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ORIGINAL PAPER

Assessment of heavy metals and associated oxidative stress in occupationally exposed workers from Bannu and Karak Districts in Pakistan

Kaleem Khan · Muhammad Tariq Rafiq · Aziz-Ur-Rahim Bacha · Iqra Nabi · Muhammad Irshad · Faridullah Faridullah · Muhammad Younas · Muhammad Daud Khan · Rukhsanda Aziz · Muhammad Amin · Awais Arifeen · Sohaib Aslam · Shabir Ahmad · Akhtar Iqbal

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Abstract Heavy metals (HMs) are extensively found in occupationally exposed miners and industrial workers, which may cause serious health-related problems to the large workforce. In order to evaluate the impact of these toxic pollutants, we have investigated the effect of cadmium (Cd), chromium (Cr), copper (Cu), and lead (Pb) concentration on exposed workers of mining, and woolen textile mill and compared the findings with unexposed individuals. From each category like exposed workers (mining, and woolen mill textile site) and unexposed individuals, 50 blood samples were taken. The occurrence of HMs in a sample was investigated through atomic absorption spectrometry while the oxidative stress marker malondialdehyde (MDA) and antioxidant enzyme statuses such as superoxide dismutase (SOD) and catalase (CAT) were analyzed in exposed and control samples. The results showed significant ($p < 0.05$) variation in Cd, Cr, Cu, and Pb levels in exposed and control samples. The concentration of Cd in the blood of WMWs, KMWs, and control group was 5.75, 3.89, and 0.42 μg/dL, respectively. On the other hand, the concentration of Pb in the blood of WMWs, MWs, and control was 32.34, 24.39, and 0.39 μg/dL

K. Khan · R. Aziz · S. Ahmad
Department of Environmental Science, Faculty of Basic and Applied Sciences, International Islamic University, Islamabad 44000, Pakistan

M. T. Rafiq (✉)
Center for Interdisciplinary Research in Basic Sciences, International Islamic University, Islamabad 44000, Pakistan
e-mail: tariq.rafiq@iiu.edu.pk

A.-U. Bacha (✉) · M. Irshad · F. Faridullah · A. Arifeen · A. Iqbal
Department of Environmental Sciences, COMSATS University Islamabad, Abbottabad Campus, Abbottabad 22060, Pakistan
e-mail: urbaziz17@fudan.edu.cn

A.-U. Bacha · I. Nabi
Department of Environmental Science and Engineering, Fudan University, Shanghai 200433, People's Republic of China

M. Younas
State Environmental Protection Key Laboratory of Soil Health and Green Remediation, College of Resources and Environment, Huazhong Agricultural University, Wuhan 430070, People's Republic of China

M. D. Khan
Department of Biotechnology and Genetic Engineering, Kohat University of Science and Technology, Kohat 26000, Pakistan

M. Amin
Department of Environmental Sciences, Shaheed Benazir Bhutto University Sheringal, Dir 18000, Pakistan

S. Aslam
Department of Environmental Sciences, Forman Christian College (A Chartered University), Ferozepur Road, Lahore 54600, Pakistan

while the concentrations of Cr and Cu in the blood of WMWs, MWs, and control group were 11.61 and 104.14 µg/dL, 4.21 and 113.21 µg/dL, 0.32 and 65.53 µg/dL, respectively. An increase in MDA was recorded in the exposed workers' group as compared to control subjects, whereas SOD and CAT activities decreased. Meanwhile, MDA was significantly and positively ($p<0.01$) correlated with HMs, while negative significant correlations were found among HMs with SOD and CAT.

