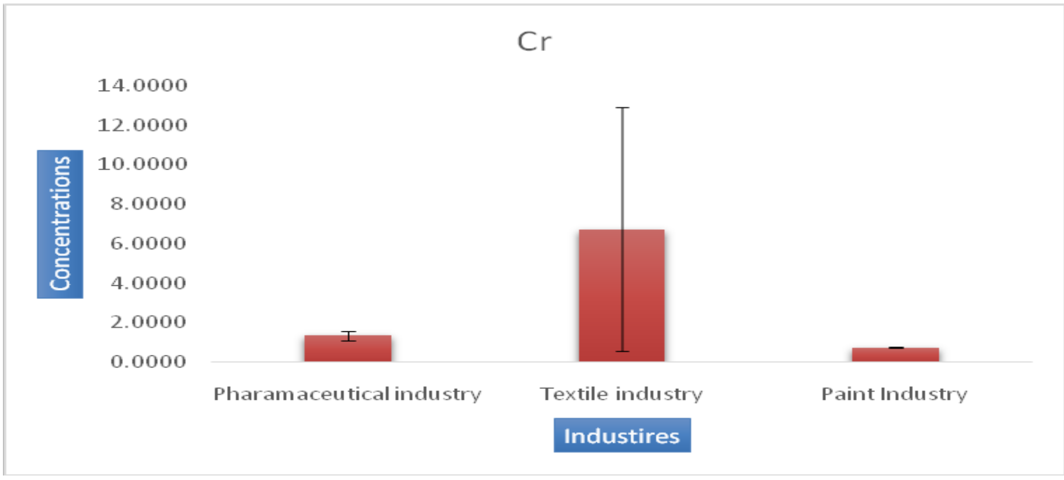

Figure 3: Concentration of Iron found in workers from different industries

Table 4: Concentration comparison of Copper with control group and people from different industries. Pairwise comparison of industries with reference group is done by Bonferoni test. ** Significant at p<0.05 . *significant at p<0.01 . NS no significant p>0.05

Groups	Mean ± S.E	N (Samples)	Minimum µg/g	Maximum µg/g
References Group	1.76±0.25	5	1.00	2.60
Pharmaceutical Industry	2.22±0.30	10	0.87	5.20
Textile Industry	0.61±0.12	8	0.12	0.94
Paint industry	9.36±8.36	8	0.93	67.90
P value	0.000**	Total # 31		

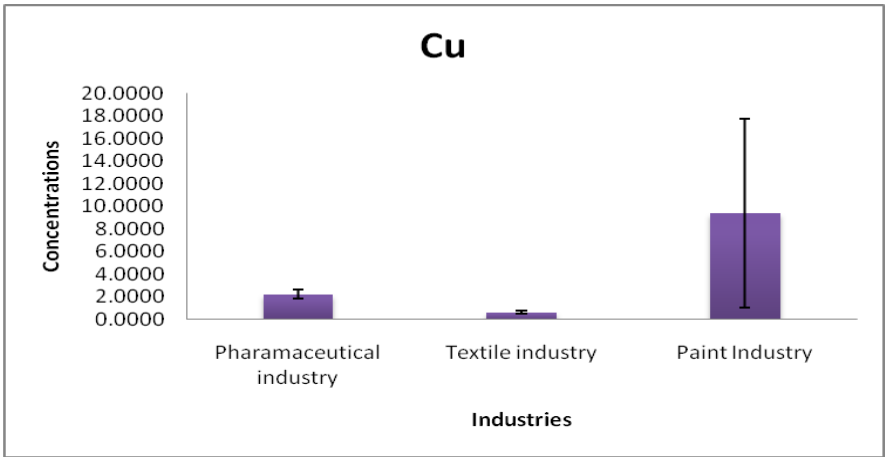

Figure 4: Concentration of Copper found in workers from different industries

Table 5: Concentration comparison of Zinc with control group and people from different industries. Pairwise comparison of industries with reference group is done by Bonferoni test. ** Significant at p<0.05 . *significant at p<0.01 . NS no significant p>0.05

