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Determination of manganese and chromium in welders and non-welders population in Lahore, Pakistan

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Abstract

The heavy metal contamination from an occupational origin is of major concern due to its accumulation in living organisms leading to long term toxic effects. The present study estimated the quantities of manganese and chromium in blood, urine, and hair of welders and non-welders (n = 25) (ages ranged from 16 to 50 years) in Lahore, Pakistan. The results indicated that both manganese and chromium were higher in all the samples compared to the international norms by world health organization (WHO), (i.e., 20-80 ppb in blood, 1-8 ppb in urine, and 300 ppb in hair for manganese while 20-50 ppb in blood, 0.24-1.8 ppb in urine and 100-1000 ppb in hair respectively). Though one of the subject was of 50 years and had 32 years of experience, his manganese and chromium amounts were less because he was the owner of the shop and was currently less exposed to the fumes. The statistical data was in accordance with the finding of the study; whereas control group showed manganese and chromium levels within limits.

Keywords: Atomic Absorption Spectrometer; Blood; Chromium; Hairs; Manganese; Urine; Welders

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Introduction

Welding of metals to form permanent bonds involve release of fine particles (i.e., 2µm) or fumes called as welding smoke (Jeffus, 2002). The constituents of fumes depend on the type of filler, i.e. metal rod and the material, used to weld the base metal. The fumes usually consist of chromium, manganese, iron, beryllium, cadmium, copper, cobalt, lead, vanadium, zinc etc. Inhalation of such fumes, especially when generated at a confined and incarcerated spaces, pose health hazard in workers, as the concentration of fumes keep on building in the breathing space (McDermott and Ness, 2004).

Manganese is one of the vital trace elements for humans, with a daily nutritional requirement of 30-50 mg/kg body weight which is used by the body for protein metabolism, bone formation, synthesis of cholesterol and mucopolysaccharides (Alloway and Selinus, 2005). However, it is more toxic in air than when ingested by mouth; as inhaled manganese is directly transported to the brain without being metabolized in the liver and therefore results into post-encephalitic Parkinsonian disease (Csuros, 2002). Exposure to high level of manganese is associated with neurological symptoms especially in those welders who have been exposed to high concentration of manganese containing welding fumes, while welding operations are performed in poorly ventilated areas (Albini et al., 2006).

Chromium is another essential trace element required by human body with daily dietary intake ranging from 50-200 µg that helps to maintain normal glucose level and helps in protein and fat metabolism (Ensminger, 1994). High levels of chromium in the body can cause gastrointestinal disorders, hemorrhagic diathesis, convulsions and may also cause bronchial carcinoma in humans when inhaled (Adriano, 2001). According to a study, among 79 electroplating workers, 16 were found to suffer from nasal septum perforation, 42 suffered from ulceration, and 10 developed skin ulcers (Chan et al. 1994); while showing symptoms related to nose (Lee et al., 2002), respiration (El–Zein et al., 2005; Sultan and Thamir, 2003), asthma (Lillianberg et al., 2008; Toumi et al., 2007), in their blood and urine (Ellingsen et al., 2006; Meo et al., 2006). On the other hand, people with chromium deficiency may suffer from impaired glucose tolerance and elevation in serum insulin, cholesterol and total triglycerides (Guertin et al., 2005).

In the present study, an attempt is made to estimate the level of manganese and chromium in the blood, urine and hairs of welders, who are frequently exposed to these two elements. Though these are vital trace elements required by human body, however, in quantities above world health organization (WHO) standards are lethal, i.e., 20-80 ppb in blood, 1-8 ppb in urine and 300 ppb in hair for manganese (Tobin, 2005; WHO, 2009); and 20-50 ppb in blood, 0.24-1.8 ppb in urine and 100-1000 ppb in hair for chromium respectively (Cocker, 2005; Nriagu and Nieboer 1988; Mertz, 1969).

Materials and Methods

Subjects and Questionnaire Survey

The cross-sectional questionnaire study was carried out in February 2009. The sample size involved 25 workers (all of them male) of each group, i.e., welders and non-welders, from Rehmanpura, Lahore, Pakistan. However, the non-welders group were occupants of Iqbal Town, Garden Town, Muslim Town, Johar Town and Wapda Town, Lahore. A self-administered questionnaire comprising to socio-demographic information of the participants was used to conduct interview based questionnaire survey. The questionnaire survey contained both open-ended and close-ended questions correlating the age, experience, duration of shift, type of welding process employed, source of ventilation and personal protective equipment as well as the health complaints and concerns of the exposed group.

