زانکۆی سەلاحەدین - هەولێر
Salahaddin University-Erbil

Air Pollution and Concentration of Heavy Meatal in blood

Research Project
Submitted to the Department of Biology in partial fulfillment of the requirements for the degree of B. A or BSc. in Biology

By

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Supervised By

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April– 2023

- قال تعالى: (اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ* خَلَقَ الْإِنسَانَ مِنْ عَلَقٍ* اقْرَأْ وَرَبُّكَ الْأَكْرَمُ* الَّذِي عَلَّمَ بِالْقَلَمِ* عَلَّمَ الْإِنسَانَ مَا لَمْ يَعْلَمْ).

صدق الله العظيم

Supervisor's Certification

I certify that this project was prepared under my supervision at the Department of Biology, College of Education, Salahaddin University-Erbil, and I do hereby recommend it to be accepted as partial fulfillment of the requirements for the BSc. in Biology**.**

Signature:

Supervisor

Assist. Prof. Dr. Zhian R. Salih

Date: 6 / 4 / 2023

Signature:

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Head of the Department of biology

I confirm that all the requirements have been fulfilled.

Date:

DEDICATION

This effort I dedicate to **Allah** Almighty, my lord, my powerful foundation, my source of inspiration, wisdom, knowledge, and understanding. Throughout this project, he was the source of my energy.

ACKNOWLEDGEMENTS

First of all, I thank Allah, who gave me strength and health and facilitated the ways for me to carry on my studies.

I would like to thank the Department of Biology, College of Education, Salahaddin University-Erbil. It is my privilege to express my most profound sense of gratitude and indebtedness to my supervisor Assist. Prof. Dr. Zhian R. Salih for their interest, close supervision, constant encouragement, and giving generously of their time, advice, and guidance to get this study completed.

Most importantly I would like to thank my family, especially my mother for making all my dreams come true and being a strong wall. It is her love for science that inspired me to follow a career in biology. Her courage, love, and strength are what crafted me into the person I am today, and I owe every success to her. I want to thank my father for his love, care, help, and support which allowed me to carry on, through both the good and difficult days. I would like to thank my brother and sisters for being supportive and loving. I am extremely lucky to have the support and love of my family.

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Air Pollution and Concentration of Heavy Metals in Blood

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Abstract

The present study aimed to investigate the effect of heavy metal pollution results from the gasoline station on some hematological parameters including red blood cells (RBC), hemoglobin (Hb), hematocrit, Total white blood cells (WBC), lymphocytes, thrombocytes, and Red Cell Distribution Width (RDW). The study included 30 male samples (15 workers worked in a different location of a gasoline station within Erbil City and the other 15 samples were taken in A Malakan region away from the pollution and used as a control group. The study has been conducted during the period from November 2022 to March 2023. The results showed significantly higher concentrations of the Fe, Co, Ni, Pd, Cu, Se, Hg, and Zn metals in the blood serum of the workers who work in gasoline stations when compared with the control group.

Regarding the years of working, the workers who work ≥26 years in gasoline stations showed significantly higher concentrations of the serum Fe, Co, Ni, Pd, Se, and Hg and lower Cu and Zn when compared with the workers who work 15-20 and 21-25 years. Workers exposed to metal pollution have a significantly lower concentration of RBC, Hb, hematocrit, Total white blood cells WBC, and lymphocytes as compared with the control males. The workers who work ≥26 years in gasoline stations showed significantly lower concentrations of the RBC, Hb, hematocrit, Total white blood cells WBC, and lymphocytes when compared with the workers who work 1520 and 21-25 years.

Keywords: gasoline station; Heavy metals; RBC; Hb, WBC, Pollution; Lymphocytes

INTRODUCTION

Air is considered a basic requirement of human health and well-being. However, air pollution continues to pose a significant threat to health worldwide(Abbas and Abbas, 2021). Air pollution is the contamination of the indoor or outdoor environment by any chemical, physical or biological agent that modifies the natural characteristics of the atmosphere(Solé et al., 2023) .The combined effects from outdoor (ambient) air pollution and indoor (household, in particular) air pollution cause approximately 7 million premature deaths every year, largely as a result of increased mortality from stroke, Ischemic heart disease (IHD), Chronic obstructive pulmonary disease (COPD), lung cancer, and acute respiratory infections (WHO, 2022). the source of air pollution are numerous and can be divided into two categories. Natural sources include volcanic eruption, pollens, windblown dust, and forest fires and Anthropogenic source include automobiles, industries, burning of fossil fuels, agricultural activities, and radioactive fallout (Ganguly et al., 2021).

