

IMPACT OF OIL AND GAS-RELATED HEAVY METAL EXPOSURE ON ANTIOXIDANT ENZYME ACTIVITY IN HUMAN POPULATIONS OF KARAK, PAKISTAN

Asad Zamir^{*1}, Sadia Ayaz², Siraj Bibi³, Muhammad Abdul Salam⁴, Afshan Farooq³,
Shahzad Rauf³

^{*1}Department of Chemistry, Government Post Graduate College Karak, KP, Pakistan,

^{*2}Department of Chemistry, Government College University Faisalabad, Pakistan,

^{*3}Department of Chemistry, Kohat University of Science and Technology, Kohat, KP, Pakistan,

^{*4}Department of chemistry, Khushal Khan Khattak University Karak, KP, Pakistan

asadzamir000@gmail.com

DOI: <https://doi.org/10.5281/zenodo.18168996>

Keywords

Heavy metals; Antioxidant enzymes;
Oxidative stress; Human
biomonitoring; Oil and gas pollution;
Karak Pakistan

Article History

Received: 03 November 2025

Accepted: 17 December 2025

Published: 31 December 2025

Copyright @Author

Corresponding Author: *

Asad Zamir

Abstract

Chronic exposure to heavy metals released from oil and gas operations poses a serious risk to human health through the induction of oxidative stress. This study quantified blood concentrations of arsenic (As), cadmium (Cd), lead (Pb), mercury (Hg), cobalt (Co), and copper (Cu) in residents living near the Nashpa oil and gas plant in Karak, Pakistan, and evaluated their effects on antioxidant enzyme activity. Blood samples were collected from 63 individuals residing within a 6 km² radius of the facility and analyzed using atomic absorption spectrophotometry. Antioxidant defense and oxidative stress biomarkers were assessed by measuring catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), reactive oxygen species (ROS), and lipid peroxidation (LPO). Mean blood concentrations of Cd (5.65 µg/L), Pb (306 µg/L), Hg (8.38 µg/L), Co (5.78 µg/L), and Cu (1963.75 µg/L) exceeded World Health Organization (WHO) permissible limits. Correspondingly, mean activities of CAT (5.13 U/ml), POD (9.33 U/ml), and SOD (35.35 U/ml) were markedly reduced, while ROS (0.17 U/ml) and LPO (16.19 U/ml) levels were elevated, indicating enhanced oxidative stress. Elevated heavy metal exposure was inversely associated with antioxidant enzyme activity, confirming oxidative imbalance in the exposed population. These findings highlight the potential health risks faced by communities residing near oil and gas facilities and underscore the urgent need for continuous biomonitoring and stricter environmental regulation in petroleum-producing regions.

INTRODUCTION

Oil and gas exploration and production are among the major industrial activities responsible for environmental contamination worldwide. These operations release toxic heavy metals such as arsenic (As), cadmium (Cd), lead (Pb), mercury (Hg), cobalt (Co), and copper (Cu) into surrounding ecosystems through drilling fluids, produced water, gas flaring, and improper disposal of industrial wastes (Budi et al., 2024;

Qaiser et al., 2018). Due to their persistence, non-biodegradable nature, and long biological half-lives, these metals readily accumulate in soil, water, and biological systems, thereby posing serious risks to human health (Li et al., 2022; D'Andrea & Reddy, 2014).

Human exposure to heavy metals occurs primarily through ingestion of contaminated drinking water and food, inhalation of polluted air, and dermal contact with contaminated

media. Once absorbed into the bloodstream, heavy metals disrupt cellular redox homeostasis by generating reactive oxygen species (ROS) and impairing antioxidant defense mechanisms (Flora et al., 2008; Valko et al., 2016). This oxidative imbalance leads to oxidative stress, a pathological condition associated with the development of cardiovascular diseases, neurological disorders, renal dysfunction, metabolic abnormalities, and carcinogenesis.

Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) play a critical role in protecting cells from oxidative damage by neutralizing superoxide radicals and hydrogen peroxide. In contrast, lipid peroxidation (LPO) serves as a key biomarker of oxidative membrane damage (Mishra & Flora, 2008; Wu et al., 2016). Numerous studies have reported that toxic metals, particularly Cd, Pb, and Hg, suppress the activities of SOD, CAT, and POD while simultaneously increasing ROS production and LPO levels (Patra et al., 2011; Al-Fartosy et al., 2017; Ajeel et al., 2021). These biochemical alterations provide strong evidence that oxidative stress is a central mechanism underlying heavy metal-induced toxicity.