Reagents and Materials

The laboratory equipment was washed with distilled water and placed in 6 molar hydrochloric acid (HCl) for 24 hours and washed again with double-distilled water. The 6% ethylenediaminetetra acetic acid (EDTA) (E Merck, Germany) was prepared by dissolving 6 g in 100 ml of distilled water. The 6 molar HCl (E Merck, Germany) was prepared by dissolving 27.3 ml in distilled water and was standardized against 0.1N sodium hydroxide (NaOH). Concentrated Nitric Acid (HNO_3), E Merck (Germany). 5 M NaOH was prepared by dissolving 20 g of NaOH in 50 ml of hot deionized water. The solution was subsequently diluted to 100 ml. Manganese stock solution 1000ppm (Aldrich, USA). 100ppm standard solution (w/v) of manganese was prepared by diluting 10 ml of the stock solution to 100 ml with distilled water. The solution was diluted further to get 100 ppb working solution. Chromium stock solution 1000 ppm (Fluka, Switzerland) was prepared by diluting 10 ml of the stock solution to 100 ml with distilled water. The solution was diluted afterwards to get 100 ppb working solution. Atomic Absorption Spectrometer, Hitachi Z 800 (Japan), equipped with graphite furnace was used for the determination of manganese and chromium. For the collection of blood, disposable syringes and vials were obtained from Vacutainer (Becton Dickinson). All chemicals and reagents were of analytical grade.

Sample Collection

Blood: A 5ml of blood sample was collected from 25 male welders, who were directly exposed to the manganese and chromium fumes, in a vial containing an anticoagulant (0.5ml 6% EDTA), with a disposable plastic syringe. The subjects were employed at various welding shops on Rehmanpura Road Lahore, Pakistan. The ages of welder participants ranged between 16-50 years, while non -welders were selected nearly with same age range and their blood samples were obtained from the occupants residing in areas Iqbal Town, Garden Town, Muslim Town, Johar Town and Wapda Town, Lahore.

Urine: A 25 ml of urine sample was collected in polyethylene bottles, containing 0.5 ml of concentrated HNO_3, from the welders who were directly exposed to the manganese and chromium fumes; as well as from non-welders accordingly.

Hairs: A 1g of hair sample was collected from the nape close to the skin, in polyethylene bags, from the same welders and non-welders group, as mentioned previously. The hair samples were washed with de-ionized water before processing in laboratory.

Experimental Procedure

The analyses were carried out in the minimum time possible after the collections of the sample from all 25 men (ages ranged from 16 to 50 years).

Determination of Manganese and Chromium in Blood: A 5ml of blood sample in 0.5 ml of EDTA was dissolved in 10 ml of distilled water. The solution was dried in an oven at 116°C for an hour in a crucible. Complete ash of the sample was obtained by transferring the sample into a muffle furnace at 540°C for 2 hours in order to evaporate all the organic content, while the inorganic content remained in the form of a rust colored ash in the crucible. The ash was then dissolved in 1 ml of concentrated HCl and the solution was heated over a water bath for 15 minutes, until the color of the solution changed from rust to yellow. The solution was filtered through Whatman filter paper number 41 into a 10 ml volumetric flask and volume was made up to the mark with distilled water. This solution was then subjected to atomic absorption spectrometer for the determination of manganese and chromium (Mutaftchiev et al., 1999).

Determination of Manganese and Chromium in Urine: A 50 ml of urine sample was transferred in a separatory funnel. The pH of the sample was alkaline with 1ml of 5 M NaOH. To this, 20 ml of chloroform-isopropyl alcohol mixture (3:1) was added subsequently. The mixture was shaken for a couple of minutes and the extract was filtered through a Wattman filter paper into a beaker. Afterwards, 5 extractions were drawn from the same solution and the extract was adjusted to pH 2 by 6 M HCl and evaporated to 10 ml. Lastly, concentrations of manganese and chromium in the samples were determined with atomic absorption spectrometer as described previously (Mutaftchiev et al.,1999).