A substance in the air that can be adverse to humans and the environment is known as an air pollutant. Pollutants can be in the form of solid particles, liquid droplets, or gases. In addition, they may be natural or man-made. Pollutants can be classified as primary or secondary, Air pollutants are either pollutant that is emitted into the air directly from a source named primary pollutants or created into the air during primary pollutants' reactions and in the presence of sunlight secondary or often named precursor (Hassan et al., 2022). Individual inhalation exposure to six air pollutants, including $PM_{2.5}$, inhalable particulate matter (PM_{10}), nitrogen dioxide (NO_2), sulfur dioxide (SO_2), carbon monoxide (CO), and ozone (O_3), was estimated by combining the daily concentrations of these air pollutants and the participants' daily breathing volume (Qiu et al., 2023).

Particulate matter represents a good index for other air pollutants. Its impact on public health is more effective than other pollutants. Regarding health effects, two particulate matter categories are considered: PM10 particles with a diameter of 10 microns or less, and PM2.5 with a diameter of 2.5 microns or less. PM2.5 has many adverse health effects since it can penetrate deep inside the lungs, and reach the blood circulation system (Abbas and Abbas, 2021). As one of the major causes of cardiovascular disease, dyslipidemia contributes to 4.40 million premature deaths worldwide (Hu et al., 2023).

Metal ions are required to keep the human body healthy because several critical biological functions in humans depend upon their presence, and their absence or scarcity may lead to diseases. However, a few metal ions, particularly the called heavy metal ions, such as mercury and lead, can be dangerous due to their toxic effects (Gupta, 2018). Heavy metals are elements with atomic weights between 63.5 and 200.6 and densities of more than 5 g/cm^3 (Ethaib et al., 2022) Heavy metals are classified into two main types: essential and non-essential (Aljanabi et al., 2022). Heavy metals are either essential (Mo, Mn Cu, Ni, Fe, Zn) or non-essential metals (Cd, Ni, As, Hg, Pb). Essential metals maintain the metabolism of the human body Cu is essential for hemoglobin formation, and carbohydrate metabolism but if these are present in excess, they cause cellular damage (Bharti and Sharma, 2022).

Human exposure to heavy metals can include the emission and absorption of fine particles through the lungs, ingestion of contaminated food or water, and dermal exposure to products containing heavy metals (Saleh et al., 2021). Heavy metals are linked to red blood cells and are eliminated mainly slowly by the urine but some of them like lead accumulated in the skeleton, and are released only slowly from this body compartment. When not digested the heavy metals accumulated in the human body become very toxic and cause many problems to human health (Azize, 2018). In human beings, heavy metal toxicity causes neurological disorders nervous system damage, multiple organ damage, Blood composition damage, Alzheimer's disease, cancer, etc.

(Pandey and Kumari, 2023). Function Previous research studies have shown high levels of heavy metals in the blood of workers of different industrial activities and locations like gasoline station workers (Saleh et al., 2021). Certain heavy metals are known to generate free radicals which may lead to oxidative stress and cause other cellular damage The mechanism of free radical generation (Engwa et al., 2019) .

Benzene is one of the hazardous factors that poison the workers exposed to it in petroleum filling stations, its severity depends on the duration of exposure, its concentration in the air, and the age and health of the person exposed (Al-Obaidi et al.) Gasoline station workers stand close to the fuel dispenser during their service period, receiving no relevant training, and working in a location where the benzene concentration is greater than 50 ppb (Tongsantia et al., 2021). If unprotected individuals are exposed for long periods, it may lead to permanent suppression of bone marrow functioning, accompanied by a reduction in the formation of new blood cells causing aplastic anemia.

