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Original Article

Quantification of Heavy Metals and Chemical Stressors in Ground Water of Coal Mining Areas and Associated Human Health Risk

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ABSTRACT

Acid mine drainage is one of the most obvious challenges in coal mining areas that is responsible for deteriorating soil and ground water quality of nearby communities thus posing serious human health risk. **Objective:** To quantify of heavy metals and chemical stressors in ground water and associated human health risk. **Methods:** Cross-sectional study with a combination of random sampling and Probability-Proportional-to-Size Sampling was used. Eight different sites were selected for water sample collection, and heavy metals were quantified. The analysis was carried out. Physicochemical properties of water were assessed using a portable photometer. Hematological parameters and antioxidants in the blood of study subjects were also measured. **Results:** Among the water samples, site S1 had the highest iron concentration at 0.354 ppm, exceeding the US limit of 0.3 ppm, with nickel being the next most abundant metal. Site S8 recorded the highest temperature at 36.4°C, while site S5 had the highest pH in the drinking water. The maximum electrical conductivity was found at S4 with 1387 s/m, and the total dissolved solvent parameter peaked at 1598 ppm in S8. Subjects exposed to acid mine drainage through water consumption displayed significant changes in antioxidant and blood parameters compared to the control group. In the exposed group, catalase (63.47), superoxide dismutase (33.26), and glutathione peroxidase (532.97) levels decreased, while malondialdehyde levels increased to 1.39. **Conclusions:** The physical and chemical properties of all water resources of mining areas were negatively altered due to heavy metals contamination thus poses a serious threat of oxidative stress in exposed subjects.

INTRODUCTION

The negative impact of coal mining on the environment is of great concern. Coal mining is kept under red category meaning it is in the top bracket of environmental as being one of the top producers of toxic substances [1]. Mining disturbs geological formations as well as natural systems and processes [2]. These disturbances may cause aquatic and terrestrial pollution [3]. The toxic nature of mining operations puts human and non-human communities of mining area are at risk from a variety of negative health impacts. The most severe and common problem caused by coal mining is caused by heavy metal contaminated water flowing from coal mines [4] to water bodies adjacent to area [5]. Coal mining and extraction are water-intensive practices [6]. For each ton of coal produced, 2.5 tons of water are contaminated [7]. Acid mine drainage (AMD) is one of the most serious environmental threat associated with mining [8]. The outcome of AMD on the surroundings includes the destruction of submersed resources, abstinence of terrestrial and wetland plant growth, impureness of groundwater, magnified water treatment costs and impairment to concrete and metal structures [9]. Acid Mine Drainage starts with the reaction of pyrite with oxygen and water. This exposure oxidizes the pyrite and results in the release of hydrogen ions, therefore

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lowering the pH [10]. During the oxidation of pyrite, ferrous sulfate and sulfuric acid which can be further oxidized to increase acidity. Coal mine drainage ranges widely in composition, from acidic to alkaline, typically with elevated concentrations of sulfate (SO4), iron (Fe), manganese (Mn) and aluminum (Al) as well as common elements such as calcium, sodium, potassium and magnesium. Acid mine drainage also dissolves toxic metals, such as copper, cadmium, arsenic, lead and mercury, from the surrounding rock [11]. People who live close to mining areas are likely to consume drinking water from surface and groundwater sources contaminated by coal mine generated waste and heavy metals [12]. These heavy metals when entered into the body they produce toxicity either by combining with bio molecules of cells or by inhibiting enzymatic activity or through endocrine disruption [13]. Some metals such as iron, chromium and copper are redox active and undergo redox cycle while other metals such as mercury, lead and cadmium are redox inactive metal and they play role in depleting cells from antioxidants [14]. Both redox active and redox inactive metals are responsible for increased production of reactive oxygen species such as hydrogen peroxides, superoxide and hydroxyl radical [15]. All these species are responsible for condition known as "oxidative stress" by either increasing the reactive oxygen species or by decreasing antioxidants [16]. Present study was designed to identify and quantify the presence and extent of health outcomes associated with chemical and non-chemical stressors in drinking water of coal mining areas.