The petroleum industry is a well-recognized source of heavy metal pollution, particularly in regions where environmental regulation and waste management practices are inadequate. Petroleum extraction and processing generate metal-rich effluents and solid wastes that contaminate terrestrial and aquatic environments (Sojinu & Ejeromedoghene, 2019). In Pakistan, several studies have documented elevated concentrations of heavy metals in industrial regions. Malik et al. (2010) reported excessive levels of Pb and Cu in soils and groundwater from industrial zones, highlighting the direct risk to human health. Similarly, Riffat et al. (2023) identified Cd, Cu, Pb, Ni, Fe, and Mn as major contaminants in surface and groundwater across the former FATA region, frequently exceeding World Health Organization (WHO) permissible limits. Although environmental contamination around petroleum facilities has been widely reported, relatively few studies have examined the biological consequences of such exposure through human biomonitoring. Studies from other oil-producing regions, such as Basra, Iraq,

have demonstrated significantly elevated blood levels of Pb, Cd, and Hg among petroleum workers, accompanied by increased malondialdehyde (MDA) and reduced antioxidant enzyme activities, confirming the link between petroleum-related heavy metal exposure and oxidative stress (Al-Fartosy et al., 2017). Comparable findings were reported by Abarikwu et al. (2017) and Oforu et al. (2021), who highlighted bioaccumulation of heavy metals and oxidative damage in organisms inhabiting petroleum-impacted environments.

In Pakistan, however, research linking heavy metal exposure from oil and gas activities to oxidative stress biomarkers in human populations remains scarce. The Nashpa oil and gas plant, located in Karak District of Khyber Pakhtunkhwa, is a major petroleum production site where local communities live in close proximity to ongoing industrial operations (Qaiser et al., 2018). Despite increasing concerns regarding environmental pollution in the region, comprehensive biomonitoring studies assessing heavy metal exposure and antioxidant enzyme disruption in residents are lacking.

Therefore, the present study aimed to quantify blood concentrations of As, Cd, Pb, Hg, Co, and Cu in individuals residing near the Nashpa oil and gas plant and to evaluate their association with antioxidant enzyme activities (CAT, POD, and SOD), reactive oxygen species (ROS), and lipid peroxidation (LPO). By integrating heavy metal biomonitoring with oxidative stress biomarkers, this study provides critical insight into the potential health risks posed by chronic exposure to oil and gas-related pollution in Karak, Pakistan.

MATERIALS AND METHODS

Study Area

The study was conducted around the Nashpa oil and gas plant, located in the Karak District of Khyber Pakhtunkhwa (KP), Pakistan. The region is one of the province's major petroleum-producing zones, hosting several active exploration and drilling operations (Qaiser et al., 2018). Continuous extraction and waste discharge from these industrial activities have raised concerns about heavy metal exposure among nearby communities. The study area covered approximately 6 km² surrounding the Nashpa plant, where residents live and work in

close proximity to oil and gas operations, potentially increasing their vulnerability to environmental pollutants.

Population and Blood Sample Collection

A total of 63 residents, aged between 18 and 60 years, volunteered to participate in the study. Participants were selected from households located within the defined 6 km² radius of the

Nashpa oil and gas plant as in figure 1. The study protocol was approved by an institutional ethical review committee and conducted in accordance with the World Medical Association Declaration of Helsinki (WMA, 2013). Informed consent was obtained from each participant after explaining the objectives and procedures of the study.