Keywords Heavy metals · Malondialdehyde · Superoxide dismutase · Catalase · Exposed miners and industrial workers

Introduction

All kinds of heavy metals (HMs) such as cadmium (Cd), chromium (Cr), lead (Pb), copper (Cu), and nickel (Ni) have gained much research attention in recent years due to their toxicity, persistent nature, and bio-accumulation (Chen et al., 2018; Li et al., 2013; Liu et al., 2017; Singh & Kumar, 2017). They are extensively used for making color pigments of textile dyes and may be released from various mining and industrial activities (Imtiazuddin et al., 2012; Singh & Chadha, 2016; Zeiner et al., 2012). Depending on the dose and persistence, HMs can bio-accumulate in the living body and become toxic (Hu et al., 2020; Xiao et al., 2021). Among routes of exposure to HMs, they can be inhaled as fume or dust and ingested through drinking water and consumption of contaminated food (Bermudez et al., 2011; Ji et al., 2013; Li et al., 2013; Maeaba et al., 2021). When a metal is absorbed, it is distributed in organs and tissues in the living body (Heo et al., 2017; Zheng et al., 2010). However, the quantity that is essentially absorbed from the digestive tract can vary, depending on the chemical form of the metals, nutritional status, body weight, duration of exposure, and age of the individual.

Mining and textile mill activities have posed several health-related issues to the large workforce. Exposure to toxic HMs has a harmful influence on the health status of occupationally exposed miners and textile mill workers (Malekirad et al., 2010; Nouioui et al., 2018). These metals and other chemical agents in the workplace cause several health-related problems and diseases owing to their adverse effects on the living system. Besides, they may cause injury to many tissues and cells including blood erythrocytes (Ruczaj & Brzóska, 2022). Abundant discharge of HMs from the mining and textile industry has become a big global issue in the last few years (Briffa et al., 2020), and studies present metals-induced toxicity in occupationally exposed workers (Wongsasuluk et al., 2021). The soil of Khyber Pakhtunkhwa has anomalous traces of Cu, Cr, and Cd (Ahmad et al., 2020; Malkani et al., 2017). The main sources of heavy metals are mining, industrial activities such as oil refineries, petrochemical plants, chemical industry, effluents, and the burning of fossil fuels (Munir et al., 2016). Meanwhile, HMs may cause the production of free radicals such as reactive oxygen radical species (ROS) inside the body which leads to oxidative stress (Fu & Xi, 2020; Omidifar et al., 2021).

Oxidative stress has been measured as one of the major indicators behind HMs toxicity. ROS are free radicals that are produced constantly through the way of normal oxidative metabolism and generated by many xenobiotic substances including HMs (Sun et al., 2022). However, ROS are not only produced mostly during normal physiological processes but produced as a result of external (heavy metals and other contaminants accumulated in the living body) and xenobiotic factors, including occupational exposure and metal pollution in the work environment (Asano et al., 2012). HMs have the potential to produce highly reactive chemical entities such as free radicals and lead to lipid peroxidation (Sharma et al., 2019). Malondialdehyde (MDA) is one of the best biomarkers of oxidative stress and the final product of lipid peroxidation. MDA is measured as an indicator in various biological samples including blood while increased levels of HMs lead to higher production of MDA (Doherty et al., 2010). Numerous studies have been conducted on the impact of HMs in exposed workers but to the best of our knowledge, this is the first on the impact of HMs concentration in exposed mining, and woolen mill textile workers, and compare with unexposed individuals of these two sites in Pakistan.

Experimental section

Sampling collection

Venous blood samples were collected from 50 exposed KMWs and WMWs. A total of 6 mL blood was collected in separate ethylene diamine tetra acetic acid (EDTA) tubes from each exposed individual and control subject. This study was based on occupationally exposed workers working at mining ($n=25$) and industrial textile mills ($n=25$) who were exposed to HMs (Fig. 1). Subjects were selected using random sampling methods from working sites such as people working at Bannu mining sites or Karak woolen industry. Both sites were selected as experimental sites due to anticipated occupational exposure of workers to HMs pollution during the industrial and mining process. The control subjects were selected from workers in offices distant from the industrial and mining areas. We noticed at that time, there were no dust and other pollutants found in the control area, and the control subjects were not directly exposed to any types of pollution as compared to exposed individuals. The location of control individuals was within the distance of 1 km from the sampling sites of exposed individuals (The control subjects were not exposed to dust, etc., on daily basis, because they were professional individuals), while they were within the distance of 5 min.

