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Interrelation of Zinc and Cadmium in the Biological Samples of Indoor and Outdoor Workers of Five Zonal Areas of Coal Mining Field

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Summary: Coal mining is still one of the most tough, nasty, and dangerous jobs in the world, with more fatalities than any other. The present study assessed zinc (Zn) and cadmium (Cd) in scalp hair and blood samples of 270 coal mining field employees, working in Hyderabad, Pakistan, aged 18-55 years. The scalp hair and blood samples of age-matched healthy volunteers (n= 70) were examined for chosen elements for a comparison analysis. For understudied metals, environmental samples (soil, water, and coal) from coal mining fields and nonindustrial areas were investigated. The Zn levels in the scalp hair and blood samples of coal mining male exposed workers were found to be lower, in the range of {88.9- 118; 100- 133 µg/g} and {3.32- 4.41; 3.33- 6.04 mg/l)}, respectively than the referent subjects {224-237; 209-221} µg/g and {6.82-7.82; 6.60-7.42 mg/l}, respectively. Whilst, the Cd concentrations in the scalp hair and blood samples different five zones of indoor and out-door coal mining field exposed workers were found to be higher, in the range of {10.1- 16.9; 9.65- 16.9} and {8.89- 12.65; 8.96- 11.7} µg/l, respectively than the biological samples of two age groups (18-35) and (36- 55) years referent subjects {3.45-4.45; 4.02-5.32} µg/g and {3.25-3.72; 3.82-4.10 µg/l}, respectively. Serum, hemoglobin, hematocrit, red blood count, and creatinine clearance counts were significantly lower in mining filed workers than in referents (p0.001), whereas erythrocyte sedimentation rate and N-acetyl- beta-glucosaminidase levels were significantly higher in workers than in referents (p0.001).The Zn concentration in water and soil samples of mining field were found to be within the WHO recommended values, whilst Zn concentration in coal samples of mining field was in between 15.5- 20.6 µg/g. Whilst, Cd levels in water and soil samples of mining field were found above the WHO recommended values, whilst Cd content in coal samples of mining field was in between 0.52- 0.75 µg/g. The findings indicate that immediate action is required to improve ventilation and hygiene measures within factories, mines, and other work environments.

Keywords: Lakhra Coal Mining, Zinc, Cadmium, Scalp Hair, Blood, Water, Soil, Coal, atomic absorption spectrophotometry.

Introduction

Coal industry has always been center of attention for the researchers [1]. Coal is also known to have to spread these elements into air when the coal is burnt, and it also produces particulates that have serious health impacts [2]. Coal mining, is known to have dangerous effects on the eco-system such as the damage the bio-diversity, formation of sinkholes, pollution of natural (ground and under-ground) water sources with contaminants from the mine and most importantly our air [3].

Zinc (Zn) is the essential most trace metal since is major ingredient of safe and strong immune system as well as safer biological membranes with that is also main requirement for numerous enzymes [4]. It has also its involvement in various stages of cellular metabolism [5] and is a critical constituent of some very imperative enzymatic systems that are Zn dependent including CO_2 elimination for blood, radox reactions progression and birthing of various proteins etc [6].

Workers in industries that utilize materials are well aware of the dangers of exposure to toxic elements. These compounds bind to bio-active sites in the human body system and modify the metabolism of numerous biological activities if the employee is subjected to higher levels than the daily permissible limit. As a result of their exposure to these components, employees are subjected to chemicals, harmful fumes, and dust, which have both short- and long-term consequences [7]. Toxic elements can also disrupt with essential and trace metals' proper activity in enzymatic activities. Toxic metals may be causing enzymes to become inactive by replacing necessary elements. Increased amounts of micronutrients, on the other hand, may aid in the removal of harmful chemicals from our biological systems [8]. Melanosomes bind metal ions proportionally to their volume and atomic weight. To supersede freshly bonded metals, toxic elements can successfully participate for the same limiting destinations as outside particles [8]. These bound heavy metals, on the other hand, are difficult to remove [9].

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Cadmium (Cd) is a recognised toxic metal that travels through the bloodstream and accumulates in the kidneys and liver when ingested by humans [10]. Toxic elements ingestion may cause pulmonary tuberculosis, atherosclerosis, and myocardial infarctions by increasing oxidative stress due to Zn deficiency — an anti-oxidant element [11, 12]. The trace elements found in coal, particularly heavy metals, comprise a vast collection of varied contaminants [13]. Their high levels of exposure have a harmful impact on human health. Some have been linked to cancer, as well as damage to children's reproductive, normal growth, brain and immune systems, and respiratory irritants that can cause asthma [13]. Coal mining generates several hazardous contaminants into the air, water, and soil [14]. As a result, people living near coal mines or mine employees exposed to such an environment are at a significant risk of becoming victims of undiscovered mine toxicity [15]. Lead, mercury, nickel, cadmium, arsenic, manganese, beryllium, and chromium, among other lethal and carcinogenic elements found in coal, are causing a variety of detrimental health effects [16].

Tons of these harmful metals are released into the air and water each year as a result of mining activities. The latter can come from groundwater aquifers abundant in drinking water wells, as well as fish, wildlife, and other food sources. Coal's effects are equally widespread, and it has been linked to respiratory ailments, different types of cancers, birth deformities, heart disease, and other major health issues [17].

The goal/aim of this research was to determine the zinc and cadmium in biological samples collected from exposed outdoor and indoor employees (official and control subjects) in five different coal mining zones. Non-diseased (referents) nonindutrial age-matched subjects were also chosen for comparison purposes. We also compared the results of non-industrial drinking water and soil samples with those of coal mining fields' water, soil, and coal samples.

Experimental

Apparatus

Atomic Absorption Spectrophotometer AA. Analyst 700 was used for for the detection of Cd and Zn. Table-1 lists the instrument conditions. The equipment's absorbance signals were collected and computed before being used. For the digestion of the materials, a domestic microwave oven (PMO23) Pel approximately 900W heating power was used. For sample storage and processing, acid-washed polytetrafluoroethylene (PTFE) bottles were chosen. In parallel, elaborate standard and reagent blanks were examined, and necessary changes were performed.

Table-1. Measurement conditions of elements in electro atomic absorption spectrometer A. Analyst 700.

Parameters	Cadmium	zinc
Lamp current (mA)	6.0	7.5
Wave length (nm)	228.8	213.8
Slit width (nm)	0.7	0..7
Drying Temp (°C)/ramp hold (s)	140/15/5	Burner height (mm) 7.5 Oxidant (Air) 1min^{-1} 17.0 Fuel (Acetylene) 1min^{-1} 2.0
Ashing Temp (°C)/ramp hold (s)	850/10/20	
Atomization Temp (°C)/ramp hold (s)	1659/0/5.	
Cleaning Temp (°C)/ramp hold (s)	2600/1/3	
Chemical Modifier	5µg Pd as Pd(NO_3)	

Sample volume (10µl), Cuvette = Cup, Carrier gas = (200ml/min), Background correction (D2 Lamp)

Reagents and glassware

Millipore supplied the ultra-pure water required in the procedures (Mili-Q USA). E. Merck, a German company that manufactures analytical chemicals such hydrogen peroxide and nitric acid, has been used. All of the samples had been checked for contamination before being used. Fluka Kamica (Buchs, Switzerland) used fully certified 1000 ppm Cd and Zn stock solutions. Working standard (stock) solutions were serially diluted with (0.2 mol/L) HNO_3. The standard and diluted solutions were stored and processed in polyethylene bottles at 4-6 °C for further analysis Certified reference materials of human hair (BCR 397, Brussels, Belgium), human blood (Clinchek® Control lypholized, Germany Munich, Recipe), and Water SRM 1643e were purchased to achieve a sensitive technique. Plastics and apparatus were immersed in 2 mol/L HNO_3 for 24 hours before being cleaned and rinsed with Mili-Q'd water.