METHODS

Cross-sectional study was conducted in Khushab district, Punjab, at eight different research sites (Table 1). At each site, drinking water samples, demographic data, and blood samples were collected from males with significant exposure for health assessment. To ensure a representative sample, a multifaceted sampling approach combining random sampling and Probability-Proportional-to-Size Sampling was employed. Initially, a subset of coal mines was randomly selected based on their distribution in the study area. Subsequently, specific groups were chosen, considering the total number of miners in these selected coal mines and their work arrangements using Probability-Proportional-to-Size Sampling. The study employs a sample size estimation formula: $n= z^2. [p*q]/d^2$; with key parameters: nn (the required sample size), dd (set at 5% acceptable error), zz (representing the value of the standard variate for a 95% confidence interval, resulting in a zz-value of 1.96), pp (an estimated proportion of 35%), and qq (the complementary proportion, calculated as 1−p1−p, which equates to 0.65 in this context).

Table 1: Dimensions of Different Sampling sites under study

Sites	S1	S2	S3	S4	S5	S6	S7	S812
Dimensions	32.6273E	32.6344E	32.6314E	32.6394E	32.6422E	32.6404E	32.656.4E	32.6555E
	72.5322N	72.4629N	72.4536N	72.4895N	72.6142N	72.4621N	72.4939N	72.4759N

Physical, chemical, and biological parameters in drinking water samples were analyzed, utilizing a portable digital meter from HANNA for pH, temperature, electrical conductivity, and total dissolved solids. Heavy metal determination was done through atomic absorption spectroscopy, involving nitric acid digestion. Blood samples were collected from the subclavian vein, and hematological investigations were conducted using an automated hematology analyzer (Figure 1). Antioxidant activities were also monitored in plasma. A control group was established for comparative purposes, comprising individuals unrelated to coal mining areas and their communities. Inclusion criteria encompassed 30 exposed and 20 control subjects aged 20 to 45, with exclusion criteria including smoking habits, drug usage, medical history, and family genetic diseases.

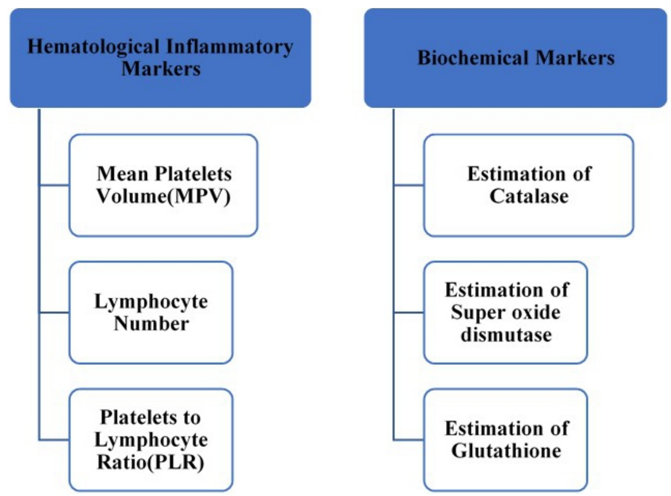

Figure 1: Human health Risk assessment using CBC and Antioxidants

Catalase level was determined in plasma by measuring the lowering of H_2O_2 concentration at wavelength of 240 nm through spectrophotometer following methods of Maehly and Chance [17]. Super Oxide dismutase in plasma was determined by measuring the percentage inhibition of Nitro Blue Tetrazole at wavelength of 560nm. Glutathione per oxidase in plasma was measured through utilization of NADPH during conversion of lipid peroxides and H_2O_2 at wavelength of 340nm. Statistical analysis was done using SPSS version 22.0. ANNOVA was used for comparative analysis of among different groups on the basis of distance. Descriptive statistics were given for all the parameters under study. The effect of distance on antioxidant level of exposed subjects was estimated by using correlation.

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RESULTS

ANOVA was employed to assess differences in physiochemical parameters among the sampling sites. Table 2 indicates a significant variation among the groups for temperature (TMP), pH, and Total Dissolved Solids (TDS) in the water samples from different sampling sites.