Determination of Manganese and Chromium in Hair: Hair sample (1g), collected in polyethylene bag, was transferred to the crucible followed by rinsing with acetone (Kucera et al., 1996). The residual was washed with distilled water leading to drying in an oven as described previously (Mutaftchiev et al., 1999).

Statistical Analysis

The Minitab v 16.0 was used for the data analyses especially box-plot comparison and descriptive analysis. Chi-square tests was employed to assess these relationships with statistical significance,

Table 1: Demographic and work-related variables for welder population and non-welder population

Welders						Non-welders		
Variable	Participants (n = 25)		Variable	Participants (n = 25)		Variable	Participants (n = 25)	
	n	%age		n	%age		n	%age
Gender			Experience in present occupation (year/s)	25		Gender		
Male	25	100	less than 1	1	4	Male	25	100
Age (years)			1 – 5	5	20	Age (years)		
10 – 20	4	16	6 – 10	1	4	10 – 20	0	0
21 – 30	12	48	11 – 15	9	36	21 – 30	14	56
31 – 40	8	32	16 – 20	5	20	31 – 40	10	40
41 – 50+	1	4	more than 20 years	4	16	41 – 50+	1	4

if α = 0.05. Moreover, PAST v4.0 was used to perform correspondence analysis. The aim was to describe the relationships among different variables in welder and non-welder population. Prior to statistical analysis, the data was normalized and an additive logarithmic transformation log (1 + X) was performed to eliminate the effects of orders of magnitude differences between different environmental variables. The corresponding analysis was carried out in order to highlight influence of age, experience on metal accumulation for each groups. Pearson Correlation Coefficient (r) was applied to measure of the linear correlation between two variables i.e. age and experience, and manganese/chromium quantities in welders.

Results

Demographic Variables

Table 1 describes the socioeconomic demographics of both the groups. The welders and non-welders were all male participants with mean age of 29.4 (± 7.91) and 29.76 (± 5.96) years respectively. From Table 1 it can be seen that 4 (16%) welders ranged between 10 – 20 years of age, 12 (48%) ranged between 21 – 30 years of age, 8 (32%) ranged between 31 – 40 years of age, and only 1 (4%) ranged between 41 – 50 years of age. Majority of the participants (80%) worked for 8 hours/day and most of them (36%) have had 15 year experience in the study occupation while 20% had a work experience for up to 20 years. The welder lived in Rehmanpura, Lahore, Pakistan.

Similarly, 14 (56%) of the non-welders non-welders ranged between 21 – 30 years of age, 10 (40%) ranged between 31 – 40 years of age, and only 1 (4%) ranged between 41 – 50 years of age. The non-welders were residents of Iqbal Town, Garden Town, Muslim Town, Johar Town and Wapda Town, Lahore where there were no welding shops and the possibility of the hazards of manganese and chromium fumes were very remote.

Determination of Manganese and Chromium

The concentrations of manganese and chromium in the blood, urine and hair samples of welders as well as non-welders are shown in Table 2. The results demonstrate that manganese and chromium level in blood, hair and urine of welders were significantly higher than those seen in non-welders. Also the levels of the two metals were beyond the thresh hold limit value described by WHO i.e. the WHO standards for manganese are 20-80 ppb in blood, 1-8 ppb in urine and 300 ppb in hairs and for chromium the WHO Standards are 20-50 ppb blood, 0.24-1.8 ppb urine and 100 - 1000 ppb hair respectively.

From Table 2 it can be seen that the mean value of manganese in blood, urine and hair of welders was calculated to be 96.30 (±14.43), 18.49 (± 2.06) and 754.4 (± 131.82) respectively, comparatively, mean value of manganese in blood, urine and hair of non-welders was estimated to be 49.26 (±14.28), 5.17 (±0.91) and 154.8 (±50.99) respectively. A significant difference can be observed between the mean values of both the groups.