Study population

Adults (18 to 35 years) and seniors (36 to 55 years) comprised up the current study population. Males comprised up the entire population, and they were given a consent form as well as a separate form, to collect their physical health and dietary habits. For a comparative investigation, a referent group of adults and seniors of similar ages was examined in a non-modern zone that had no previous work-related exposure. LCDC (Lakhra coal development company), HCM (Hashim coal mine), ICM (Indus coal mine), BCM (Bismillah coal mine), and PMDC (Pakistan mine development company) are the five sub industrial zones examined in the coal mining sector. There are two main regions for coal mining in these sub-industrial zones; one is indoor mining while other is outdoor mining. The majority of workers worked normally 48 hours per week. Some employees worked additional shifts for extra money, putting in a total of 12 hours each day. Prior to their sampling day, the people in the sample had employed in the mining for 10 to 30 years. Prior to sample collection, all individuals of the population were thoroughly surveyed. Table 2 shows the demographic data used in the sampling. All coal miners had their biochemical data and physical examinations performed, as well as their blood pressure, height, and weight (Table 3). Each member of the group underwent a thorough medical examination with the assistance of on-site toxicologists and epidemiologists, as well as university employees and public health officials. Standard operating procedure was used to send 3 mL of blood samples from each coal miner to the hospital's pathological laboratories for biochemical tests (Table 3), which was ratified by Pointe Scientific Test (Laboratory Procedure Manual, 2015) [18] for haemoglobin, hematocrit, red blood count, ESR, Creatinine clearance, and urinary N - acetyl - beta -glucosaminidase tests.

Each mine worker who took part in the study signed a consent form stating that they were satisfied with the samples being taken and analysed. Each worker was given a complete questionnaire with information about their national origin, physical characteristics, duration of exposure, health condition, dietary habits, type of job, age, cigarette smoking habits, and length of service. The physical examination covered the individuals' BMI (with the help of height and weight), systolic and disystolic blood pressure, and other biochemical data. There were no statistically significant differences in height and weight amongst all coal miners (p0.05) (Table 3). All of the works in this study followed the ILO-1999 (International Labor Organization) rules [19]. Many mining companies employ adolescent boys underneath the age of 18 to just save money and avoid the infancy stages generated by adult labour; nevertheless, the Lakhra coal mine follows the ILO-199 guideline (as prescribed above). Another fact is that no personal protective equipment (PPE) was provided to the workers, such as safety shoes and eyewear. Everyone on the job, however, was provided a safety helmet.

Sample collection

Blood sample collection

With the use of a syringe, blood was extracted aseptically from each volunteer's veins. A registered male nurse used a 5ml sterile – one time use only – syringe to execute the blood drying procedure. The puncture site was cleansed with an alcohol swab that contained no chemicals and was sealed in plastic. This particular alcohol swab was selected solely to avoid infection and contamination. Blood samples were collected by the protocol, which is described in previous study [8].

Scalp hair sample collection

Hair samples weighing about 0.5g (0.5–2.0 cm long) were collected from each diseased and healthy subject using stainless steel scissors. Before and after cutting the hair of each member, the scissors were properly cleansed and sanitised with an alcohol swab. Scalp hair samples were collected by the protocol, which is described in previous study [8].

Environmental sample collection

Water samples were taken three times in 2019 (N=10 from each subdivision) from the storage tanks including all five sub-divisions at the coal mining site (N=50). Whereas, the MT water was also collected (N=100). All water samples were tested for pH before being filtered and refrigerated at 4 degrees Celsius for further analysis. Zn and Cd concentrations analysis were computed following previously established protocol [20, 21].

Table-2: The number of subjects as control and coal mining workers.

Mining Areas workers	Age groups	Referents	Workers				
			PMDC	LCDC	ICD	HCM	BCM
Indoor Workers	18-35	------	17	18	15	13	15
	36-55	------	13	09	14	10	12
Outdoor Workers	18-35	29	19	09	21	07	18
	36-55	28	15	08	17	05	15
Total		57	64	44	67	35	60

Table-3: Clinical and biochemical characteristics of male coal mining workers, working in five sub divisions of Lakhra coal mining Area

	Referents	PMDC	LCDC	ICD	HCM	BCM	Normal range
Indoor Workers							
Serum ferritin (mg/l)	________	27.6±3.34	24.6± 1.58	22.8± 1.12	26.3± 3.03	24.7± 2.11	≥30
Hemoglobin (g/dl)	________	12.1±0.55	10.8 ±0.32	12.5 ±0.58	11.3 ±0.34	10.8 ±0.49	11.5-16.5
Hematocrit (%)	________	36.8±2.81	33.4±2.37	31.2±1.85	35.0±1.97	33.0±1.89	35-55
Red blood count (M/mm^3)	________	3.26±0.38	3.21±0.36	2.99±0.27	3.17±0.26	3.16±0.13	3.5-5.5
Systolic blood pressure (mm Hg)	________	130 ± 4.09	135 ± 5.04	138 ± 7.52	147 ± 6.21	153 ± 6.52	80-120
Diastolic blood pressure (mm Hg)	________	89.1 ± 3.56	91.2 ± 2.56	90.9 ± 2.68	96.9 ± 3.59	93.2 ± 2.93	
ESR (mm/1hr)	________	34.2 ± 2.75	33.9± 3.92	33.8 ± 1.65	35.2 ± 2.39	32.4 ± 1.56	
Creatinine clearance (mL/min)	________	68.1± 4.03	57.9± 4.17	52.7± 4.21	53.9± 7.14	51.6± 4.51	
Urinary N -acetyl - beta -glucosaminidase (NAG) (IU/L)	________	24.6±0.73	25.4±1.33	26.1±1.30	24.2±1.56	25.3±0.33	
36-55							
Serum ferritin (mg/l	________	27.2±1.99	23.0±3.41	21.5± 1.91	23.6± 1.14	23.0± 0.95	≥30
Hemoglobin (g/dl)	________	10.4±0.42	9.2±0.35	11.0±0.21	10.3±0.31	9.64±0.35	11.5-16.5
Hematocrit (%)	________	36.8±3.47	36.0±2.06	33.2±2.80	35.7±2.05	36.50±1.32	35-55
Red blood count (M/mm^3)	________	3.15±0.22	3.26±0.12	3.17±0.37	3.02±0.18	3.03±0.08	3.5-5.5
Systolic blood pressure (mm Hg)	________	123 ± 6.74	119 ± 7.33	128 ± 3.60	131 ±6.36	135 ± 5.23	80-120
Diastolic blood pressure (mm Hg)	________	81.8 ±1.99	82.9 ±2.29	82.8 ±1.69	83.5 ±1.24	84.2 ±1.48	
ESR (mm/1hr)	________	34.5 ± 2.87	36.1 ± 3.05	34.9 ± 1.84	36.4 ± 2.28	34.1 ± 2.06	
Creatinine clearance (mL/min)	________	71.2± 8.36	59.2± 4.44	55.1± 6.99	55.3± 5.76	51.1± 5.37	
Urinary N -acetyl - beta -glucosaminidase (NAG) (IU/L)	________	27.7±0.99	25.7±2.89	28.6±2.04	27.8±1.90	27.0±0.72	
Out door							
18-35							
Serum ferritin (mg/l)	43.4±3.27	25.9±2.64	22.8± 0.67	22.0± 1.20	23.6± 1.97	23.8± 1.04	≥30
Hemoglobin (g/dl)	13.7±0.53	9.65±0.35	8.81±0.24	10.3±0.21	9.62±0.28	9.1±0.27	11.5-16.5
Hematocrit (%)	48.7±2.36	34.7±1.81	32.5±1.52	28.8±1.17	33.2±0.60	33.2±1.15	35-55
Red blood count (M/mm^3)	4.35±0.41	3.24±0.29	3.16±0.18	3.28±0.31	3.09±0.16	3.09±0.12	3.5-5.5
Systolic blood pressure (mm Hg)	115 ± 3.37	124 ± 3.71	118 ± 4.82	128 ± 7.66	129 ± 7.70	133 ± 4.67	80-120
Diastolic blood pressure (mm Hg)	77.0 ± 2.34	81.1 ±1.65	83.2 ±1.76	82.9 ±2.26	83.0 ±0.66	84.7 ±1.06	
ESR (mm/1hr)	8.57 ± 0.42	18.3 ± 1.10	15.8 ± 0.67	16.9 ± 0.85	16.9 ± 1.16	17.4 ± 1.97	
Creatinine clearance (mL/min)	86.1±6.82	42.0± 7.55	37.3± 4.55	38.0± 6.28	36.1± 5.28	38.7± 2.62	
Urinary N -acetyl - beta -glucosaminidase (NAG) (IU/L)	3.45±0.39	15.4±1.88	14.1±0.88	12.86±1.17	13.8±2.63	13.5±1.74	
36-55							
Serum ferritin (mg/l)	43.2±2.54	28.3±1.64	25.7±2.15	27.8±1.07	27.1±1.20	27.8±1.48	≥30
Hemoglobin (g/dl)	12.4±0.93	8.35±0.48	7.93±0.72	7.70±0.75	7.55±0.48	7.16±0.64	11.5-16.5
Hematocrit (%)	47.3±1.21	35.7±1.00	34.1±0.50	34.1±1.37	32.9±1.98	31.1±1.15	35-55
Red blood count (M/mm^3)	4.18±0.57	3.24±0.60	2.89±0.58	2.81±0.33	3.06±0.81	3.06±0.37	3.5-5.5
Systolic blood pressure (mm Hg)	122±8.95	137±7.46	128±6.89	139±7.92	136±7.72	138±8.97	80-120
Diastolic blood pressure (mm Hg)	84.7±4.30	93.2±3.32	95.9±4.14	92.7±5.33	93.8±3.30	91.9±5.72	
ESR (mm/1hr)	10.3 ± 1.11	23.3 ± 2.37	22.8 ± 2.51	24.0 ± 0.62	23.6 ± 1.62	21.2 ± 3.23	
Creatinine clearance (mL/min)	110± 11.0	34.1± 3.74	31.1± 6.55	33.8± 2.83	33.3± 4.88	32.6± 3.23	
Urinary N -acetyl - beta -glucosaminidase (NAG) (IU/L)	5.07±0.54	16.5±1.84	15.30±1.04	14.6±1.70	15.7±1.09	15.3±2.16	