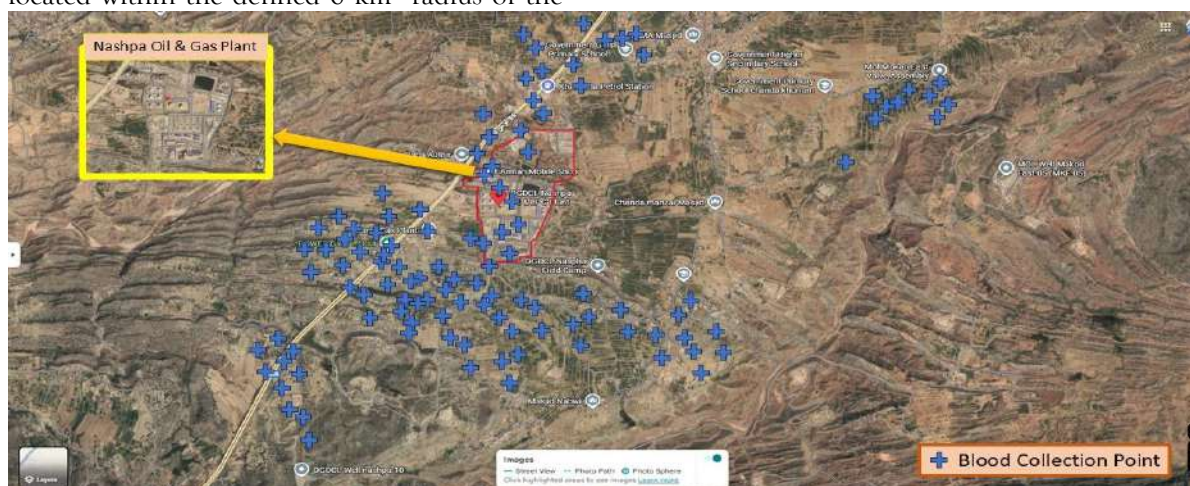


Figure-1: Blood Collection points near Nashpa Plant of Oil and Gas Karak, KP, Pakistan

From each participant, a 3 ml venous blood sample was collected using standard aseptic techniques by a certified phlebotomist. Samples were drawn into EDTA tubes, gently mixed, and immediately transported in an ice box to Thyrocare Technologies Limited Laboratory, Islamabad. Whole blood and serum were separated and stored at -20 °C until analysis. The concentrations of selected heavy metals – arsenic (As), cadmium (Cd), lead (Pb), mercury (Hg), cobalt (Co), and copper (Cu) – were determined using Atomic Absorption Spectrophotometry (AAS) in accordance with USEPA (2007) guidelines. All analyses were performed in triplicate for precision and reliability.

Determination of Heavy Metals in Blood

Blood concentrations of arsenic (As), cadmium (Cd), lead (Pb), mercury (Hg), cobalt (Co), and copper (Cu) were determined using atomic absorption spectrophotometry (AAS) following United States Environmental Protection Agency guidelines (USEPA, 2007). Calibration curves were prepared using certified standard solutions, and reagent blanks were included to ensure analytical accuracy. All samples were analyzed in

triplicate, and results were expressed in micrograms per liter (µg/L).

Assessment of Antioxidant Enzyme Activities and Oxidative Stress Biomarkers

Serum antioxidant enzyme activities, including superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), along with oxidative stress biomarkers—reactive oxygen species (ROS) and lipid peroxidation (LPO)—were measured using commercially available assay kits (ZellBio GmbH, Germany; CAT No. ZB-96A). All assays were performed strictly according to the manufacturer's protocols.

Quality Assurance and Quality Control

Quality assurance and quality control procedures were maintained throughout the analytical process following the protocol described by Armonk et al. (2013). Reagent blanks, duplicate samples, and certified reference materials were included in each analytical batch. Analytical precision for heavy metal determination remained within ±1.0–1.5%, ensuring data reliability and reproducibility.

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics version 22. Descriptive statistics were calculated for heavy metal concentrations and antioxidant enzyme activities. Pearson's correlation coefficient (r) was used to assess relationships between heavy metals and oxidative stress biomarkers. Statistical significance was defined at $p < 0.05$.

RESULT

Heavy Metals Contamination in Human Blood

The statistical parameters of blood heavy metal concentrations, including minimum, maximum, mean, median, standard deviation, and skewness, are presented in Table 1, while comparison of mean metal concentrations with World Health Organization (WHO) permissible limits is shown in Table 2. The distribution and relative mean values of heavy metals are graphically illustrated in Figure 2 and Figure 3, respectively.

Table 1. Statistical parameters of selected heavy metals (µg/L) in blood.

Parameters	As	Cd	Pb	Hg	Co	Cu
Minimum	2.7	1.6	70.7	3.6	0.7	472.3
Maximum	7.3	8.6	528.7	14.3	11.8	3188.4
Mean	5.4	5.65	306	8.383333	5.783333	1963.75
Median	5.75	6.2	310.5	8.2	5.7	2157.4
Standard deviation	1.939071943	2.601346	179.5153	4.231981	4.782224	1119.867
Skewness	-0.39525927	-0.63269	-0.0864	0.249411	0.129868	-0.36632

Fig-2. Statistical parameters of selected heavy metals (µg/L) in blood.