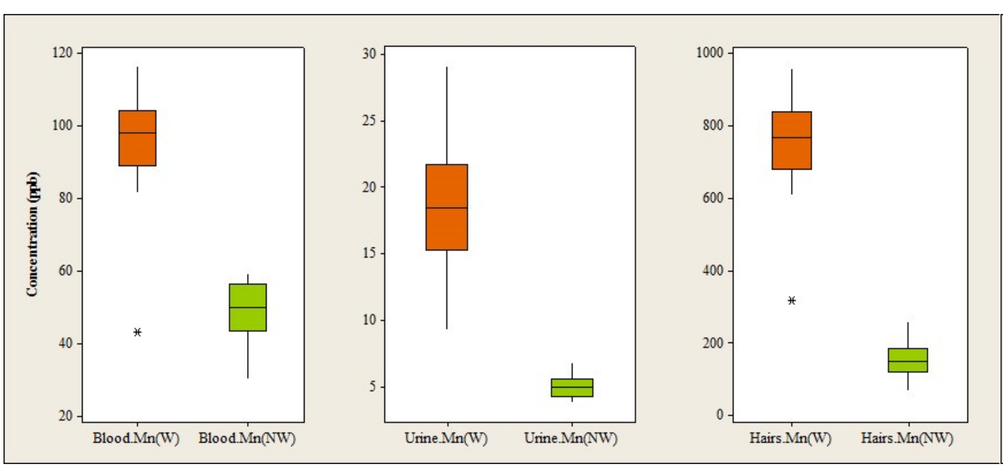
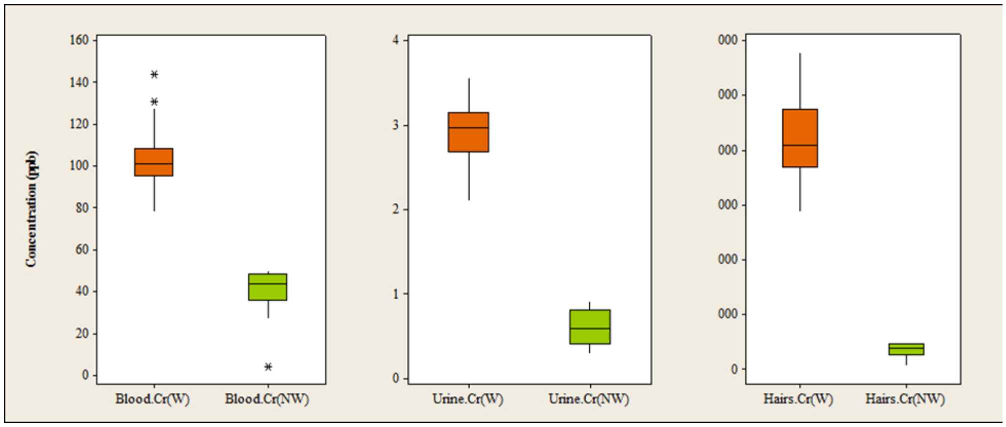


Fig. 1: Comparison of manganese concentration in welder and non-welder population

Fig. 1: Comparison of chromium concentration in welder and non-welder population



Similar trend was witnessed in case of chromium where the mean value in blood, urine and hair of welders was estimated to be 754.4 (±131.82), 2.92 (±0.36) and 8443.6 (±1353.36) respectively, whereas for the non-welders the mean value was found to be 41.01 (±9.78), 0.61 (±0.21) and 714.8 (±232.78) respectively. The mean values for welders also fall far from the guidelines demonstrated by WHO for both manganese and chromium.

Table 3 shows correlation coefficient (r) between age, experience, and manganese/chromium quantities in welders. The value of *r* was calculated for all the three biological specimens i.e. blood, hair and urine and presented positive values which strongly signifies that the attributes, age and experience, relates with elevated levels of the concerned heavy metals.

Statistical Analysis

The correspondence analysis elucidates stronger influence of

Table 2: Manganese and Chromium present in the blood, urine and hair of welders and non-welders