Collection of Soil and Coal samples

Samples of coal and adjacent soil were collected from the five subdivisions of Thar coal mining field (n= 20), with varying depth range of 120-280 meter, by the help from Sindh coal authority. All collected samples of soil and coal were sealed in air-tight plastic bags to avoid any moisture and other contamination. At laboratory all the collected samples were dried for one week at room temperature. Later the samples were crushed to obtain grain size of <200 µm diameter with the help of motor and pestle. The crushed samples were stored in desiccator so that moisture contamination may be avoided. All ISO recommendations were followed for sample preparation. For comparison purpose, the soil samples of Hyderabad were also collected, and treated it the mentioned protocol [22].

Microwave-assisted Acid Digestion Method (MWDM)

With the use of MWDM, duplicates of each sample were made for elemental analysis. Six replication samples of the certificated reference materials (CRMs) (blood and human hair, water, and soil) were obtained. For each sample, 200 mg (~0.2 g) of hair, soil, coal, and 0.5 ml of human blood were combined with 1 ml of newly made H_2O_2 – HNO_3 mixture (1:2, v/v) in a PTFE flask. While 10 mL of the mixture flask was placed in a MW oven, it was microwaved for 3 minutes at 950MW power until the sample digestion was complete. The digested samples were allowed to cool to room temperature before being diluted with Mili-Q water to a final amount of 10ml. AAS was used to examine these samples for elements. For the preparation of blank samples, the same procedure was used. The CRMs of hair, blood, water and soil were employed to achieve a sensitive of method.

Data and Statistical Analyses

The statistical analysis of data was performed using Excel X state and Minitab software. The method's validity was shown to be the best in terms of certified element values, with 99.4 - 99.5 percent recovery (Table-4). The samples required less than 5 minutes to digest completely, and there was only a 1-2 percent variation in mean values of each element, with a relative standard deviation (RSD) of 2%.

We calculated the Z-score, U-score, and relative bias (RB) to assess the laboratory's performance. These parameters were designed according to these equations,

$$U_{\text{score}} = \frac{|X_{\text{Lab}} - X_{\text{Ref}}|}{\sqrt{\mu_{\text{Lab}}^2 + \sigma_{\text{Ref}}^2}}$$

$$Z_{\text{score}} = \frac{|X_{\text{Lab}} - X_{\text{Ref}}|}{\mu_{\text{Lab}}}$$

$$\text{Relative bias (RB)} = \frac{X_{\text{Lab}} - X_{\text{Ref}}}{X_{\text{Ref}}} \times 100$$

where XLab is laboratory results, µLab is standard deviation, XRef is recommended uncertainty, and σ Ref is standard uncertainty.

With the help of these formula, we evaluated the laboratory performance. In our results, U-score ≤ 1 and Z-score ≤ 2, then our method is satisfactory

Table-4: Determination of Cd and Zn in certified samples by microwave digestion method (N=6).

Elements	Certified values	MWD Mean ± SD	(%) Recovery	Paired t-test[a] $t_{\text{Experimental}}$	*U*-score	Z-score	Bias
	Certified sample of whole blood (µg /l)						
Cd	1.2±0.4	1.189± 0.10 (8.41)[b]	99.1	0.00636	0.02668	0.0275	0.9167
Zn*	2.27 ± 0.06	2.19±0.15 (6.61)	96.5	0.260	0.495	1.33	3.52
	Certified sample of human hair (µg/g)						
Cd	0.52±0.024	0.515±0.042 (8.15)	99.04	0.145	0.103	0.208	0.97
Zn	199 ± 5.0	197.8±7.29 (3.68)	99.4	0.678	0.136	0.240	0.603
	Water SRM 1643e (µg /l)						
Cd	6.568 ± 0.073	6.495± 0.52 (8.00)	98.9	0.418	0.137	1.00	1.11
Zn	78.5 ± 2.2	77.85 ± 6.01 (7.01)	99.2	0.389	0.101	0.295	0.828
	Soil amended with sewage sludge BCR 483 (µg/g)						
Cd	24.3 ± 1.3	23.95 ± 2.07 (8.64)	98.5	0.549	0.143	0.269	1.44
Zn	612.0 ± 19.0	606.5 ± 24.6 (4.06)	99.1	0.490	0.176	0.289	0.898

[a] Paired t-test between certified values vs. found values, degree of freedom (n−1)= 5.
t_{Critical} at 95% confidence limit= 2.57.
[b] Values in parenthesis RSD.
* mg/l

Table-5: Cadmium contents in water, soils of and coal samples of five sub zones of Coal mining area and non - exposed areas

Sampling areas		pH	Water (µg/L)	Soil ((µg/g)	Coal (µg/g)
		Zinc			
Recommended values [c]			**5.0***	**150–300**	-------
Non industrial area	**Municipal -treated water**	**6.9-7.1**	**0.56± 0.05***	**195± 10.6**	-------
Coal mining area	**PMDC**	**5.9- 6.3**	**0.24± 0.03***	**132± 8.55**	**16.6±0.63**
	LCDC	**5.8- 6.4**	**0.17± 0.03***	**128± 7.08**	**15.5± 1.09**
	ICD	**6.0- 6.3**	**0.21± 0.05***	**135± 9.02**	**18.3± 1.95**
	HCM	**6.1- 6.6**	**0.19± 0.03***	**135± 11.5**	**20.6± 2.53**
	BCM	**6.2- 6.8**	**0.18± 0.03***	**124± 6.99**	**19.0± 1.09**
		Cadmium			
Recommended values [c]			**3.0**	**1–3**	-------
Non industrial area	**Municipal -treated water**	**6.9-7.1**	**1.52±0.17**	**3.15± 0.45**	-------
Coal mining area	**PMDC**	**5.9- 6.3**	**18.2± 2.05**	**11.2± 0.98**	**0.62±0.12**
	LCDC	**5.8- 6.4**	**17.5± 1.62**	**10.8± 0.57**	**0.75±0.19**
	ICD	**6.0- 6.3**	**19.8± 0.70**	**10.6± 0.68**	**0.53±0.14**
	HCM	**6.1- 6.6**	**20.7± 1.05**	**10.3± 0.73**	**0.55±0.15**
	BCM	**6.2- 6.8**	**19.2± 0.89**	**10.5± 0.72**	**0.52±0.17**

Keywords: - Lakhra coal development company (LCDC), Hashim coal mine (HCM), Indus coal mine (ICM), Bismillah coal mine (BCM), and Pakistan mine development company (PMDC)
* mg/l
[c] WHO (2004).
WHO, 2004. Guideline for drinking water quality, third ed. In: Recommendation World Health Organization, Geneva.