Groups	Mean ± S.E	N (Samples)	Minimum µg/g	Maximum µg/g
References Group	3.08±0.15	5	2.70	3.40
Pharmaceutical Industry	3.30± 0.55	10	0.00	5.70
Textile Industry	3.76±1.28	8	0.00	10.10
Paint industry	1.31±0.25	8	0.64	2.90
P value	0.000**	Total # 31		

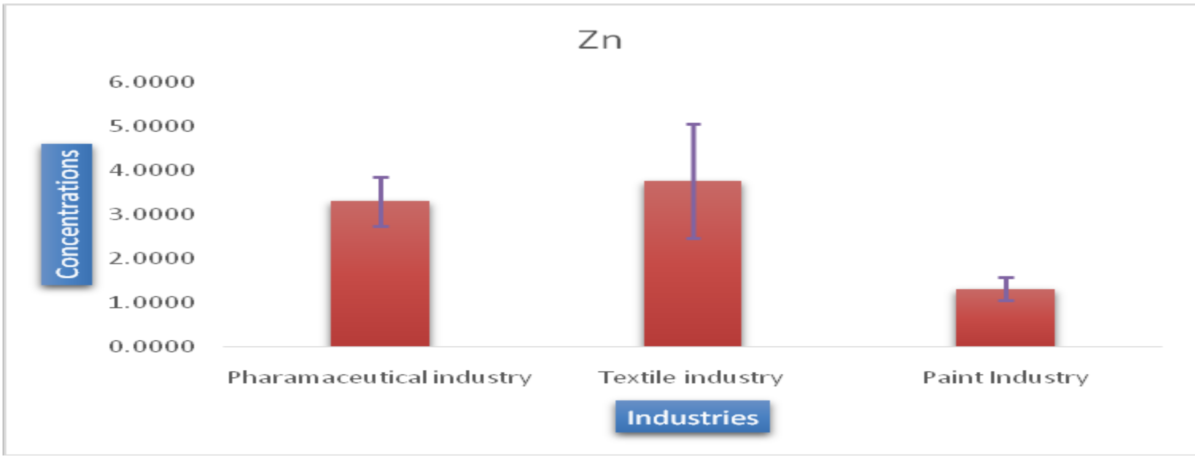

Figure 5 : Concentration of Zinc found in workers from different industries

Table 6: Concentration comparison of Nickel with control group and people from different industries. Pairwise comparison of industries with reference group is done by Bonferoni test. ** Significant at p<0.05 . *significant at p<0.01 NS no significant p>0.05

Groups	Mean ± S.E	N (Samples)	Minimum µg/g	Maximum µg/g
References Group	2.52±0.15	5	2.10	2.90
Pharmaceutical Industry	1.44±0.21	10	0.50	3.10
Textile Industry	8.93±8.29	8	0.26	67.0
Paint industry	0.76±0.01	8	0.69	0.80
P value	0.000**	Total # 31		