Blood sample analysis

In order to analyze the HMs, the blood digestion of collected samples was carried out according to the reported study of Memon et al. (2007). Blood samples (0.5 mL) were taken in 5 mL beakers added with 3 mL of nitric acid (15.7 M) and hydrogen peroxide (9.8 M) mixture (Lab Alley and Bob's Best) (Memon

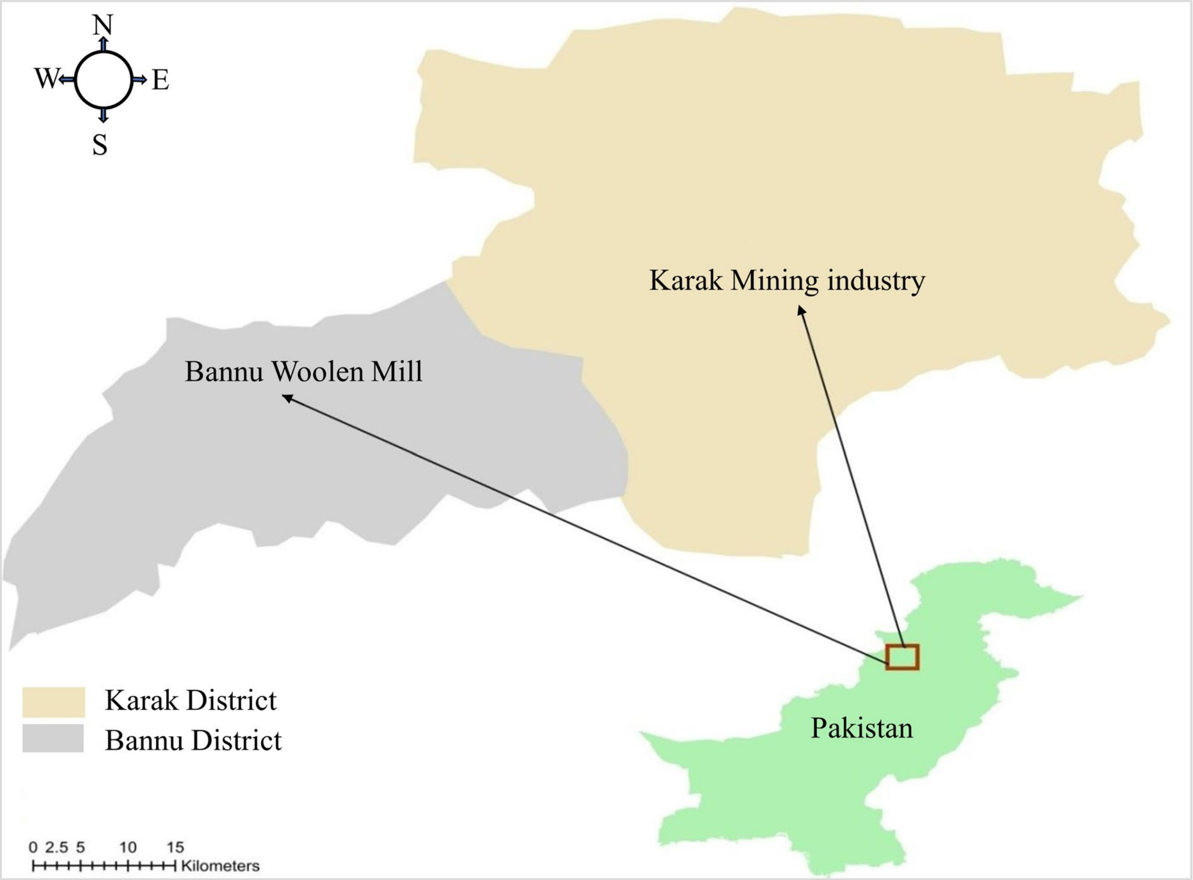

Fig. 1 Study map of mining and industrial textile mill in Pakistan

et al., 2007). The solution was kept for 10 min to gain equilibrium Thereafter, the samples were digested at 60 °C following the addition of 2 mL HNO_3 and H_2O_2. Solution was heated until it changed its color and the obtained solution was stored in plastic bottles. The concentrations of HMs (Cd, Cr, Cu, and Pb) were analyzed by using an atomic absorption spectrometer AAS (PerkinElmer A Analyst 700, USA).