Table 2: Comparative analysis of Physiochemical parameters of water samples under study

ANOVA		Sum of Squares	df	Mean Square	F	Sig.
TMP	Between Groups	492.17	7	70.31	4.98	.000*
pH	Between Groups	17.42	7	2.48	7.92	.000*
EC	Between Groups	2760154.60	7	394307.80	1.93	.077
TDS	Between Groups	4503596.20	7	643370.887	8.58	.000*

The mean values for temperature, pH, Electrical Conductivity (EC), and Total Dissolved Solids (TDS) from various sampling sites are presented in Table 3. Site S8 recorded the highest temperature at 36.4°C, while the highest pH was observed in the drinking water from S5. Site S4 had the maximum electrical conductivity at 1387 s/m, and TDS levels peaked at S8, reaching 1598 ppm.

Table 3: Mean values of different physiochemical parameters in drinking water samples of different sampling sites under study

Mean Levelof Physiochemical Parameters					
Sampling sites		TMP	pH	EC S/m,	TDS mg/l
S1	Mean±SD	29.46±4.43	6.74±.341	974.94±607.12	608.11±244.53
S2	Mean±SD	26.73±3.34	6.59±.34	815.83±175.23	453.33±105.94
S3	Mean±SD	28.37±2.05	6.76±.37	1313.75±759.792	579.00±236.38
S4	Mean±SD	29.85±3.66	6.80±.346	1387.50±600.54	690.00±299.42
S5	Mean±SD	32.15±1.73	7.76±.31	1067.33±252.85	445.16±155.47
S6	Mean±SD	26.20±6.27	6.85±1.47	790.50±162.55	387.00±78.68
S7	Mean±SD	33.04±3.30	6.12±.24	1013.80±148.32	994.40±555.57
S8	Mean±SD	36.40±.000	6.10±.00	956.00±.00	1598.00±.000

ANOVA was applied to compare the means of metals and physical parameters among the sampling sites. Table 4 demonstrates a significant difference among the groups based on their spatial distribution.

Table 4: Comparative Analysis Of Heavy Metals At Sampling Sites

Heavy metals		Sum of Squares	Mean Square	F	Sig.
Cadmium	Between Groups	.001	.000	10.074	.000*
Chromium	Between Groups	.002	.000	8.533	.000*
Copper	Between Groups	.003	.000	5.476	.000*
Iron	Between Groups	.633	.070	8.709	.000*
Lead	Between Groups	.003	.000	1.677	.112
Nickel	Between Groups	.016	.002	9.058	.000*

Metal concentrations at various sampling sites were assessed, revealing that cadmium (0.0065 ppm), copper (0.221 ppm), iron (0.358 ppm), and lead (0.249 ppm) were most elevated at S1, the primary mining site. Conversely, nickel reached its highest level at S2 (0.337 ppm), while S4 exhibited the highest chromium concentration (0.190 ppm) among all sampling sites (Table 5).

Table 5: Mean values in ppm/mg/l of Heavy metals in drinking water samples of different sampling sites under study

Distance		Cd	Cr	Cu	Fe	Pb	Ni
S1	Mean±SD	.0065± .0045	.011± .007	.0221± .0081	.358± .116	.0249± .0032	.0090± .0093
S2	Mean±SD	.0051± .0022	.0154± .0093	.0121± .0031	.3424± .1304	.0216± .0303	.0337± .0423
S3	Mean±SD	.0058± .0037	.0152± .0071	.0228± .0106	.2129± .0940	.0095± .0046	.0199± .0145
S4	Mean±SD	.0061± .0031	.0190± .0042	.0185± .0092	.2939± .0623	.0133± .0158	.0194± .0126
S5	Mean±SD	.0033± .0028	.0177± .0014	.0185± .0096	.1490± .0524	.0193± .0161	.0162± .0029
S6	Mean±SD	.0007± .0002	.0158± .0054	.0058± .0031	.2503± .1474	.0199± .0097	.0068± .0073
S7	Mean±SD	.0025± .0019	.0012± .0035	.0094± .0043	.2313± .1183	.0056± .0058	.0102± .0101
S8	Mean±SD	.0022± .0000	.0017± .0000	.0072± .0000	.0839± .0000	.0007± .0000	.0016± .0000

Hematological analysis alternations among the control subjects and control group. Hemoglobin was higher among the control group as compared to the ones exposed in coal mining sites. Number of platelets was higher among exposed group.