Sample No.	Welders						Non-welders					
	Manganese (ppb)			Chromium (ppb)			Manganese (ppb)			Chromium (ppb)		
	Blood	Urine	Hair	Blood	Urine	Hair	Blood	Urine	Hair	Blood	Urine	Hair
1	105.40	16.23	840	88.34	2.39	7350	58.09	5.62	220	33.47	0.34	380
2	96.13	11.08	780	78.15	2.1	5740	59.32	6.81	250	48.30	0.81	940
3	100.50	18.45	790	103.6	3.01	9960	54.39	4.21	170	4.23	0.55	810
4	43.25	9.30	320	96.13	2.71	7150	50.10	4.98	130	48.46	0.86	910
5	116.00	25.63	940	124.5	3.34	8820	30.35	3.87	090	50.0	0.92	990
6	87.13	14.78	670	80.05	2.26	6470	49.55	4.54	120	45.3	0.49	750
7	99.00	18.24	780	105.9	3.11	7020	57.69	5.62	180	48.61	0.78	960
8	96.16	17.65	730	96.17	2.74	8190	58.42	6.77	230	27.14	0.30	150
9	81.63	13.51	610	104.6	3.09	8930	59.37	4.86	260	49.16	0.89	970
10	84.48	16.69	630	144.0	3.56	11540	49.28	4.21	140	49.98	0.91	980
11	96.35	20.36	750	98.22	2.84	8240	52.26	5.10	160	35.21	0.38	420
12	98.01	21.33	770	131.02	3.47	10710	48.74	4.96	140	44.01	0.63	760
13	102.43	26.47	830	102.67	2.99	7690	39.45	4.07	110	41.69	0.53	680
14	98.68	19.72	760	101.47	2.97	7430	54.32	5.52	150	39.54	0.44	540
15	91.24	15.89	690	90.17	2.58	7580	43.62	4.67	120	42.63	0.59	710
16	109.56	22.17	860	128.09	3.38	10320	47.54	4.36	130	49.06	0.82	930
17	85.14	16.82	620	100.85	2.96	8670	42.13	4.18	120	36.53	0.34	550
18	95.47	19.53	720	94.56	2.66	7920	50.02	6.78	160	47.29	0.74	860
19	82.32	12.26	610	114.30	3.21	9780	53.23	6.86	190	37.80	0.48	540
20	112.69	28.43	930	91.29	2.61	7290	44.19	5.32	180	45.24	0.69	870
21	108.32	23.21	870	107.66	3.14	9370	59.3	4.71	210	44.23	0.60	810
22	97.78	14.27	760	109.31	3.15	9620	38.42	5.33	070	33.12	0.32	360
23	103.11	20.49	840	104.54	3.07	9080	43.14	6.26	100	48.44	0.88	920
24	100.04	18.52	800	99.11	2.91	8170	55.09	5.38	170	34.20	0.34	390
25	116.59	29.11	960	97.05	2.77	8050	33.50	4.23	070	41.52	0.52	690
Mean (Standard Deviation)	**96.30 (+14.43)**	**18.49 (+2.06)**	**754.4 (+131.82)**	**754.4 (+131.82)**	**2.92 (+0.36)**	**8443.6 (+1353.3)**	**49.26 (+14.28)**	**5.17 (+0.91)**	**154.8 (+50.99)**	**41.01 (+9.78)**	**0.61 (+0.21)**	**714.8 (+232)**