Results & Discussion

Water, Soil and Coal Samples of Coal Mining Areas

The domestic treated water supplies of non - uncovered regions have pH between 6.9-7.1, however the drinking water samples tanks/ coalers of coal minning field have pH in the ranged of 5.8 -6.8. the Zn concentrations in drinking water tests accomplished from city treated water supply framework were found in the level of 0.53 -0.58 mg/L, which is less than permissible limit of WHO, (5.0 mg/L) [23], whilst Cd concentration was in between 1.47- 1.65 µg/L, which is than WHO level, 3.0 µg/L (Table-5). The water tankers of five different zones of coal minning areas had Zn levels in the range of 0.17- 0.24 mg/ L , which are twenty time lower than the WHO guideline for drinking water, whilst Cd concentrations were in between 17.5-20.7, which is 6 to 7 fold higher than WHO limits [24]. The Zn concentration in soil samples of coal mining area was in between 124 -135 µg/g , whilst Zn level in soil samples of non -exposed areas was found in the range of 190 -200 µg/g , which was lower than the obtained results from soil samples of coal mining area, whilst Cd concentration in non-exposed areas was found to be 2.90- 3.38 µg/g in soil samples of non exposed areas, where as the soil of coal mining areas had Cd concentration in the range of 10.3 – 11.2 µg/g (Table-5). The concentration of Cd in coal samples obtained from coal minning areas was found to be between 0.52 and 0.75 mg kg^{-1}, whilst Zn concentrations were found to be in the range of 15.5 to 20.6 mg/ kg (Table-5).

The Municipal -treated water of nonindustrial area has pH in between 6.9-7.1, whereas the drinking water samples of five sub divisions of coal mining field have ranged in between 5.8- 6.8.

The values of Zn and Cd in drinking water samples obtained from municipal water supply water, used in the houses of nonindustries area, was in the ranged from 0.53 to 0.58 mg/l and 1.44 to 1.60 µg/L, respectively which are lesser than recommended permissible limit of WHO (Table-5). The water containers of five zones of coal mining field contain Zn and Cd levels at the range of 0.17– 0.24 mg/l and 17.5–20.7 µg/l, respectively. The Zn levels in the water samples of five zones of coal mining field are within the WHO permissible limit [23]. The Cd levels in water samples collected from five distinct subzones of the coal mining sector were found to be six to seven times higher than WHO recommended Cd limits.

It is found that drinking water, used in coal mining area is a source of harmful elemental exposure for employees of both ages [25].

The soil samples of coal mining field and nonindustrial zones have Zn and Cd levels in the range of (124–135; 10.3- 11.2) and (189-201 2.69- 3.27) µg/g, respectively. The Zn levels in the soil samples of five zones of coal mining field and nonindustrial areas are within the WHO permissible limit (WHO, 2004) [24], (150–300 µg/g WHO recommended value for Zn). Whilst the Cd levels in soil samples, collected from five different sub zones of coal mining field were three to four folds upper than WHO recommended Cd levels (WHO, 2004) [24].

The Zn and Cd contents in coal samples, obtained from these five zones of coal mining field, were found in the range of 15.5 –20.6 and 0.52– 0.75 mg/kg, respectively.

The following aspects in coal are known for causing air pollution, either directly or indirectly. Because the impact of follow metals on human health is dependent on the living being, measures, and concoction structure [25, 26], it is impossible to classify them as dangerous or cancer-causing. Many of the following ingredients are necessary for human digestion, but if consumed in excess of the body's requirements, they can be fatal. These have been classified depending on their level of risk, which is based on actual negative health impacts or their prevalence in coal [27]. The focus, conveyance, and techniques of elements event in coal were influenced heavily via topographical variables including such depositional circumstances, the connection among natural issue and basinal liquids, residue digenesis, and semi-active volcanic sources of information [27- 30].*Elemental concentrations in the Biological Samples of Workers and referent subjects*

This study delivers information on essential trace and toxic element levels in biological samples collected from outdoor and indoor workers of coal mining areas of five different zone sufferers and non-diseased subjects of (18-35 and 36-55) years age groups.

The Zn concentrations in the scalp hair samples of male referent subjects were found to be higher at 95 percent confidence limits (CI: 222-240 µg /g) and (CI: 207-220 µg /g) than those Zn values observed in the scalp hair samples of outdoor and indoor coal mining workers (CI: 106-133 g /g) and (CI: 100-123 µg /g) and (CI: 92.3 -118 µg /g) (CI: (Table-6). In the case of Cd, however, the opposite pattern was seen (Table-6).

In comparison to male outdoor and indoor workers of coal mining areas (CI: 4.21-5.25), (CI: 3.33-4.50) mg /l and (CI: 3.75-4.35) & (CI: 3.32-4.41) mg /l in age groups (18- 35) and (36- 55), the Zn concentrations in blood samples of male out door referent subjects, age ranged (18- 35) and (36- 60) years, were found to be higher (CI: 6.80-7.0) (Table-6). While Cd levels in blood samples of male out door referent subjects aged 18 to 35 years and 36 to 60 years were found to be lower (CI: 3.20-3.78) and (CI: 3.9-4.05) µg/L, respectively, as compared to male outdoor and indoor coal mining workers aged 18 to 35 years and 36 to 55 years (Table-6).

Table 6. Zinc and Cadmium Contents in biological (scalp hair and blood) samples of Mining Areas workers

Mining Areas workers	Age groups	Referents	Workers				
			PMDC	LCDC	ICD	HCM	BCM
			Zinc				
			Scalp hair (µg/g)				
	18-35	230±15.6	110±6.20	125±8.15	120±10.5	115±7.05	128±6.30
Outdoor Workers		224-237	106-133	118-130	114-125	112-120	124-133
Indoor workers		____	105±7.13	109±9.02	101±6.42	99±9.23	107±9.02
			100-111	104-118	96.5-108	92.3-106	101-113
	36- 55	215±10.7	104±6.33	119±9.22	109±6.72	108±9.20	118±10.2
outdoor Workers		209-221	100-110	114-123	105-112	103-115	112-123
Indoor workers		______	99.0±6.83	100±9.22	97.2±7.82	95.2±8.44	95.5±10.2
			93.0-105	94.5-109	90.5-104	89.6-102	88.9-105
			Blood (mg/l)				
	18-35	7.53±1.52	5.03±0.45	4.25±0.33	4.45±0.62	4.29±0.95	4.42±0.39
Outdoor Workers		6.82-7.82	4.80-5.25	4.51-6.04	4.23-4.79	3.85-4.70	4.21-4.60
Indoor workers		______	4.04±0.25	4.15±0.32	4.17±0.25	4.02±0.15	4.15±0.19
			3.75-4.17	4.00-4.35	4.05-4.31	4.02-4.17	4.06-4.27
Outdoor Workers	36- 55	6.90±0.62	4.08±0.65	3.80±0.42	3.66±0.57	4.05±0.85	4.02±0.33
		6.60-7.42	3.88-4.49	3.65-4.05	3.33-3.95	3.75-4.50	3.81-4.37
Indoor workers		______	4.03±0.35	3.74±0.41	3.77±0.29	3.67±0.45	4.00±0.21
			3.89-4.41	3.38-3.92	3.45-3.91	3.32-4.07	3.79-4.21
			Cadmium				
			Scalp hair (µg/g)				
	18-35	3.95±0.62	9.15±1.75	10.5±1.44	8.99±1.05	9.37±0.92	8.85±1.37
Outdoor Workers		3.45-4.45	7.65-11.2	7.29-9.98	7.82-9.95	8.53-10.2	7.62-10.2
Indoor workers		______	12.3±2.95	11.9±1.49	12.7±1.45	13.2±1.40	12.6±0.99
			10.5-14.4	10.6-13.3	11.3-14.3	12.0-14.5	11.7-13.5
	36- 55						
outdoor Workers							
		4.62±0.75	10.8±2.75	11.7±1.21	11.5±1.05	12.0±1.55	10.9±0.99
		4.02-5.32	9.65-12.2	11.2-12.4	11.0-12.2	11.3-13.0	10.4-11.5
Indoor workers		______	14.3±3.05	13.8±1.59	14.9±1.89	15.4±1.80	13.9±0.99
			10.1-15.4	11.2-15.9	12.3-16.40	12.9-16.9	12.8-15.6
			Blood (µg/l)				
	18-35	3.15±0.40	9.50±0.92	9.85±1.25	9.52±0.75	9.85±0.54	9.45±0.70
Outdoor Workers		3.25-3.72	8.69-10.0	9.15-10.6	9.28-9.99	9.45-10.39	9.00-9.82
Indoor workers		______	10.60±0.98	11.05±1.65	9.92±0.85	10.55±0.84	10.25±0.80
			8.89-11.36	10.25-11.70	9.08-10.99	9.49-11.09	9.60-10.98
	36- 55	3.95±0.25	10.3±0.70	10.3±0.95	10.3±0.82	10.5±0.69	9.85±0.67
outdoor Workers		3.82-4.10	9.85-10.8	9.85-10.9	9.89-10.8	10.1-10.9	9.41-10.3
Indoor workers		______	11.64±1.08	11.85±1.75	10.95±1.02	10.98±0.98	10.85±0.95
			10.89-12.06	10.95-12.65	10.05-11.69	9.89-11.79	9.90-11.33

Keywords: - Lakhra coal development company (LCDC), Hashim coal mine (HCM), Indus coal mine (ICM), Bismillah coal mine (BCM), and Pakistan mine development company (PMDC)

Student t-test (unpaired) was used to compare all of the groups studied at different probabilities. At 95 percent confidence intervals, the estimated t-value surpasses the critical t-value, indicating that the difference between mean values of Cd and Zn in referents and coal mining area employees was significant (p 0.001).