Long-term exposure to gasoline at gas stations affected RBC parameters and platelet count (Teklu et al., 2021). Our pilot study showed that even at low exposures to benzene, some gasoline station workers had an increased carcinogenic risk based on long-term exposure. Benzene released from fuel vapors can be harmful when inhaled, especially among people working in gasoline stations

The most common symptoms reported by gasoline station workers were headache, fatigue, throat irritation, nose irritation, nausea, dizziness, and depression, Mild symptoms of benzene exposure, include irritation of the respiratory system, skin, and eyes. Prolonged exposure affects the nervous system, causing headaches, dizziness, fatigue, etc., and also affects blood circulation, causing symptoms, such as bleeding spots, epistaxis, and leukemia (Tongsantia et al., 2021).

The present study aimed to:

1- Determination of concentration of heavy metals in blood serum for workers in gas stations.
2- The determination of the concentration of heavy metal in blood serum depends on the year of workers working in the gasoline station.
3- Evaluation of the effect of heavy metals pollution on some hematological parameters of exposed workers.

2. Materials and Methods

2.1. Area of study

The study was conducted from Ankawa gasoline station (polluted site) which is located in a crowded street with cars causing high amounts of Pollutants in Ankawa of Erbil City and the study was also conducted in the mountainous areas of Malakan (control site).

2.2. Questionnaire and Data Collection

Data were collected over one month by questionnaire interviews focusing on (years of exposure, working time, smoking habits, medication history, and alcohol consumption ,age).

2.3. Study design

The blood samples were taken from fifteen gasoline station workers, in addition to taking blood samples from fifteen people as a control group living at Malakan without a history of gas station work. The control group is not different from the group of workers who works in the gasoline station in terms of gender and age groups.

2.4. Sampling collection

This study was carried out on people who work in gasoline stations. Samples were taken from 15 gas station workers15 samples were taken in a control group. About 10 ml of the blood was drawn from the forearm vein of each worker. 5ml blood was added into EDTA-containing polypropylene tubes and shacked gently to be used for the determination of hematological parameters and 5ml of blood was collected in serum separator tubes and placed in a centrifuge until the separation was done at 4000rpm.

2.5. Estimation of heavy metals

Estimation of heavy metals by Flame Atomic Absorption Spectrophotometer (FAAS- Phoenix 986 AA United Kingdom -UK.) was done according to the procedure of the manufacturer (Wu, 2023).

2.6. Hematological parameters

RBC, Hb, hematocrit, Total white blood cells WBC, lymphocytes, and RDW were done by an automated digital counter, which was a fully automated hematology analyzer performing complete blood count (CBC). (Getu et al., 2020).

2.7. Statistical analysis

Analysis of the data was performed using SPSS (Version 17). Results are expressed as means ± standard error. An independent t-test was used for the comparison of the studied parameters between control and workers who were exposed to heavy metal pollution. Analysis of variance (ANOVA) and Tukey's post-Hoc test were used for the comparison of the studied parameters between 1520, 21-25, and ≥ 26 years of working. A p-value equal to or less than 0.05 was considered to be statistically significant

3.Result and Discussion

3.1. Concentration of heavy metals in serum

Benzene, which is a major organic component of gasoline stations, is known as one of the predominant toxic air pollutants in the atmosphere. Environmental exposure to benzene has long been known as a carcinogen of human blood components.(Chehrehei et al., 2023).

The results which are presented in Table 1 showed the comparison of various trace elements and heavy metals in the blood of workers with the healthy control group. Iron concentration was increased in the blood serum of gasoline station workers to (0.3%) while the level of Iron in the control group was (0.2%). The main cause of the iron increase is car fuel combustion and direct inhalation of iron.

The increase of iron is confirmed for workers at the gas stations than in control. This increase may affect the red blood cells, which may cause the breakdown of blood cells and iron exit in the bloodstream (Maktoof et al., 2019).