Isolation of erythrocytes from blood samples

For biochemical analysis, red blood erythrocytes were separated from the plasma in test tubes using a centrifuge spanned for 40 min at 4000 rpm at 4 °C and the plasma was washed and removed. After separation, erythrocytes were washed with a phosphate buffer solution of 0.2 mol/L, pH 7.5, and centrifuged further for 40 min. Finally, the erythrocytes were packed in Falcon tubes containing (phosphate buffer pH 7.4; 0.1 M) and stored at − 20 °C. The determination of antioxidant enzyme activities and MDA levels was performed within 1 week.

Determination of malondialdehyde (MDA) level in blood erythrocytes

MDA was determined using a modified method of Stocks and Dormandy (1971). 2.0 mL of reaction mixture was prepared to comprise 35 mg of trichloroacetic acid (Splendora) and 0.08 mg of thiobarbituric acid (Sigma-Aldrich). The reaction mixture was added to 2 mL of extracted erythrocytes and incubated for 20 min in the water bath at 95 °C. After cooling, reaction mixture was centrifuged for 20 min at 4000 rpm and absorbance was measured by spectrophotometer (Shimadzu) at 532 nm.

Determination of superoxide dismutase (SOD) activity in blood erythrocytes

SOD activity was determined using the modified method of Beauchamp and Fridovich (1971). For substrate preparation, NBT 15.5 mg (RPI), riboflavin 0.2 mg (nutricost), Na EDTA 100 mg (RPI), and methionine 485 mg (Wego chemicals) were mixed in a reagent bottle while distilled water was added to make the final volume of 250 mL. The absorbance was measured on a spectrometer (Shimadzu) and read at 560 nm.

Determination of catalase (CAT) activity in the blood erythrocytes

CAT activity was analyzed according to Sinha (1972). Mixture contained 0.8 mL of phosphate (Finuchem) and 0.5 mL of H_2O_2. This was added to erythrocytes extract and the reaction process was stopped after a few seconds by the addition of 2 mL of dichromate acetic acid (J K Enterprises Chemical). The sample was kept in a water bath boiled for 20 min at 95 °C and cooled properly until color changed. The absorbance was measured at 530 nm by AAS (PerkinElmer A Analyst 700, USA).

Hematological and biochemical analysis of blood samples

RBCs count, hemoglobin (Hb) concentration, mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), and mean corpuscular volume (MCV), alanine aminotransferase (ALT), alkaline phosphatase (ALP), albumin (Alb), phosphorus (P), uric acid (mg/dL), blood urea nitrogen (BUN), and iron (Fe) were determined in the miners and control subjects' samples using automatic hematological assay analyzer (Medonic hematology assay analyzer, USA). Range of variations of thresholds for biochemical parameters are as follows: MCHC: 32–36 (g/dL), MCH: 27–31 picograms/cell, MCV: 80–100 femtoliter, ALT: 7–55 U/L, ALP: 40–129 U/L, RBCs: 4.3–5.9 million/mm^3, Hb: 13.5–17.5 g/dL. All laboratory tests were performed in a standard and approved medical laboratory (Anwar clinical laboratory).

Statistical analysis

Data were analyzed using graph pad prism version (5.01) software. Analysis of variance ANOVA followed by least significant difference (LSD) was performed at 0.05 significance level. Data significance level ($p < 0.05$) analysis of exposed and control groups was carried out through ANOVA. Microsoft excel office (2007) software was used for the statistical calculation of data expressed as the mean ± standard deviation (SD). Pearson's correlations were performed between HMs and oxidative and antioxidant parameters.