The concentrations of Zinc in biological samples of coal mining field workers were found to be lower as compared to referents. As discussed above, induced expression due to metallothionein binding has important role in Zn homeostasis. The Zn is additionally significant for immuno-fitness of upper respiratory framework, as diggers are maximally influenced in their air sections because of mining dusty air, hence this component is healthfully significant for them [31]. The Zn is extremely noteworthy for skin wellbeing, the moist climate of under mines hastens different skin contamination in coal diggers, a large number of our examination subjects are discovered influenced with this issue, Zn can be the solution for this skin illness [32]. Whilst the Cd levels in biological samples of coal mining field were found to be higher than referent subjects.

Cadmium's half-life in the human body is between 13.6 and 23.5 years. Exposure to cadmium, which is unsafe in nature, raises blood, serum and urine concentrations and may trigger pro-inflammatory immunological responses. High Cd exposure is linked to a variety of physiological problems, including decreased pulmonary function, cardiovascular problems and different types of cancer.

Because of its prolonged half-life, cadmium may accumulate in the human body as a result of smoking (Richter et al., 2009). The increase in Cd concentration in human lung tissues has been related to smoking history, which is higher in 5th lobes of lungs of smokers [29, 30]. Cadmium is easily transported through cigarette smoke, unlike other toxic elements present in tobacco. After inhalation, cadmium might make its way in lung and subsequently disseminate to other tissues [31, 32].

Coal mining smoke may disturb bioavailability of Zn via nonselective binding with metallothionein during Cd related biosynthesis of metallothionein [33-35]. Cadmium and Zn have inverse toxicological relationship [36, 37]. Cadmium and Zn may compete for same binding sites. Hence Zn supplements may decrease bad effects of Cd probably due Zn induced biosynthesis of metallothionein [38, 39]. The metallothionein also disrupts the action of metals-assisted enzymes by substituting critical elements like Zn [40]. A large number of studies on Cd show that it has negative health impacts [41, 42].

Correlation in between clinical parameters and zinc levels in scalp hair and blood samples of Coal Mining employees

The serum ferritin, haemoglobin, hematocrit, and red blood count levels of Coal Mining workers were found to be lesser in serum compared to referent subjects. Whilst, the values of ESR and NAG in the serum of workers were higher. Our findings are consistent with those of earlier studies, which found that cadmium-exposed rats had lower haemoglobin, red blood cell counts, and ferritin levels. The negative effects of Cd on haematological parameters, such as shortened erythrocyte lifespan and decreased heme production, have been documented.

The majority of coal mining field workers, both indoor and outdoor, were anaemic, with haemoglobin levels ranging from 9.64 to 12.1 percent, which were lower than healthy non-workers. The high levels of hazardous elements (Cd and Pb) are directly proportional to anaemia, particularly in adults and older persons [43]. Because these employees did not use face shields, goggles, or hand covers/gloves, the younger age group workers of both indoor and outdoor coal mining fields claimed stomach illnesses, and these individuals were anaemic as well as having eye difficulties. In addition, improper waste disposal or storage, as well as poor hygiene, may increase their vulnerability to harmful metals.

The mean creatinine clearance values in indoor and outdoor coal miners, particularly middle-aged miners with renal issues, were significantly lesser than recommended values (p 0.05). Urinary Nacetyl-beta-glucosaminidase (NAG) levels were significantly greater in both outdoor and indoor coal mining field employees ($p = 0.001$). Increased NAG levels in urine samples of the coal mining workers can be utilised to diagnose nephritis syndrome and nephropathy-related illnesses [44, 45]. The erythrocyte sedimentation rate (ESR) in coal mining field workers was found to be higher than permitted levels, indicating the presence of a variety of clinical illnesses.

The correlation (r) in between Zn values in scalp hair and blood samples and biochemical parameters (Hemoglobin, Serum ferritin, ESR, RBC, and NAG) of referents and coal mining workers was observed. The correlation (r) in between Zinc content in scalp hair and blood samples vs biochemical/ clinical parameters (serum ferritin, haemoglobin, RBC) of non-exposed referents is (r=0.52-0.71), whereas the correlation (r=0.06-0.23) was observed in biological samples of the coal mining workers (Table-7). The Zn content in scalp hair and blood samples correlates with biochemical markers (ESR and NAG) of referents (r= 0.08-0.18) (Table 7). Employees of the coal mining were shown to have a correlation of (r=0.52-0.71).

Table 7. Pearson's coefficient for zinc versus different biochemical parameters in referents and coal mining workers, working in five sub divisions of Lakhra coal mining Area

Biological Samples	biochemical parameters	Referents	PMDC	LCDC	ICD	HCM	BCM
		18- 35 years					
		Zinc					
		Indoor Workers					
	Serum ferritin	---------	y = 0.0663x + 20.7 r= 0.18	y = -0.0377x+28.5 r= 0.22	y=-0.0388x+26.3 r=0.22	y=-0.0429x + 31.1 r=0.15	y =0.048x +19.2 r=0.19
	Hemoglobin	---------	y = 0.0039x +11.5 r=0.06	y =0.0048x +10.35 r=0.17	y =0.0091x +11.7 r = 0.13	y =0.0072x +10.53 r=0.19	y =0.0068x+10.18 r=0.13
	RBC	---------	y = -0.0116x + 4.37 r=0.22	y =-0.004x + 3.71 r=0.16	y =0.0072x +2.24 r=0.16	y =-0.0042x+ 3.49 r=0.18	y = 0.0027x + 2.87 r=0.19
	ESR	---------	y = 0.0688x + 25.9 r=0.17	y = 0.0535x + 28.5 r=0.14	y =0.0333x+30.6 r=0.21	y =0.0272x + 33.2 r=0.18	y = -0.0317x + 35.3 r=0.21
	NAG	---------	y = 0.0176x+ 22.8 r=0.23	y =-0.0218x+27.99 r=0.17	y =0.0278x + 23.0 r=0.14	y = 0.0332x + 21.4 r=0.23	y = -0.004x + 25.7 r=0.15
		Outdoor Workers					
	Serum ferritin	y=0.078x+26.3 r= 0.71	y = 0.0642x + 19.2 r= 0.16	y =0.0102x +21.64 r=0.10	y =-0.0184x+23.8 r=0.22	y = -0.0265x +25.3 r=0.21	y = -0.0237x+27.1 r=0.153
	Hemoglobin	y =0.020x+ 9.1 r= 0.71	y = -0.0124x + 10.9 r=0.23	y = -0.0043x +9.38 r=0.19	y =0.0039x + 9.87 r=0.19	y = -0.004x + 10.3 r=0.11	y =-0.0043x +9.592 r=0.10
	RBC	y =0.015x+1.15 r=0.62	y = 0.0093x + 2.21 r=0.24	y = -0.0046x+ 3.77 r=0.16	y =0.0043x + 2.65 r=0.16	y = 0.002x + 2.99 r=0.14	y = 0.0037x + 2.60 r=0.23
	ESR	y =-0.0017x+9.1 r=0.08	y = 0.1217x + 4.88 r=0.71	y =0.0828x+ 5.44 r=0.67	y =0.0523x + 10.6 r=0.64	y = 0.0731x + 8.15 r=0.60	y = 0.1426x - 0.397 r=0.55
	NAG	y = -0.002x +3.9 r=0.09	y = 0.1751x - 4.361 r=0.63	y =0.148x -5.09 r=0.64	y = 0.0441x +7.58 r=0.64	y = 0.2111x- 9.39 r=0.69	y = 0.1455x - 5.19 r=0.59
Scalp hair		36-55 years					
		Indoor workers					
	Serum ferritin	---------	y = 0.0676x + 20.8 r=0.24	y = -0.04x + 25.2 r=0.12	y =-0.0702x+28.3 r=0.23	y =0.0133x+22.9 r=0.16	y =-0.0217x +25.02 r=0.22
	Hemoglobin	---------	y =0.0132x + 9.17 r=0.22	y =-0.0049x + 9.55 r=0.14	y =0.0053x +10.5 r=0.24	y = -0.0059x +10.9 r=0.13	y =0.0058x + 8.90 r=0.17
	RBC	---------	y = -0.0088x + 3.89 r=0.23	y = -0.0021x +3.47 r=0.22	y =0.0048x+ 2.67 r=0.10	y =-0.0037x +3.42 r=0.15	y =-0.0009x + 3.14 r=0.13
	ESR	---------	y = 0.0848x +26.12 r=0.19	y = -0.0808x +43.2 r=0.22	y =-0.1425x+36.2 r=0.26	y =0.0477x +32.13 r=0.16	y =-0.0214x +36.14 r=0.11
	NAG	---------	y = 0.0189x + 26.3 r=0.13	y = 0.0735x+16.35 r=0.21	y=0.0226x +25.9 r=0.11	y =-0.0403x+31.9 r=0.23	y=0.0107x + 26.24 r=0.14
		Outdoor workers					
	Serum ferritin	y=0.138x +13.2 r=0.69	y =-0.0527x + 33.4 r=0.16	y =-0.0096x + 27.1 r=0.13	y =0.0734x + 19.9 r=0.10	y =-0.0348x+30.19 r=0.19	y =-0.0335x + 32.4 r=0.16
	Hemoglobin	y=0.056x+0.34 r= 0.59	y =0.0152x+ 6.89 r=0.15	y = 0.016x + 6.17 r=0.22	y =0.0131x + 6.06 r=0.10	y= -0.0071x + 8.18 r=0.09	y = -0.0163x + 9.21 r=0.22
	RBC	y =0.027x-1.98 r= 0.56	y = 0.0137x + 1.33 r=0.12	y =-0.0064x+ 3.39 r=0.10	y =-0.0105x+4.06 r=0.18	y = 0.0114x + 1.66 r=0.09	y = -0.0033x + 3.33 r=0.12
	ESR	y=0.0103x+8.5 r=0.11	y = 0.216x - 1.1197 r=0.63	y =-0.0953x+34.75 r=0.62	y =0.0678x+ 16.8 r=0.67	y =-0.1566x + 40.7 r=0.61	y = 0.2364x - 5.995 r=0.56
	NAG	y =-0.0077x+6.9 r=0.16	y = 0.1863x - 2.32 r=0.61	y =0.0288x+ 11.82 r=0.50	y = 0.1428x-1.26 r=0.61	y = 0.0457x + 10.9 r=0.61	y = 0.1721x - 4.441 r=0.63
Blood		18- 35 years					
		Indoor Workers					