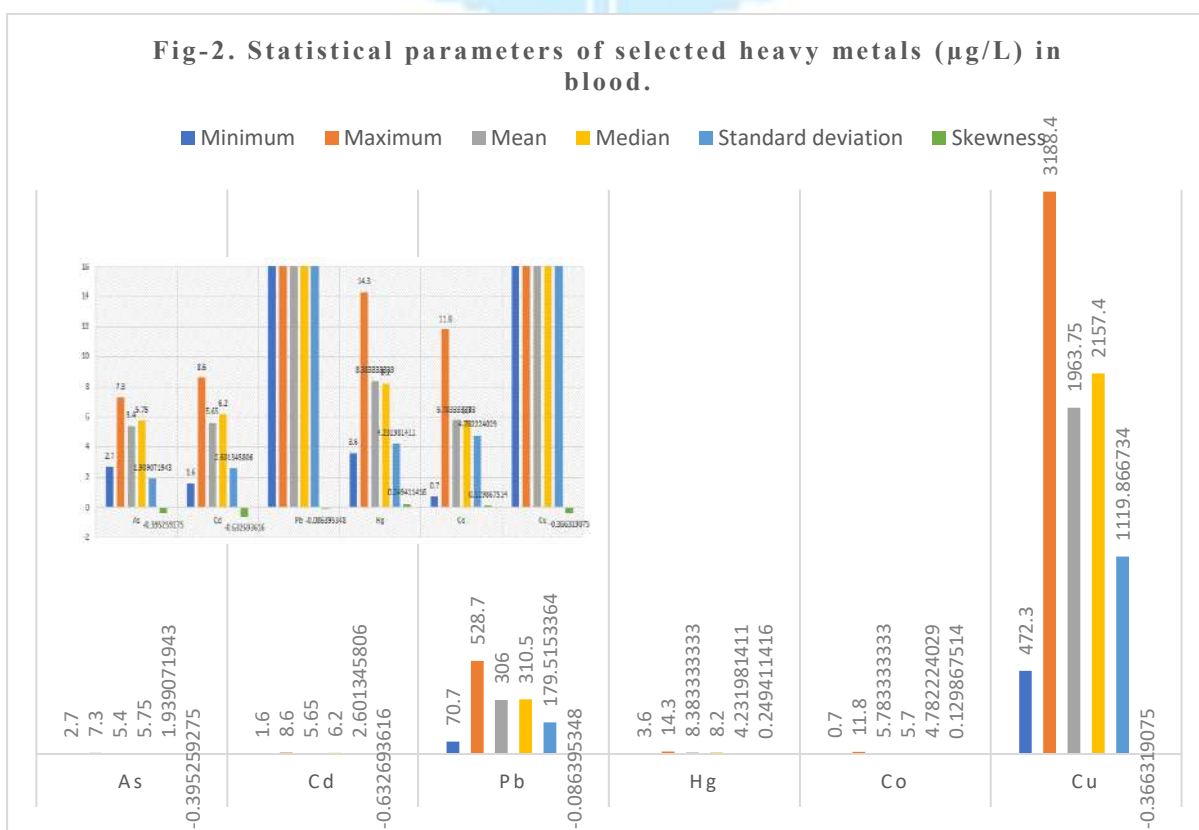
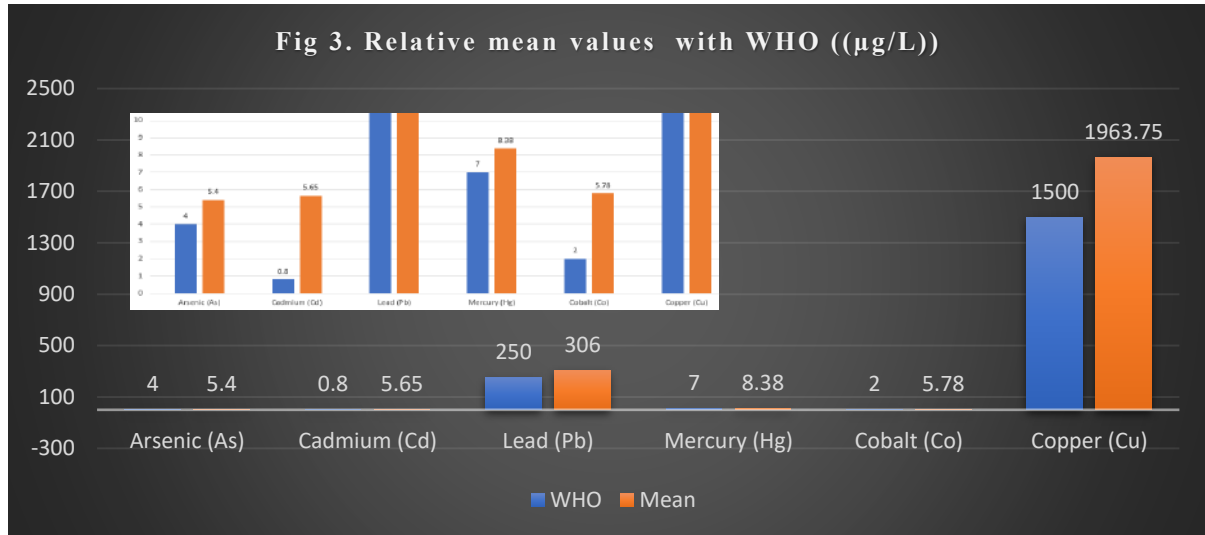