Results and discussion

HMs concentration in blood samples

Statistically significant ($p < 0.05$) HMs concentrations in blood samples of exposed and controlled groups are shown in Table 1. The HMs levels exceeded the permissible limits in both exposed workers and unexposed groups. The mean Cd (3.89 µg/dL) and Pb (24.39 µg/dL) were increased in Karak mining workers (KMWs) as compared to their control subjects (Cd: 0.42 µg/dL, Pb: 0.39 µg/dL). Moreover, the mean Cr was higher (4.21 µg/dL) in KMWs as compared to control group (0.32 µg/dL) which agreed with the report of Huang et al., (2011). Similarly, elevated levels of Cu were recorded in exposed KMWs (113.21 µg/dL) while their levels were decreased in control subjects (65.53 µg/dL). The most probable source of Cu at the workplace is Cu ore extraction during mining and improper disposal of Cu-based material. The difference in the HMs level of exposed and control groups was significant ($p < 0.05$).

Increased levels of these metals were observed in the exposed group of textiles WMWs as compared to that of control group. Mean Cd was almost 18-fold higher in WMWs (5.75 ± 0.11 µg/dL) than in the control group (0.32 ± 0.8 µg/dL). Conversely, mean Cd was greater than in steel industry workers reported by a previous study (Gil et al., 2011). Moreover, the probable explanation for the high Cd concentration is its emission from automobiles, mining, and the tear of automobiles (Hamzeh et al., 2011). Meanwhile, the mean concentration of Cr was approximately 10 times higher in textile WMWs (11.61 ± 0.24 µg/dL) than that in the control group (1.12 ± 0.3 µg/dL). However, our findings showed a low Cr concentration in occupationally exposed welders (WMWs) which are in good agreement with the literature (Danadevi et al., 2004). High Cr concentration may be detected after its discharge at the working site due to the production of surgical instruments in Sialkot, Pakistan. Various activities take place in the production of surgical instruments which leads to chromium (Cr) pollution in the work environment (Sughis et al., 2012) while exposure may lead to several health-related problems (Hessel et al., 2021). Furthermore, the Cu concentration was more than twofold higher in the textile WMWs' group (104.14 ± 1.8 µg/dL) than in the control subjects (45.83 ± 0.18 µg/dL). Nevertheless, the mean Cu remained below the permissible limit (150 µg/dL). The Pb concentration was also about 100 times higher in the WMWs (32.45 ± 0.12 µg/dL) group than in the control group (0.32 ± 0.4 µg/dL) and more than threefold higher than the permissible limit (Table 1). Previous studies reported that elevated blood Pb higher than 10 µg/dL led to neurological disorders and hypertension (Jiménez-Rodríguez et al., 2009).

Oxidative stress marker (MDA) in the blood samples

The result showed an increase in MDA levels and a significant decrease in enzymatic activities of SOD and CAT. Oxidative stress occurred due to the high production of free radicals and reactive oxygen species or insufficient accessibility of antioxidant enzymes and our findings are similar to that of Juan et al., (2021). MDA was the final product of lipid peroxidation due to which the oxidative stress level of MDA increased which lead toward damaging of cellular membrane (Bergsma et al., 2022). The results showed increased MDA levels in both tested workers' groups. The MDA mean content was 1.12 times higher in KMWs (24.43 µmol/L) and 2.27-fold higher

Table 1 HMs concentration (µg/dL) in blood samples of exposed Karak mining workers (KMWs) and textile woolen mill workers (WMWs) with their control group

Metals	Concentration (µg/dL)					
	KMWs	Control	WMWs	Control	$p < 0.05$	Permissible limit (µg/dL)
Cd	3.89 ± 0.46	0.42 ± 0.4	5.75 ± 0.11	0.32 ± 0.8	0.05	0.03–0.12
Cr	4.21 ± 0.36	0.32 ± 0.6	11.61 ± 0.24	1.12 ± 0.3	0.01	0.01–0.016
Cu	113.21 ± 15.99	65.53 ± 0.85	104.14 ± 1.8	45.83 ± 0.18	0.005	150
Pb	24.39 ± 0.68	0.39 ± 0.4	32.45 ± 0.12	0.32 ± 0.4	0.01	0–10