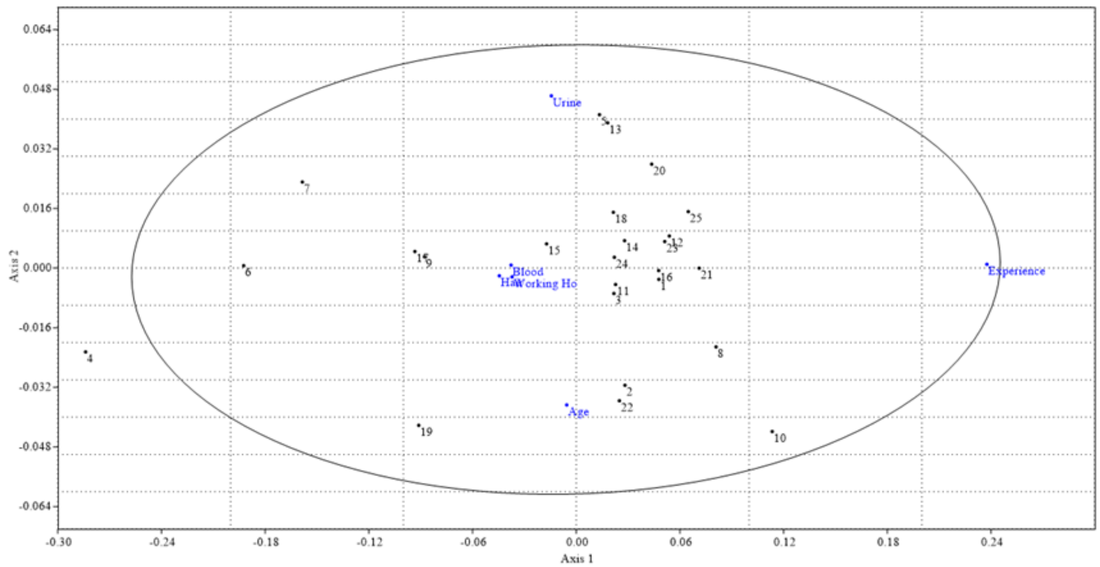


Fig. 3: Correspondence analysis ordination diagram projecting variables on the factor-planes (axis 1x2) for manganese concentration

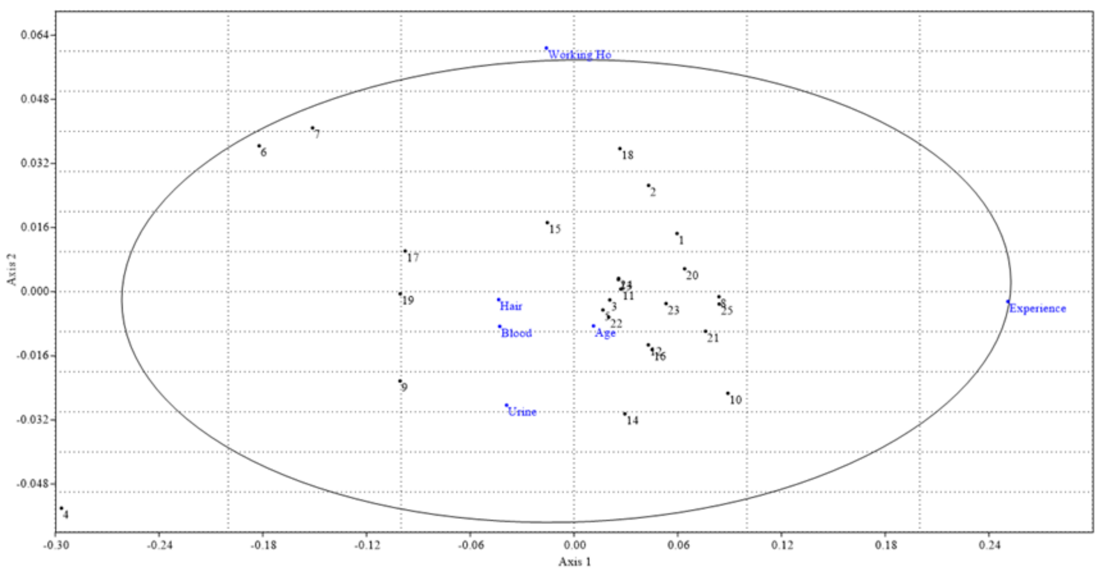


Fig. 4: Correspondence analysis ordination diagram projecting variables on the factor-planes (axis 1x2) for manganese concentration

working hours on bioaccumulation of manganese in blood and hairs whereas experience did not show any significant correlation. Nevertheless, age and urine are observed to have mild effects on bioaccumulation potential of manganese. On the other hand, for chromium, age factor appeared to be strongly influencing the bioaccumulation whereas working hours and experience had least significant effects (Figure 1 & 2).

Discussion

All of welders had higher quantities of manganese present in the blood, urine and hair than threshold limit given by WHO standards (i.e., 20-80 ppb blood, 1-8 ppb urine and 100 - 1000 ppb hair respectively except for subject number 4 in which the manganese level was found to be 43.25 ppb in blood, 9.30 ppb in urine and 320 ppb in hair respectively. This might be due to the reason that worker had an experience of six months in the profession of welding and his working hours were also less (4 hours) as compared to others and hence less exposure to the fumes of welding. While, in subject number 25, the quantity of manganese in blood, urine and hair was maximum i.e. 116.59 ppb, 29.11 ppb and 960 ppb, respectively, as he had an experience of 25 years. Though subject number 10 was of 50 years and had 32 years of experience, the quantity of manganese in blood, urine and hair were comparatively lesser (84.48 ppb, 16.69 ppb and 630 ppb respectively) as compared to subject number 25,who was the owner and was currently less exposed to the fumes of manganese. From this study it was found that the quantity of manganese increased especially when