Table 2. Relative mean values with WHO ((µg/L))

Metal	Mean	WHO
Arsenic (As)	5.4	1 - 4
Cadmium (Cd)	5.65	0.2 - 0.8 (non-smokers); 1.4 - 4.5 (smokers)
Lead (Pb)	306	100 - 250

Mercury (Hg)	8.38	0.1 – 7
Cobalt (Co)	5.78	0.1 – 2
Copper (Cu)	1963.75	700 – 1500



Catalytic Activities of Enzymes

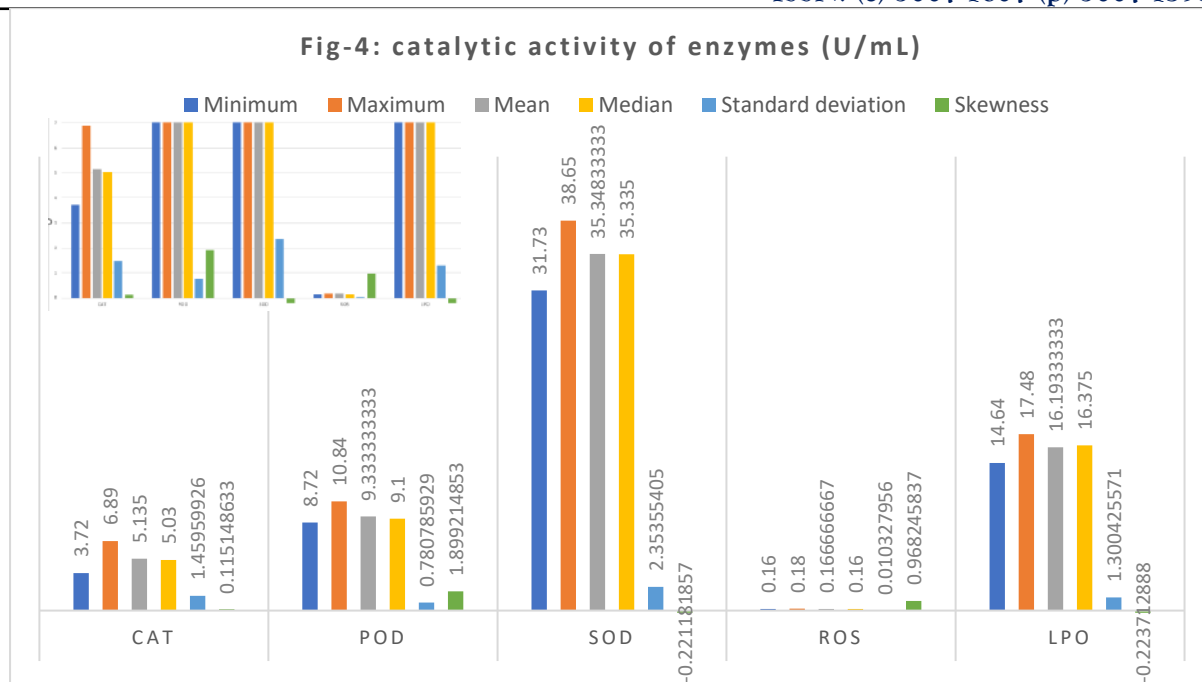
Absorbance values were recorded using an ELISA microplate reader at the following wavelengths: SOD (420 nm), CAT (405 nm), POD (490 nm), ROS (540 nm), and LPO (500

nm). Enzyme activities were expressed as units per milliliter (U/mL).