Values are mean ± SD; statistically significant level: $p < 0.05$ on ANOVA

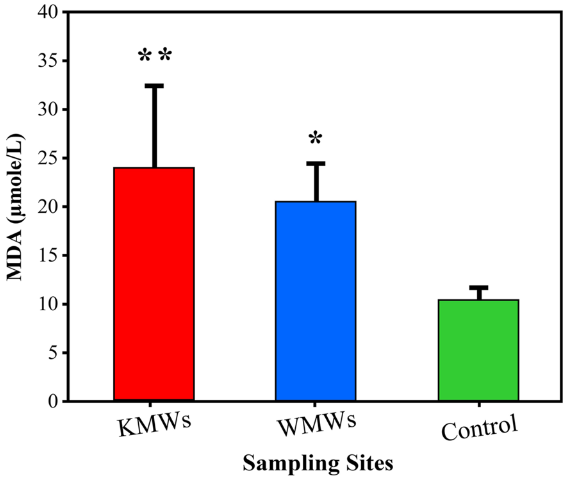

Fig. 2 Effects of HMs on MDA levels (μmol/L) in Karak mining workers (KMWs; $n = 25$) and wool mill workers (WMWs) as compared to the control group (mean ± SD). The asterisks ** represent highly significant and * present significant

in WMWs (21.64 μmol/L) as compared to that of the control group (10.8 μmole/L) (Fig. 2). The previous study by Ahamed et al. (2006) also reported similar results for MDA contents in occupationally exposed workers (Ahamed et al., 2006). The presence of high heavy metal levels in the blood leads to increased oxidative stress, intensive lipid peroxidation, and high MDA level as a final product of cellular membrane damage (Manivasagam et al., 2020). It has been shown that HMs increase blood MDA levels in exposed workers which then leads to oxidative stress (Fig. 2).

Status of antioxidant enzymes (SOD and CAT) in blood samples

SOD and CAT are primary antioxidant enzyme that works as scavengers and defense system against the effect of toxic and xenobiotic substances which protect living cells from injury. Generally, they react with superoxide radicals leading to their conversion into H_2O_2 which ultimately produces water molecules by catalase. SOD and CAT have been found in various tissues to protect cells and tissues from injury (Yuan et al., 2023). In this work, a significant variation in SOD and CAT activity was noticed in exposed groups as compared to controls. However, SOD showed a reduction in enzymatic activity in exposed mining workers (3.22 U/ml) and textile mill workers (3.75 U/ml), respectively (Fig. 3a). Similarly, lower CAT activity was observed in KMWs (3.42 U/ml) and WMWs (2.68 U/ml) as compared to control (8.57 U/ml) (Fig. 3b). The presence of high levels of heavy metals in the blood is a meaningful cause of ROS generation leading to excessive demand for antioxidant enzyme activities. Long-term exposure of both workers' groups to pollutants has most probably led to a collapse of enzyme synthesis and then depletion of their activities (Fig. 3b).