Serum ferritin	---------	y = -1.9349x + 35.5 r=0.14	y = -0.821x +27.9 r=0.15	y = 0.6396x+ 19.8 r=0.11	y = 4.1767x +9.61 r=0.16	y = 1.79x +16.6 r=0.14
Hemoglobin	---------	y = -0.4663x + 13.8 r=0.17	y = -0.1186x+ 11.4 r=0.13	y = -0.477x+ 14.6 r=0.21	y =-0.597x + 13.7 r=0.17	y = 0.3774x+ 9.29 r=0.15
RBC	---------	y =-0.376x + 4.67 r=0.16	y =-0.136x+3.85 r=0.16	y =-0.2228x+3.89 r=0.16	y = -0.251x+4.09 r=0.12	y = -0.08x + 3.485 r=0.11
ESR	---------	y = 9.69x - 6.03 r=0.59	y = 5.984x + 8.568 r=0.57	y =2.86x+ 22.2 r=0.61	y = -8.478x+ 70.7 r=0.61	y = 3.768x + 16.2 r=0.51
NAG	---------	y = 1.8099x + 17.3 r=0.56	y =2.507x+ 14.83 r=0.62	y = 3.439x +11.6 r=0.56	y =7.867x- 7.56 r=0.61	y =0.6983x + 22.4 r=0.54
			Outdoor Workers			
Serum ferritin	y=0.71x +38.5 r=0.61	y = -1.1181x + 31.8 r=0.19	y =-0.285x + 24.15 r=0.12	y =-0.2745x+22.9 r=0.16	y =-0.511x + 24.72 r=0.22	y = -0.243x + 25.09 r=0.11
Hemoglobin	y =0.17x+12.2 r=0.59	y = 0.116x + 8.978 r=0.16	y = -0.074x+ 9.175 r=0.14	y = 0.073x + 9.99 r=0.17	y= -0.104x + 10.33 r=0.15	y = 0.0963x + 8.61 r=0.18
RBC	y =0.153x+3.26 r=0.61	y = 0.0758x + 2.85 r=0.13	y = 0.137x + 2.593 r=0.19	y =0.1246x+ 2.59 r=0.21	y = 0.0472x + 2.99 r=0.18	y = -0.0302x + 3.21 r=0.15
ESR	y =-0.045x+9.02 r=0.18	y = 1.443x + 10.96 r=0.59	y = 1.5973x + 8.65 r=0.56	y = 1.005x + 12.1 r=0.58	y = 1.2202x + 10.6 r=0.52	y =1.7458x + 10.22 r=0.53
NAG	y =0.032x +3.27 r=0.17	y = 2.788x + 0.85 r=0.69	y = 3.2325x - 1.05 r=0.60	y =0.925x + 8.482 r=0.63	y = 3.7272x - 3.29 r=0.64	y = 1.8374x + 5.39 r=0.58
			36- 55 years **Indoor Workers**			
Serum ferritin	---------	y = -0.732x + 30.25 r=0.14	y = 1.729x + 14.56 r=0.16	y =1.621x + 15.37 r=0.17	y = 0.111x + 23.85 r=0.11	y = -0.6824x + 25.6 r=0.14
Hemoglobin	---------	y = 0.203x +9.64 r=0.18	y =0.1924x+ 8.319 r=0.17	y = 0.140x + 10.5 r=0.20	y = 0.105x + 9.975 r=0.17	y = -0.1957x + 10.2 r=0.11
RBC	---------	y = 0.090x + 2.673 r=0.13	y = -0.036x + 3.39 r=0.13	y =-0.229x+ 3.99 r=0.15	y = -0.068x + 3.3 r=0.22	y = 0.052x + 2.839 r=0.16
ESR	---------	y = 5.085x + 13.99 r=0.64	y = 6.153x + 11.76 r=0.56	y =5.062x + 16.34 r=0.63	y = 2.3136x + 28.7 r=0.61	y = -6.862x + 61.39 r=0.65
NAG	---------	y = 1.808x + 20.9 r=0.64	y = 5.3571x+ 3.86 r=0.52	y = 3.31x + 15.9 r=0.53	y =1.1699x+ 23.97 r=0.53	y = 2.722x + 16.44 r=0.67
			Outdoor Workers			
Serum ferritin	y =1.75x +31.0 r=0.52	y = 0.4235x+ 26.33 r=0.16	y =-0.418x + 27.62 r=0.13	y =-0.286x + 28.9 r=0.10	y =0.1142x+ 25.94 r=0.10	y = -1.1651x + 33.3 r=0.18
Hemoglobin	y =0.89x+6.44 r=0.55	y = -0.1481x + 9.04 r=0.17	y = -0.619x + 10.6 r=0.20	y =0.2778x + 6.52 r=0.16	y =-0.0807x + 3.23 r=0.10	y = -0.5196x + 9.33 r=0.21
RBC	y=0.425x+1.04 r=0.52	y = -0.503x + 4.639 r=0.16	y = 0.718x - 0.247 r=0.22	y =-0.081x +3.20 r=0.11	y = 0.281x + 22.6 r=0.18	y =-0.124x+3.43 r=0.14
ESR	y = -0.244x+12.3 r=0.15	y = 1.781x + 15.02 r=0.61	y = 4.2756x + 6.2 r=0.65	y = 0.761x + 21.6 r=0.60	y = 0.5376x + 0.65 r=0.69	y = 7.6866x - 8.35 r=0.53
NAG	y =0.106x+4.53 r=0.13	y = 1.630x + 11.26 r=0.63	y = 1.4409x + 9.55 r=0.57	y = 1.673x + 8.51 r=0.57	y = -0.24x + 16.85 r=0.52	y = 5.77x - 6.84 r=0.62

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Keywords: - Lakhra coal development company (LCDC), Hashim coal mine (HCM), Indus coal mine (ICM), Bismillah coal mine (BCM), Pakistan mine development company (PMDC), RBCs= Red blood count, SBP= Systolic blood pressure , DBP= Diastolic blood pressure , ESR= erythrocyte sedimentation rate , NAG= Urinary N -acetyl - beta -glucosaminidase