The descriptive statistical analysis of antioxidant enzymes and oxidative stress biomarkers is summarized in Table 3, and their overall distribution is illustrated in Figure 4.

Table 3. Statistical parameters of various catalytic activity of enzymes found in blood (U/mL).

Parameters	CAT	POD	SOD	ROS	LPO
Minimum	3.72	8.72	31.73	0.16	14.64
Maximum	6.89	10.84	38.65	0.18	17.48
Mean	5.135	9.333333	35.34833	0.166667	16.19333
Median	5.03	9.1	35.335	0.16	16.375
Standard deviation	1.459599	0.780786	2.353554	0.010328	1.300426
Skewness	0.115149	1.899215	-0.22118	0.968246	-0.22371



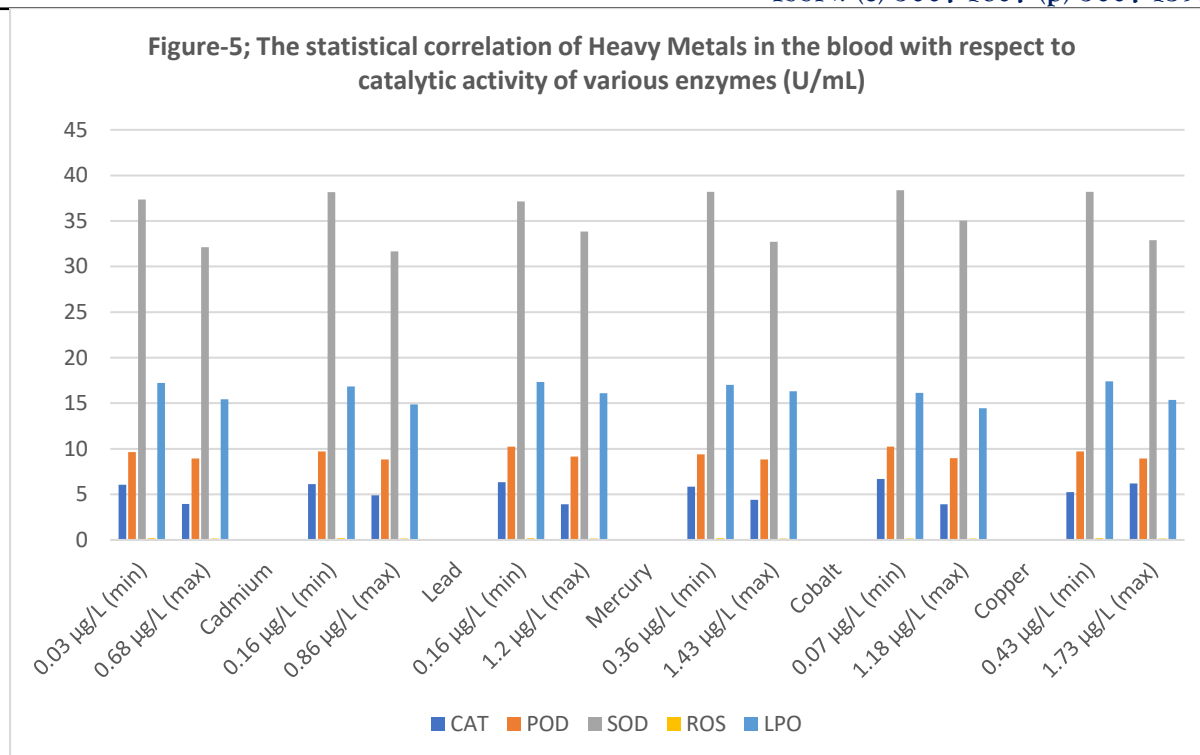
Correlation Analysis Between Heavy Metals and Antioxidant Enzymes

To assess the relationship between heavy metal exposure and oxidative stress response, correlations between individual metal concentrations (As, Cd, Pb, Hg, Co, and Cu)

and antioxidant enzyme activities (CAT, POD, SOD, ROS, and LPO) were evaluated.

Correlation results are presented collectively in Table 4, and the overall relationship between heavy metals and antioxidant enzyme activities is illustrated in Figure 5.