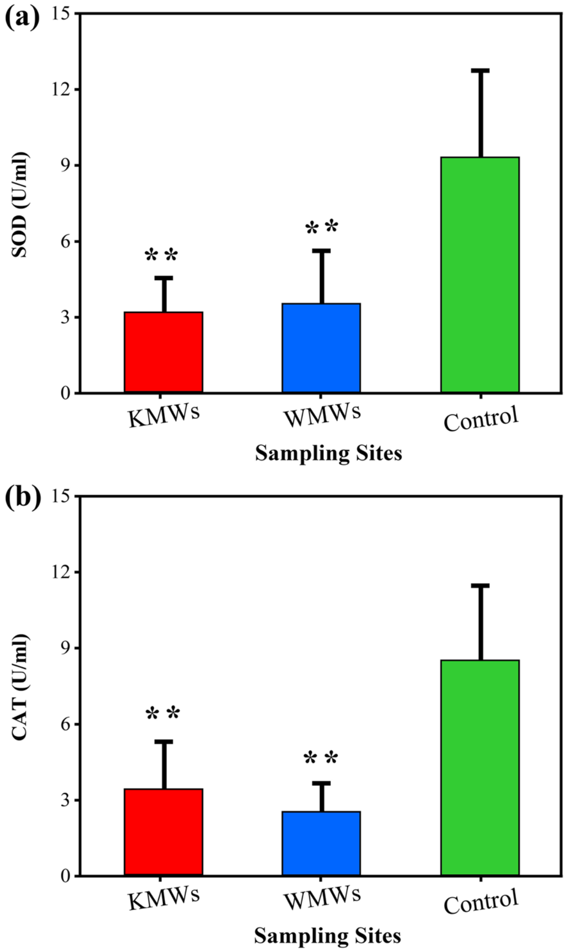

Fig. 3 Effects of heavy metals (HMs) on **a** SOD (U/mL), **b** CAT (U/mL) activity in exposed group of Karak mining workers (KMWs), wool mill workers (WMWs), and control group. Data are mean ± SD while one-way ANOVA was performed for the different statistical significances

Pearson's correlation of HMs with MDA, SOD, and CAT

Pearson correlation was carried out to analyze the response of MDA and enzymatic activities of SOD and CAT. Generally, Pearson's correlation was carried out to know about the positive, negative, and linear relationship between the variables. Pearson's correlations between MDA and Cd, Cr, Cu, and Pb were significantly ($p < 0.01$) positive, while negative and significant correlations were observed between SOD and CAT activities and HMs in blood samples of WMWs (Table 2) and KMWs (Table 3). Hence, these findings are in line with the report of Hormozi et al., (2018).

Hematological and biochemical analysis of blood samples

The results of the hematological parameters in the workers and control group are shown in Table 4. The results showed that mean values of red blood cells (RBCs) $p = 0.001$ and hemoglobin (Hb) $p = 0.05$ were higher in the exposed group as compared to the control samples. Other parameters such as mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH) were significantly lower in workers while their values were found higher in that of control $p = 0.001$. Similarly, insignificant decreases $p = 0.029$ were observed in mean corpuscular volume (MCV) in the worker's group as compared to that of the control.

Furthermore, ALT and ALP and Alb values were significantly ($p < 0.05$; $p < 0.001$; $p < 0.001$) increased in the exposed workers than in control group. The difference between groups in uric acid was weakly ($p = 0.031$) significant. The P and Fe concentrations were revealed to be not significantly ($p = 0.095$ and 0.103, respectively) different in KMWs (Table 5).

Conclusion

This study revealed that mining activities and textile industries may increase the risk of exposure to toxic HMs in the workplace. Furthermore, it showed that levels of selected HMs (Cd, Cr, Cu,

Table 2 Pearson's correlation among HMs, MDA, SOD, and CAT in the blood of wool mill workers (WMWs; $n = 25$)

Parameters	Cd	Cr	Cu	Pb	MDA	SOD	CAT
Cd	1.00						
	15.00						
Cr	0.119	1.00					
	0.00						
	15.00	15.00					
Cu	0.135	0.93	1.00				
	0.00	0.00					
	15.00	15.00	15.00				
Pb	0.178	0.78	0.72	1.00			
	0.00	0.00	0.00				
	15.00	15.00	15.00	15.00			
MDA	0.95**	0.99**	0.96**	0.78**	1.00		
	0.00	0.00	0.00	0.00			
	15.00	15.00	15.00	15.00	15.00		
SOD	−0.84**	−0.75**	−0.85**	−0.83**	−0.91**	1.00	
	0.00	0.00	0.00	0.00	0.00	0.00	
	15.00	15.00	15.00	15.00	15.00	15.00	
CAT	−0.91**	−0.74**	−0.68**	−0.81**	−0.96**	0.89	1.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	15.00	15.00	15.00	15.00	15.00	15.00	15.00

**Pearson's correlation is significant at the 0.01 level (2-tailed)

Table 3 Pearson's correlation among HMs, MDA, SOD, and CAT in the blood of Karak mining workers (KMWs; $n = 25$)