Table 6: Hematological Parameters of subjects under study

Hematological Parameters under study	Control Group	Exposed	Sig.
Haemoglobin (median±SE)(g/dL)	13.90±0.16	11.40±0.22*	p<0.05
Platelets (median±SE)(x10^3/μL)	251±14.13	480±6.34*	p<0.05
Lymphocyte percentage (median±SE)	36±1.32	13±0.47	-
Neutrophil percentage (median ±SE)	54.20±1.29	77±0.99	-
NLR (median±SE)	1.41±0.05	4±0.25*	p<0.05
PLR (median±SE)	96.09±6.50	249±10.566*	p<0.05
MPV (median±SE)(fL)	9.76±0.19	8.43±0.08	-

Assessing the antioxidant levels in the study subjects, a decrease in catalase (63.47), SOD (33.26), and GPX (532.97) levels was observed in the exposed group in comparison to the control group. Conversely, the level of MDA increased to 1.39 in the exposed group. Additionally, other oxidative stress parameters, TP and Oxidative Stress Index, also showed elevated values (Table.7)

Table 7: Antioxidant levels of different enzymes measuring oxidative stress of subjects under study

Parameters Levels	Non-exposed Group n=20	Exposed Group n=30	Significance
SOD (U/L)	123.56±15	33.26±20*	p<0.05
Catalase (U/mL)	123.48±12	63.57±17*	p<0.05
MDA (μmol/l)	0.80±0.2	1.39±0.2	-
GPX (U/L)	313.56±81	532.97±44*	p<0.05
TAC(μmol/l)	2926.96±689	1212.51±218	-
OSI	0.2±0.1	1.26±0.5	-
TP (μmol/l)	6.14±1	15.17±5	-

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DISCUSSION

Our study aimed to assess water quality in coal mining areas by analyzing specific metals and physiochemical parameters. We also investigated potential health impacts by examining hematological parameters and antioxidant levels in consumers' blood. Concentrations of metals, including cadmium, chromium, lead, copper, iron, and nickel, varied across different sampling sites in our study area, reflecting local conditions and proximity to coal mines. Research by Tiwary in Brazil reported enriched iron, manganese, copper, and nickel in acid mine water and groundwater samples, consistent with our findings [18]. Ray and Dey found that coal mine drainage often exhibited acidity and higher levels of heavy metals, like iron, lead, manganese, and copper, aligning with our research [19]. We observed an increase in heavy metal levels in water from coal mining sites and nearby areas, indicating heavy metal pollution, consistent with the notion that mining waste contributes to such pollution [20]. Das *et al.*, found higher iron levels in underground water samples, with changes in physiochemical parameters, mirroring our findings of varying metal levels at different sites, with iron being most prominent among the metals studied [21]. Exposure to coal mine dust and contaminants can lead to the generation of reactive oxygen species and changes in antioxidant levels. Batool *et al.*, investigated coal-induced systemic hypoxia and redox imbalance, leading to alterations in antioxidant levels, consistent with our observations of differences in antioxidant enzyme levels between control and exposed groups [22]. Our findings emphasize that various non-essential heavy metals can alter antioxidant levels in the blood of exposed subjects, consistent with Abudawood *et al.*, report on the association between heavy metals in the blood and decreased antioxidant activity [23]. This study enhances our understanding of the environmental and health implications of coal mining activities.

CONCLUSIONS

The water quality of all the sampling sites was not good and the values for heavy metals and ions were higher than the permissible limits that were causing potential intestinal and water related problems especially diarrhea among the minors and the community people living in that area. As the distance of community areas increased from the main mining sites a gradual decrease in metallic, ionic and microbial content was observed. Moreover, the physical properties of all water resources of mining areas were negatively altered.

Authors Contribution

Conceptualization: FI
Methodology: FUR, AK
Formal Analysis: NHN
Writing-review and editing: FI, AIB, AA, SSH, HB

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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