Heavy Metals	CAT	POD	SOD	ROS	LPO
Arsenic					
0.03 µg/L (min)	6.07	9.64	37.35	0.18	17.21
0.68 µg/L (max)	3.94	8.94	32.12	0.17	15.45
Cadmium					
0.16 µg/L (min)	6.14	9.72	38.16	0.18	16.83
0.86 µg/L (max)	4.89	8.84	31.65	0.16	14.89
Lead					
0.16 µg/L (min)	6.34	10.22	37.16	0.18	17.33
1.2 µg/L (max)	3.91	9.13	33.83	0.17	16.12
Mercury					
0.36 µg/L (min)	5.83	9.41	38.21	0.18	17.03
1.43 µg/L (max)	4.39	8.84	32.72	0.16	16.31
Cobalt					
0.07 µg/L (min)	6.68	10.22	38.36	0.17	16.13
1.18 µg/L (max)	3.92	8.97	35.02	0.16	14.46
Copper					
0.43 µg/L (min)	5.24	9.71	38.21	0.18	17.41
1.73 µg/L (max)	6.19	8.94	32.88	0.16	15.36



DISCUSSION

The present study provides comprehensive biomonitoring evidence of heavy metal exposure and its biochemical consequences in human populations residing near an oil and gas facility in Karak, Pakistan. The elevated blood concentrations of Cd, Pb, Hg, Co, and Cu observed in this study exceed WHO permissible limits and reflect chronic environmental exposure, likely originating from petroleum extraction activities, industrial waste discharge, and contamination of local water and food sources.

Lead and copper were detected at particularly high concentrations, suggesting prolonged and continuous exposure. Similar elevated metal levels have been reported in petroleum-impacted regions in Pakistan and other developing countries, where inadequate environmental regulation and waste management facilitate the transfer of contaminants into the human food chain (Malik et al., 2010; Riffat et al., 2023). The findings are also consistent with studies conducted in oil-producing regions such as Basra, Iraq, where petroleum workers exhibited significantly elevated blood levels of Pb, Cd, and Hg (Al-Fartosy et al., 2017).

A key finding of this study is the marked suppression of antioxidant enzyme activities (CAT, POD, and SOD) in individuals exposed

to elevated heavy metal concentrations. These enzymes constitute the primary cellular defense system against oxidative damage, and their inhibition compromises the body's ability to neutralize reactive oxygen species. The observed increase in ROS and lipid peroxidation further confirms the presence of oxidative stress in the exposed population.

The inverse correlation between heavy metals and antioxidant enzyme activities, together with the positive association with ROS and LPO, supports the well-established mechanism of metal-induced oxidative toxicity. Toxic metals such as Cd, Pb, and Hg are known to bind sulfhydryl groups of enzymes, displace essential metal cofactors, and disrupt mitochondrial electron transport, leading to excessive ROS generation (Flora et al., 2008; Valko et al., 2016). These biochemical disturbances have been linked to cellular membrane damage, protein oxidation, DNA damage, and apoptosis.

Although cobalt and copper are essential trace elements, excessive concentrations can exert pro-oxidant effects. Elevated copper levels, as observed in this study, may catalyze Fenton-like reactions, further enhancing oxidative stress. Similar findings have been reported by Ajeel et al. (2021), who demonstrated oxidative imbalance in populations exposed to petroleum-

related metals, even when essential elements were involved.

The findings of this study have important public health implications. Chronic oxidative stress induced by heavy metal exposure is associated with an increased risk of renal dysfunction, neurological disorders, cardiovascular diseases, and carcinogenesis. Communities residing near oil and gas facilities, such as those in Karak, may therefore face long-term health risks if environmental contamination is not adequately controlled.

Overall, this study highlights the urgent need for continuous environmental and human biomonitoring in petroleum-producing regions of Pakistan. Implementation of stricter industrial waste management practices, regular assessment of drinking water quality, and public health surveillance are essential to mitigate the adverse health effects associated with chronic heavy metal exposure.

ACKNOWLEDGMENTS

All praise and gratitude are due to Allah Almighty for providing me with the strength, patience, and guidance to complete this research. I am deeply grateful to my parents for their unwavering support, encouragement, and prayers throughout this work. I would also like to extend my sincere appreciation to Afshan Farooq for her valuable assistance and guidance during the study. Special thanks are due to Ayesha Hayat for her support and constructive feedback, which greatly contributed to the successful completion of this research.