Correlation of cadmium concentration in water, soil and coal samples of Lakhra Coal Mining field with the cadmium level in scalp hair and blood *samples of Lakhra Coal Mining workers*

The correlation (r) in between Cd values in scalp hair and blood samples of non industrial referents normal subjects VS. Cd level in drinking water samples of non industrial areas was found to be (0.04-0.08) and (0.06- 0.08) respectively. The correlation (r) between Cd contents in biological samples of unexposed referent VS. Cd concentration in soil of unexposed areas was found to be (0.06-0.08) and (0.04- 0.05) respectively. Whilst the correlation (r) between Cd contents in biological samples of coal mining workers VS. Cd concentration in drinking water samples of coal mining tankers was found to be (0.15-0.17) and (0.10- 0.14) respectively (Table 8). The correlation (r) in between Cd contents in biological samples of coal mining area workers VS. Cd concentration in soil of coal mining was found to be (0.10-0.20) and (0.10- 0.19) respectively (Table 8). The correlation (r) in between Cd contents in scalp hair samples of Lakra coal mining workers and Cd concentration in coal samples of Lakhra coal mining was found to be (0.48-0.65) and (0.46- 0.56) respectively (Table 8). Whilst the correlation (r) in between Cd levels in blood samples of Lakra coal mining workers and Cd concentration in coal samples of Lakhra coal mining was found to be (0.39-0.64) and (0.50- 0.67) respectively (Table 8).

We observed lower the Zn/Cd mole ratio in biological samples of coal mining workers of both age groups (Table-9).

Table-8: Pearson's coefficient for Cadmium in biological samples of referents and workers of different zone of coal mining industry versus Cadmium concentration in the soil, water and coal of coal mining area

Biological Samples vs. biochemical parameters		Referents	PMDC	LCDC	ICD	HCM	BCM
				18- 35 years			
				Indoor Workers			
Scalp hair	Water	---------	r= 0.17	r= 0.17	r=0.17	r=0.15	r=0.15
	Soil	---------	r=0.17	r=0.16	r = 0.17	r=0.14	r=0.14
	Coal	---------	r=0.65	r=0.54	r=0.55	r=0.53	r=0.53
				Outdoor Workers			
	Water	r= 0.04	r= 0.10	r=0.14	r=0.10	r=0.13	r=0.14
	Soil	r= 0.06	r=0.17	r=0.16	r=0.12	r=0.15	r=0.15
	Coal	---------	r=0.48	r=0.48	r=0.48	r=0.55	r=0.55
				36-55 years			
				Indoor workers			
	Water	---------	r=0.25	r=0.28	r=0.27	r=0.26	r=0.30
	Soil	---------	r=0.15	r=0.16	r=0.16	r=0.20	r=0.19
	Coal	---------	r=0.54	r=0.51	r=0.48	r=0.55	r=0.50
				Outdoor workers			
	Water	r=0.08	r=0.15	r=0.14	r=0.16	r=0.17	r=0.15
	Soil	r= 0.06	r=0.15	r=0.15	r=0.15	r=0.13	r=0.14
	Coal	---------	r=0.46	r=0.56	r=0.53	r=0.51	r=0.55
				18- 35 years			
				Indoor Workers			
Blood	Water	---------	r=0.14	r=0.12	r=0.10	r=0.14	r=0.13
	Soil	---------	r=0.16	r=0.15	r=0.18	r=0.17	r=0.12
	Coal	---------	r=0.39	r=0.44	r=0.47	r=0.44	r=0.48
				Outdoor Workers			
	Water	r=0.06	r=0.14	r=0.19	r=0.11	r=0.12	r=0.13
	Soil	r=0.04	r=0.10	r=0.13	r=0.18	r=0.19	r=0.17
	Coal	---------	r=0.54	r=0.59	r=0.55	r=0.66	r=0.54
				36- 55 years			
				Indoor Workers			
	Water	---------	r=0.13	r=0.14	r=0.11	r=0.17	r=0.17
	Soil	---------	r=0.17	r=0.16	r=0.11	r=0.12	r=0.18
	Coal	---------	r=0.45	r=0.46	r=0.55	r=0.64	r=0.55
				Outdoor Workers			
	Water	r=0.08	r=0.15	r=0.15	r=0.15	r=0.11	r=0.15
	Soil	r=0.05	r=0.12	r=0.17	r=0.17	r=0.12	r=0.17
	Coal	---------	r=0.67	r=0.60	r=0.57	r=0.59	r=0.61

Keywords: **-** Lakhra coal development company (LCDC), Hashim coal mine (HCM), Indus coal mine (ICM), Bismillah coal mine (BCM), Pakistan mine development company (PMDC)

Table-9: Zn/Cd mole ratio in biological samples of referents and workers of different zone of coal mining industry versus Cadmium concentration in the soil, water and coal of coal mining area

Mining Areas workers	Age groups	Referents	Workers				
			PMDC	LCDC	ICD	HCM	BCM
Zinc / Cadmium Mole ratio							
Scalp hair (µg/g)							
Outdoor Workers	18-35	100	20.7	20.5	20.5	22.9	21.1
Indoor workers		-----------	14.7	15.7	13.7	13.7	12.9
Outdoor Workers	36- 55	79.9	16.1	17.2	17.2	14.6	14.1
Indoor workers		______	11.9	12.4	11.2	11.2	10.6
Blood (mg/l)							
Outdoor Workers	18-35	4108	910	741	774	776	804
Indoor workers		______	655	6455	722	701	755
Outdoor Workers	36- 55	2741	701	610	611	663	813
Indoor workers		______	693	542	592	601	698

Keywords: **-** Lakhra coal development company (LCDC), Hashim coal mine (HCM), Indus coal mine (ICM), Bismillah coal mine (BCM), Pakistan mine development company (PMDC).

Conclusion

Toxic element (Cd) concentrations were higher in scalp hair and blood samples of coal mining workers, although essential element (Zn) concentrations were lower than nonexposed referent participants. The exposure to toxic elements should be monitored on a regular basis to ensure that the workers' health is not jeopardised. This implies that work environment ventilation and industrial cleanliness procedures must be improved.

These data imply that workers had stomach problems, kidney problems, osteoporosis, reduced immune system, diarrhoea, late sexual maturation, hypogonadism in men, neurological and psychological problems, all these were extensively tracked throughout the workers' procedure. The unpackaged of coal available is of poor class and in vast numbers, and is frequently used in the energy business; however, certain coal mines are owned by private corporations unwilling to invest in developing technologies to enhance coal quality. **Funding:** This study was not funded by any funding agency.

Conflict of Interest/ Competing Interests

The authors declare that they have no competing interests.

References

1. Mineral deposits of Pakistan" (PDF). pakboi.gov.pk. (2012). Archived from the original (PDF) on 2014-03-27. Retrieved 2013-07-23.
2. A. M. Spooner (2015). in dummies.com/education/science/environmental-science/what-is-the-environmentalimpact-of-mining-and-burning-coal/.
3. M.P. Ortega-Larrocea, B. Xoconostle-Cazares. I.E.C. Maldonado-Mendozaet al.. Plant and fungal biodiversity from metal mine wastes under remediation at Zimapan, Hidalgo, Mexico. *Environ. Poll.*, **158**, 1922 (2010).
4. T.G. Kazi, A.R. Memon, H.I. Afridi, M.K. Jamali, M.B. Arain, N. Jalbani, , et al. Determination of cadmium in whole blood and scalp hair samples of Pakistani male lung cancer patients by electro thermal atomic absorption spectrometer. *Sci. Total Environ.*, **389**, 270 (2008).
5. H.G. Classen, U. Grober, D. Low, J. Schmidt, H. Stracke. Zinc deficiency: Symptoms, causes,. diagnosis and therapy. *Medizinische. Monatsschriftfuer. Pharmazeuten*., **34**, 87 (2008).
6. E. Biljana, V. Boris, D. Cena Veleska, D. Stefkovska. Matrix metalloproteinases (with accent to collagenases). *J. Cell and Animal Biol.,* **5**, 113 (2011).
7. B.A. Fowler, H. Yamauchi, E.A. Conner, M. Akkerman. Cancer risks for humans from exposure to semiconductor metals. *Scand. J. Work Env. Health*, **19**, 101 (1993).
8. T.G. Kazi, H.I. Afridi, N. Kazi, et al. Distribution of zinc, copper and iron in biological samples of Pakistani myocardial infarction (1st, 2nd and 3rd heart attack) patients and controls. *Clin. Chim. Acta*, **389**, 114 (2008).
9. R. Saraymen, M. Soylak, I. Narin. Serum cadmium levels of people living in Kayseri-Belsin region, Turkey. *Fresen. Environ. Bull*, **7**, 403 (1998).
10. T. G. Kazi, N. Jalbani, M.B. Arain, M.K. Jamali, H.I. Afridi, R.A. Sarfraz, et al. Toxic metals distribution in different components of Pakistani and imported cigarettes by electrothermal atomic absorption spectrometer. *J. Hazard. Mater.,* **163**, 302 (2009).