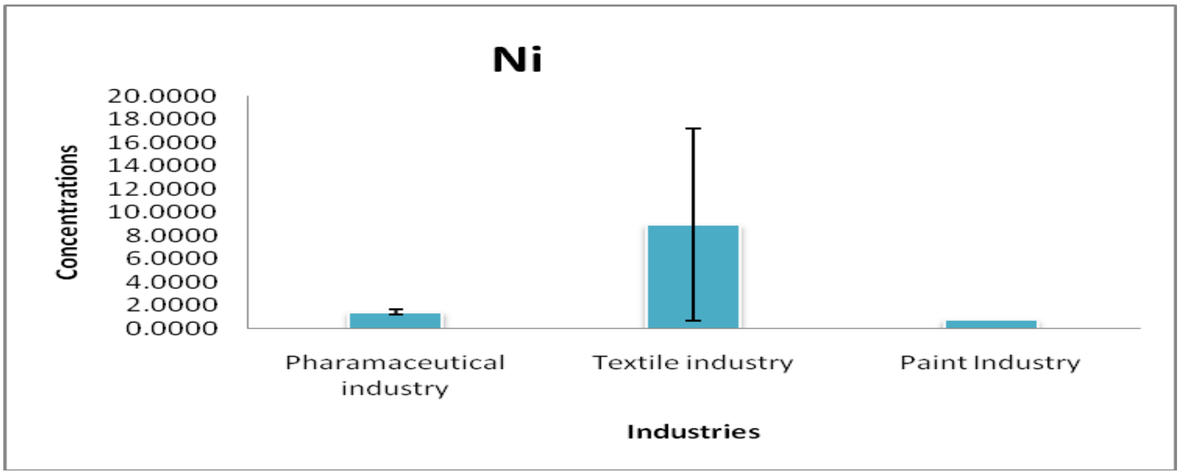

Figure 6: Concentration of Nickel found in workers from different industries

In a study [16] to monitor exposure and effects in workers from Selenium processing plants concluded that in spite of high external exposure to elemental Selenium ,they did not observe effect of glutathione peroxidase (GPx) in their blood but in current study high concentration of Selenium was detected in hair of workers from pharmaceutical industry. The effect of this exposure yet to be studied. A study using hair of workers to assess occupational and environmental exposure to heavy metals in area of southern Italy was conducted [17] and observed highest median values of Aluminum, Barium, cadmium, lead, mercury and uranium. In current study no such elements were detected from workers of paint, textile and pharmaceutical industries.

While studying cause of hyperpigmentation from people working in tannery industry in Bangladesh [18] found the chromium as reason for that. But in this study, chromium was detected in higher concentration from people working in textile industry but no visible signs of hyperpigmentation were observed. A study [19] to determine level of toxicity of essential trace elements from hair of people working in petrochemical industry and found iron in higher concentration. But in present research, highest concentration of iron was detected from hair of people working in paint industry. Researchers found that people working in leather industry [20] were having excessive amount of copper. They utilized the hair and nail samples for this purpose. Comparing to this, in recent study copper was detected in higher concentration in workers from paint industry. A research was accomplished [21] to evaluate health risk and concentration of heavy metals in workers involved in surgical instrument making factories in Sialkot, Pakistan. They found Chromium, cadmium, Nickel, copper, Lead, iron manganese and zinc in hair, saliva and blood in higher concentration. In our study, Zinc and nickel were detected in higher levels from workers of textile industry.

CONCLUSION

In current study, a considerable difference was found for presence of trace elements between hair of normal people (not working in any industry) and the people working in different industries. Also, PIXE is proved to be a valuable tool for metal and trace element detection from hair having applications in criminal investigations. This techniques is useful to play its role in forensic toxicology to predict even six months old hair profile which could shed light not only about work place of source of sample but also could give hint about the life style of person and potential information about area of residence. The recent research and development on concept of heavy and trace metals analysis from human hair can largely be attributed to the expansions made in Forensic Toxicology.

Conflict of Interest Statement

The authors declare that there is no conflict of interest regarding the publication of this paper.

REFERENCES

[1] Kuroda S, Saunders T, Nakamura T, Sera K, and Yoshiaki Nose. Epidemiological Assessment of Significance for Hair Minerals Measured by PIXE Method. Proceedings of the World Congress on Engineering and Computer Science, (2010); II: October 20-22, San Francisco, USA.

[2] Stadlbauer C, Prohaska T, Reiter C, Knaus A, Stingeder G. Timeresolved monitoring of heavy-metal intoxication in single hair by laser ablation ICP–DRCMS. Analytical and bioanalytical chemistry, (2005); 383(3): 500-508.

[3] Murao S, Daisa E, Sera K, Maglambayan V, Futatsugawa S. The role of pixe in environmental monitoring and education of small-scale miners of gold. International of PIXE, (2002); 12:175-180.