REFERENCES

- Budi, S., Rahman, A., & Prasetyo, Y. (2024). Environmental impacts of oil and gas activities: Heavy metal contamination and human health risks. *Journal of Environmental Management*, 345, 118742.
- Kaiser, M., Khan, S., & Rehman, A. (2018). Environmental impacts of oil and gas exploration in Karak District, Pakistan. *Environmental Monitoring and Assessment*, 190(6), 356.

- Li, Z., Ma, Z., van der Kuijp, T. J., Yuan, Z., & Huang, L. (2022). A review of soil heavy metal pollution from mines in China: Pollution and health risk assessment. *Science of the Total Environment*, 468–469, 843–853.
- D'Andrea, M. A., & Reddy, G. K. (2014). Health consequences among subjects involved in Gulf oil spill clean-up activities. *American Journal of Medicine*, 127(10), 966–974.
- Flora, S. J. S., Mittal, M., & Mehta, A. (2008). Heavy metal-induced oxidative stress and its possible reversal by chelation therapy. *Indian Journal of Medical Research*, 128(4), 501–523.
- Valko, M., Jomova, K., Rhodes, C. J., Kuča, K., & Musilek, K. (2016). Redox- and non-redox-metal-induced formation of free radicals and their role in human disease. *Archives of Toxicology*, 90(1), 1–37.
- Mishra, S., & Flora, S. J. S. (2008). Differential oxidative stress in rat brain regions following arsenic exposure and its modulation by antioxidants. *Toxicology and Applied Pharmacology*, 230(3), 311–318.
- Wu, H., Zhang, C., Zeng, G., Liang, J., Dai, J., & Liu, Z. (2016). Mechanisms of heavy metal-induced oxidative stress and toxicity in biological systems. *Ecotoxicology and Environmental Safety*, 134, 324–334.
- Patra, R. C., Rautray, A. K., & Swarup, D. (2011). Oxidative stress in lead and cadmium toxicity and its amelioration by antioxidants. *Veterinary Medicine International*, 2011, 457327.
- Al-Fartosy, A. J., Awad, N. A., & Al-Derzi, N. A. (2017). Oxidative stress biomarkers in petroleum workers exposed to heavy metals. *Journal of Environmental Science and Health, Part A*, 52(9), 860–868.
- Ajeel, S. G., Al-Mayahi, B. A., & Al-Fartosy, A. J. (2021). Heavy metal exposure and oxidative stress biomarkers in populations living near oil fields. *Environmental Science and Pollution Research*, 28(14), 17425–17436.

- Sojnu, S. O., & Ejeromedoghene, O. (2019). Environmental pollution from petroleum activities: Heavy metals contamination and ecological risk assessment. *Marine Pollution Bulletin*, 145, 106–115.
- Malik, R. N., Jadoon, W. A., & Husain, S. Z. (2010). Metal contamination of surface soils of an industrial city Sialkot, Pakistan: A multivariate and GIS approach. *Environmental Geochemistry and Health*, 32(3), 191–203.
- Riffat, A., Khan, M. T., & Ullah, H. (2023). Assessment of heavy metal contamination in surface and groundwater of former FATA region, Pakistan. *Environmental Monitoring and Assessment*, 195(2), 211.
- Abarikwu, S. O., Iserhienrhien, B. O., & Badejo, T. A. (2017). Petroleum hydrocarbon exposure and oxidative stress responses in biological systems. *Environmental Toxicology and Pharmacology*, 52, 74–81.
- Ofosu, A., Akoto, O., & Darko, G. (2021). Bioaccumulation of heavy metals and oxidative stress in petroleum-impacted environments. *Environmental Science and Pollution Research*, 28(9), 11234–11245.
- World Medical Association. (2013). World Medical Association Declaration of Helsinki: Ethical principles for medical research involving human subjects. *JAMA*, 310(20), 2191–2194.
- World Health Organization. (2011). *Evaluation of certain food additives and contaminants: Seventy-third report of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)*. WHO Press.
- U.S. Environmental Protection Agency. (2007). *SW-846 Test Method 7000B: Flame atomic absorption spectrophotometry*. Washington, DC.
- IBM Corp. (2013). *IBM SPSS Statistics for Windows (Version 22.0)*. Armonk, NY: IBM Corp.