Parameters	Cu	Cd	Cu	Pb	MDA	SOD	CAT
Cr	1.00						
	15.00						
Cr	0.193	1.00					
	0.00						
	15.00	15.00					
Cu	0.114	0.93	1.00				
	0.00	0.00					
	15.00	15.00	15.00				
Pb	0.127	0.78	0.72	1.00			
	0.00	0.00	0.00				
	15.00	15.00	15.00	15.00			
MDA	0.98**	0.97**	0.91**	0.94**	1.00		
	0.00	0.00	0.00	0.00			
	15.00	15.00	15.00	15.00	15.00		
SOD	−0.82**	−0.87**	−0.83**	−0.79**	−0.91**	1.00	
	0.00	0.00	0.00	0.00	0.00	0.00	
	15.00	15.00	15.00	15.00	15.00	15.00	
CAT	−0.93**	−0.77**	−0.68**	−0.85**	−0.96**	0.83	1.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	15.00	15.00	15.00	15.00	15.00	15.00	15.00

**Pearson's correlation is significant at the 0.01 level (2-tailed)

Table 4 Hematological parameters in workers group and control subjects

Parameters	KMWs	Control group	$p <$ value	RR
RBCs (mil/UL)	8.70 ± 13.19	4.94 ± 2.59	0.001	
HB (g/dL)	19.75 ± 1.31	13.72 ± 1.42	0.05	
MCHC (g/dL)	34.58 ± 3.89	41.22 ± 2.92	0.001	
MCH (pg)	31.96 ± 4.04	31.83 ± 5.68	0.004	
MCV (fL)	78.3 ± 6.60	82.41 ± 7.68	0.029	

± Standard deviation, $n = 50$, RR = Reference range

and Pb) were increased in occupationally exposed workers from both study sites as compared to the control individuals. Our findings revealed that these metals have an adverse impact on blood erythrocytes and MDA levels. It also disturbs the enzymatic status in occupationally exposed workers who are at high risk of HMs at working sites. So, the lack of basic knowledge and safety precaution is a cause of metal exposure which ultimately effect workers' health. Furthermore, the poor health status of workers has a great impact on their families as well as on the labor force. To avoid the

Table 5 Biochemical parameters in workers group and control

Parameters	KMWs	Control group	$p <$ value	RR
ALT (U/L)	43.38 ± 23.57	31.89 ± 11.32	0.05	4–36
ALP (U/L)	189.11 ± 41.26	167.42 ± 47.51	0.001	44–147
Alb (g/dL)	6.90 ± 0.56	3.94 ± 0.32	0.001	3.4–5.4
P (mg/dL)	3.93 ± 0.67	3.61 ± 0.15	0.095	2.8–4.5
Uric acid (mg/dL)	4.79 ± 0.89	5.15 ± 1.16	0.031	3.5–7.2
Bun (mg/dL)	36.29 ± 6.196	26.33 ± 6.64	0.05	6–24
Fe (µg/dL)	83.56 ± 27.45	76.44 ± 23.29	0.103	60–170

± Standard deviation, $n = 50$, RR = reference range negative effect of these chemicals on the health status of workers, they must need to take safety precautions and their health status and activities should be checked on a daily basis.

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Authors contribution KK was involved in formal analysis and writing—original draft. MTR helped in conceptualization, data curation, and writing—original draft preparation. A-U-RB contributed to data curation, writing (original draft preparation, reviewing, and editing). IN was involved in writing—reviewing and editing. MI helped in review and revision of manuscript suggested by reviewers. FF contributed to review and revision of manuscript suggested by reviewers. MY was involved in writing—reviewing and editing. MDK helped in writing—reviewing and editing. RA contributed to writing—reviewing and editing. MA helped in review and revision of the manuscript. AA helped in in review and revision of the manuscript. SA helped in review and revision of the manuscript. AI helped in review and revision of the manuscript. All authors read and approved the final manuscript.

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Declarations

Conflict of interest The authors declare no competing financial interest.

Ethics approval and consent to participate The study was approved by the ethics committee of the International Islamic University Islamabad (IIUI). Consent was obtained from all the individual participants included in the study.

Consent for publication All the authors mentioned in the manuscript agreed to publish the work, read and approved the manuscript, and gave consent for submission and subsequent publication of the manuscript.

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