11. T. S. Nawrot, L. Thijs, E.M. Den Hond, H.A. Roels, J.A. Staessen. An epidemiological reappraisal of the association between blood pressure and blood lead: a meta analysis. *J. Hum. Hypertens.*, **16**, 123 (2002).
12. K. K. A. Witte, N. P. Nikitin, A. C. Parker, S. von Haehling, H.D. Volk, S.D. Anker, et al. The effect of micronutrient supplementation on quality-of-life and left ventricular function in elderly patients with chronic heart failure. *Eur. Heart J.*, **26**, 2238 (2005).
13. Environmental Protection Agency. (2007). Toxic Release Inventory 2007. Available: http://www.epa.gov/triexplorer.
14. United Nations Environment Program. (2000). Mining facts, figures and environment. *Mining and Sustainable Developed II: Challenges and Perspectives.*
15. M. Lee, I. Paik, I. Kim, H. Kang, S. Lee. Remediation of heavy metal contaminated groundwater originated from abandoned mine using lime and calcium carbonate. *J. Hazar. Mater.,* **144**, 208 (2007).
16. P. Epstein. (Full cost accounting for the life cycle of coal in Ecological Economics Reviews. Robert 1219. *Annals of the New York Academy of Sciences 73 at 74* 2011).
17. M. Hendryx. Mortality from heart, respiratory and kidney disease in coal mining areas of Appalachia. *Int. Arch. Occup. Environ. Health,* **82**, 243 (2009).
18. Laboratory Procedure Manual, 12-HB936-156 - Pointe Scientific, Inc. 2015.
19. International Labor Organization. (1999). Convention concerning the prohibition and immediate action for the elimination of the worst forms of child labour (No. 182).

a. J. Ali, T.G. Kazi, J. B. Baig, H.I. Afridi, et al. Monitoring of arsenic fate with proximate parameters and elemental composition of coal from thar coalfield, Pakistan. *J. Geochem. Explor.,* **159**, 227 (2015).

20. M. K. Jamali, T.G. Kazi, M.B. Arain, H.I. Afridi, N. Jalbani, A.R. Memon, A. Shah. Heavy metals from soil and domestic sewage sludge and their transfer to Sorghum plants. *Environ. Chem. Lett.*, **5**, 209 (2007).
21. M. Bilal, T.G. Kazi, H.I. Afridi, M.B. Arain, J.A. Baig, M. Khan. Application of conventional and modified cloud point extraction for simultaneous enrichment of cadmium, lead and copper in lake water. *J. Ind. Eng. Chem.,* **40**, 137 (2016).
22. WHO, 1996. Guidelines for Drinking-Water Quality. 2. WHO, Geneva.
23. WHO, 2004. Guideline for drinking water quality, third ed. In: Recommendation World Health Organization, Geneva.
24. J. N. Sommar, M.K. Svensson, B.M. Bjor, S.I. Elmstahl, G. Hallmans, T. Lundh, S.M. Schön, S. Skerfving, I.A. Bergdahl. End-stage renal disease and low level exposure to lead, cadmium and mercury; a population-based, prospective nested case-referent study in Sweden. *Environ. Health*, **12**, 9 (2013).
25. R. A. Baan. Carcinogenic hazards from inhaled carbon black, titanium dioxide, and talc not containing asbestos or asbestiform fibers: recent evaluations by an IARC Monographs Working Group. *Inhalation Toxicol.*, **19**, 213 (2007).
26. C. A. Demetriou, K. Straif, P. Vineis. From testing to estimation: the problem of false positives in the context of carcinogen evaluation in the IARC Monographs. *Cancer Epidemiol. & Prevention Biomarkers*, **21**, 1272 (2012).
27. M. Lenz, P.N. Lens. The essential toxin: the changing perception of selenium in environmental sciences. *Sci. Total Environ.* **407**, 3620 (2009).
28. H. Tian, K. Cheng, Y. Wang, D. Zhao, L. Lu, W. Jia, J. Hao. Temporal and spatial variation characteristics of atmospheric emissions of Cd, Cr, and Pb from coal in China. Atmospheric Environ., **50**, 157 (2012).
29. Z. Cong, S. Kang, Y. Zhang, X. Li, Atmospheric wet deposition of trace elements to central Tibetan Plateau. *App Geochem.* **25**, 1415 (2010).
30. K. Dabhadker, R. Shrivastva, , A. Sharma. Nutrition of coal mine workers (a case study of korba coal mines, Chhattisgarh). *Int. J. Scientific & Technol. Res.*, **5**, 278 (2013).
31. H. I. Afridi, T. G. Kazi, G. H. Kazi, M. K. Jamali, G. Q. Shar. Essential trace and toxic element distribution in the scalp hair of Pakistani myocardial infarction patients and controls. *Biol. Trace Elem. Res.*, **113**, 19 (2006).
32. D. Bernhard, A. Rossman, G. Wick. Metals in cigarette smoke. *I.U.B.M.B. Life,* **57**, 805 (2005).
33. D. R.Winge. Limited proteolysis of metallothionein method. *Methods Enzymol.* **205**, 438 (1991).
34. R. Bofill, M. Capdevila, S. Atrian. Independent metal-binding features of recombinant metallothioneins convergently draw a step gradation between Zn- and Cu-thioneins. *Metallomics*, **1**, 229 (2009).
35. F. Tsuchiyama, N. Hisanaga, E. Shibata, T. Aoki, H. Takagi, T. Ando, Y. Takeuchi. Pulmonary metal distributions in urban dwellers. *Int. Arch. Occup. Environ. Health*, **70**, 77 (1997).

36. K. Hoffmann, K. Becker, C. Friedrich, D. Helm, C. Krause, B. Seifert. The German environmental survey 1990/1992 (GerES II): cadmium in blood, urine, and hair of adults and children. *J. Expo. Anal. Env. Epid.*, **10**, 126 (2000).
37. M. P. Waalkes. Cadmium carcinogenesis. Mutation Research-Fundamental and Molecular Mechanisms of Mutagenesis. *Mut. Res.***533**, 107 (2003).
38. C. A. Suzuki, H. Ohta, A. Albores, J. Koropatnick, M.G. Cherian. Induction of metallothionein synthesis by zinc in cadmium pretreated rats. *Toxicology*, **63**, 273 (1990).
39. C. Reimann, P. De Caritat. Chemical elements in the environment: factsheets for the geochemists and environmental scientists: Springer Science & Business Media (2012).
40. S. Ghazy. Removal of cadmium, lead, mercury, tin, antimony, and arsenic from drinking and seawaters by colloid precipitate flotation. *Separation Sci. & Technol.*, **30**, 933 (1995).
41. M. P. Benavides, S. M. Gallego, M. L. Tomaro. Cadmium toxicity in plants. *Brazilian J. Pant Physiol.*, **17**, 21 (2005).
42. F. Shah, T.G. Kazi, H.I. Afridi, J.A. Baig, S. Khan, N.F. Kolachi, S.K. Wadhwa, A.Q. Shah. Environmental exposure of lead and iron deficit anemia in children age ranged 1–5 years: a cross sectional study. *Sci. Total Environ.*, **408**, 5325 (2010).
43. O. Liangos, M.C. Perianayagam, V.S. Vaidya, W.K. Han, R. Wald, H. Tighiouart, R.W. MacKinnon, L. Li, V.S. Balakrishnan, B.J. Pereira, J.V. Bonventre. Urinary N-acetyl-β-(D)-glucosaminidase activity and kidney injury molecule-1 level are associated with adverse outcomes in acute renal failure. *Clin. J. Am. Soc. Nephrol.*, **18**, 904 (2007).
44. A. H. Panhwar, T. G. Kazi, H. I. Afridi, S. A. Arain, M. S. Arain, K. D. Brahaman, S. S. Arain. Correlation of cadmium and aluminum in blood samples of kidney disorder patients with drinking water and tobacco smoking: related health risk. *Environ. Geochem. Health*, **38**, 265 (2016).