[4] Warren M, Falsetti AB, Kravchenko I, Dunnam F,. Van Rinsvelt HA, Maples WR. Elemental analysis of bone: proton-induced X-ray emission Testing in forensic cases. Forensic science international,(2002);125(1): 37-41.

[5] Vadrucci M, Bazzano G, Borgognoni F, Chiari M, Mazzinghi A, et al. A new small-footprint external-beam PIXE facility for cultural heritage applications using pulsed proton beams. Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms, (2017); 406: 314-317.

[6] Ryabukhin Y S, Parr, R.M. Nuclear activation techniques in life sciences. Atomic energy review, (1978); 16 (3):547- 553.

[7] Zucchiatti A, Galán P, Prieto JE. A procedure to correct for target thickness effects in heavy-ion PIXE at MeV energies Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms, (2017); 407: 1-4.

[8] Cloete K, Šmit Ž. A Nuclear Analytical Method for Unraveling elemental Profiles in Human Scalp Hair. Marie Curie Alumni Association, (2016); 59-60.

[9] Chojnacka K, Saeid A, Witkowska Z ,Tuhy L. Biologically active compounds in seaweed extract .The prospects for application The Open Conference Proceedings Journal, (2012);3: 20-28.

[10] Bilo F, Borgese L, Cazzago D, Zacco A, Bontempi E, Guarneri R , et al. TXRF analysis of soils and sediments to assess environmental contamination. Environmental Science and Pollution Research, (2014); 21 (23): 13208–13214.

[11] Tuzen M, Onal A and Soylak M .Determination of trace heavy metals in some textile products produced in turkey. Chemical society of Ethiopia, (2008); 22(3):379-384.

[12] Calligaro T, Gonzalez V, Pichona L,.PIXE analysis of historical paintings: Is the gain worth the risk? Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms, (2015); 363:135-143.

[13] Kurdi M, Eslamkish T, Ferdows M,.Assessment of Environmental and Heavy metal pollution at the Gorgan Gulf and the QarehSou River. Conference: National conference on geography, tourism, natural resources and sustainable development, (2014).

[14] Wołowieca P, Michal I, Mikulewicz M .Hair Analysis in Health Assessment. Clinica Chimica Acta ,(2012);419: 139-171.

[15] Chojnacka K, Saeid A, Witkowska Z and Tuhy L. Biologically Active Compounds in Seaweed Extracts - the Prospects for the Application. The Open Conference Proceedings Journal, (2012); 3, (Suppl 1-M4) 20-28.

[16] Göen T, Schaller B, Jäger T, Bräu-Dümler C, Schaller H, Drexler H. Biological monitoring of exposure and effects in workers employed in a selenium-processing plant. Int Arch Occup Environ Health, (2015); 88(5): 623-630.

[17] Buononato V, Luca D, Galeandro C, Congedo L, Cavone D, Intranuovo G, Guastadisegno M, Corrado V, Ferri M. Assessment of environmental and occupational exposure to heavy metals in Taranto and other provinces of Southern Italy by means of scalp hair analysis. Environmental Monitoring and Assessment, (2016); 188: 337.

[18] Hossain M, Yajima I, Tazaki A, Xu H, Saheduzzaman M, Ohgami N *et al*. Chromium-mediated hyperpigmentation of

skin in male tannery workers in Bangladesh. Chemosphere, (2019); 229: 611-617.

[19] Skalny V, Kaminskaya G, Krekesheva T, Abikenova S, Skalnaya M, Berezkina E *et al*. The level of toxic and essential trace elements in hair of petrochemical workers involved in different technological processes. Environ Sci Pollut Res ,(2017); 24(6): 5576-5584.

[20] Junaid M, Hashmi M, Malik R. Evaluating levels and health risk of heavy metals in exposed workers from surgical instrument manufacturing industries of Sialkot, Pakistan. Environ Sci Pollut Res, (2016); 23(18): 18010-18026.