Table 3: Correlation coefficient (r) between age, experience (years) and manganese/chromium levels in welders

Subject	Samples	Correlation Coefficient (r) between age and quantities	Correlation Coefficient (r) between experience and quantities
Welders	Blood	0.41	0.57
	Urine	0.30	0.53
	Hair	0.38	0.57

welding operations were performed in poorly ventilated areas as in this case. Whereas the manganese present in the blood, urine and hair of non-welders (control group), was well within permissible limits because they had no exposure to welding. However the quantities of manganese in blood, urine and hair of non-welders might be due to the effects of environment, especially drinking water (Table 2).

All of welders had higher quantities of chromium in the blood, urine and hair as compared to the standards (20-50 ppb blood, 0.24 -1.8 ppb urine and 300 ppb hair respectively). In subject 2, the value for chromium in blood, urine and hair was minimum, i.e. 78.15ppb, 2.1 ppb, and 5740 ppb, respectively. While, in subject 10, the value for chromium in blood, urine and hair was maximum i.e. 144 ppb, 3.56 ppb and 11540 ppb, respectively. These higher quantities were due to very significant concentrations of total soluble chromium mainly hexavalent chromium in welding fumes. This shows that chromium accumulates in the blood, urine and hair of welders when exposed to chromium fumes. Whereas the chromium present in the blood, urine and hair of non-welders (Table 2) was well within permissible limits, because the subjects had no exposure to welding. It is presumed that the levels of chromium in blood, urine and hair may be due to the effects of environment.

Figure 1 and Figure 2 depicts the comparison of manganese concentration in welder and non-welder population, respectively. The difference can visibly be seen among the levels of the two heavy metals between both the groups. Both figures show elevated values for the manganese and chromium for welders while these levels drop down for the non-welders ranging with the acceptable limits.

From further tests, it was found that in the blood samples the value of (r) between age, experience and manganese levels were 0.41, 0.57. In the samples of urine, the quantities were 0.30, 0.53, and in the samples of hair the value of (r) were 0.38 and 0.57, respectively. Similarly the correlation coefficient (r) between age, experience, and chromium levels in welders it was found that in the blood samples the value of (r) between age, experience and chromium levels were 0.49, 0.37. In the samples of urine, the values were 0.42, 0.23, and in the samples of hair the value of (r) were 0.23 and 0.43 respectively. This positive value of correlation coefficient in age and experience represents that high manganese and chromium levels were interrelated i.e. advancement in age and experience shows higher accumulation in the bodies of the welders. (Table 3).

Lastly, the correspondence analysis elucidates that, for manganese, working hours and age plays vital role while, whereas for chromium, only age is important in the accumulation of both the heavy metals in the subject over time and exposure. The results are interpreted considering certain conditions and therefore may have undermined the influence of other socioeconomic factors, i.e., monthly income, literacy rate, etc. Nevertheless, the study presents first report in the region and recommends further investigations leading to adoption of occupational measures.

Conclusions

The welding operations generate perilous fumes of manganese and soluble chromium (mainly hexavalent chromium) which enters the human body and accumulate in blood, urine and hair of welders; hence acts as bio-indicators of these heavy elements in the biological specimens of the body. Exposure to manganese and chromium fumes and dust particles during the welding of metals can pose various toxicological health effects. Exposure to high levels of manganese present in the air is more toxic to humans than when it is ingested by mouth, since inhaled manganese is directly transported to the brain by blood without it being metabolized in the liver, causing neurological dysfunction (Csuros, 2002) and exposure to chromium causes hemorrhagic diathesis and perforation of the nasal septum (Guertin et at., 2005). Analysis of the blood, urine and hair by atomic absorption spectrometer indicated that the manganese and chromium levels in the welders were elevated in blood up to 34% and 44% respectively, in urine 56% and 66% respectively, and hair 66% and 84% respectively than in the non-welders. The study also depicts a positive relationship between the ages and the experiences of the welders with regard to manganese and chromium.

Compliance with ethical standards

Conflict of Interest

The authors declare that they have no conflict of interests.

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