Results obtained in this study showed a significant decrease in copper and zinc metal levels in comparison to the control group. The level of Zinc in the blood of workers in gasoline stations was (20ppm) while the level of control was (26ppm).and also level of copper in the blood of gasoline station workers was (21ppm) while that of the control group was (28ppm). These results are consistence with those who reported that; long to moderate benzene-exposed individuals are under oxidative stress due to decreased levels of antioxidants, including copper and zinc, in the plasma and red blood cells (Ajeel et al., 2021).

The concentration of lead in the whole blood of workers during the study period was (5.3ppm) and in control (1.4ppm). Lead in workers' blood serum in gasoline stations have high levels compared to control because of direct exposure to lead. Lead is a non-essential metal and is toxic to humans even at low concentrations (Husien et al., 2020).

The result showed a significant increase level of Hg in the blood of gasoline station workers (0.31ppm) compared to the control group (0.031ppm). The inhalation of mercury vapor can produce harmful effects on the nervous, digestive, and immune systems, lungs, and kidneys, and may be fatal (Basu et al., 2023).

The result showed a significant increase level of Nickel in the blood of gasoline station workers (11.11ppm) compared to the control group (8.07ppm). Workers may be harmed by exposure to nickel, Exposure to nickel may lead to cancer of the respiratory tract (Genchi et al., 2020). The result showed a significant increase level of cobalt (Co) in the blood of gasoline station workers (3ppm) compared to the control group (1.5ppm) Exposure to cobalt may cause cancer. Workers may be harmed by exposure to cobalt and cobalt-containing products (Alacabey et al., 2017).

Results obtained in this study showed a significant increase in selenium metal levels in the blood of gasoline station workers (0.3ppm) compared to the control group (0.1ppm). Excessive amounts of selenium commonly cause gastrointestinal effects (eg. diarrhea and vomiting) (Genchi et al., 2023).

Table 1. The concentration of heavy metals in the blood serum of gasoline station workers and the control group.

Heavy metals	Workers	Control	P-value
Fe (%)	0.3	0.2	0.05
Co (PPM)	3	1.5	0.01
Ni (PPM)	11.11	8.07	0.01
Pd (PPM)	5.3	1.4	0.001
Cu (PPM)	21	28	0.001
Se (PPM)	0.3	0.1	0.01
Hg (PPM)	0.31	0.031	0.001
Zn (PPM)	20	26	0.01

3.2. Concentration of heavy metals in worker's blood serum according to the period of working.

Table 2 show a significant increase in the level of (Fe, Co, Ni, Pb, Se, and Hg) in the blood of gasoline station worker as years of exposure increased and show a significant decrease in Zn and Cu as years of exposure increased.

Table 2. Effect of years of working on heavy metals concentration in the blood of gasoline station workers.

Heavy metals	Years of Working		
	15-20 (n=5)	**21-25(n=5)**	**≥26 (n=5)**
Fe (%)	0.3 [c]	0.4 [b]	0.6[a]
Co (PPM)	1.4 [c]	2.1 [b]	3.2 [a]
Ni (PPM)	9.5 [c]	10.3 [b]	11.5 [a]
Pd (PPM)	4.1 [c]	4.8 [b]	5.4 [a]
Cu (PPM)	36 a	25	20
Se (PPM)	0.1 [c]	0.3 [b]	0.5 [a]
Hg (PPM)	0.1 [c]	0.2 [b]	0.6 [a]
Zn (PPM)	21 a	17 b	15 c

3.3The hematological parameters

The results of this study showed that there are significant differences in RBC, the mean of RBC for the workers and the control groups was (5.6 and 4.9×10^6 cell/mm^3 respectively), and also The study showed significant differences in the concentration of blood Hb, the mean of Hb concentration for the workers and the control groups was (14.9 and 13.8 mmHg), and also there are significant differences in the level of hematocrit was (38 and 34%). In general, the possible reason for an

increment in RBC parameters of petrol filling workers might be because petrol is known to contain high concentrations of carbon monoxide which can enter the blood via the respiratory system. The molecule had a high affinity for Hb up to 200 times compared with oxygen to form carboxyhemoglobin which interferes with blood's oxygen transport capacity and results in tissue hypoxia. Tissue hypoxia stimulates erythropoiesis which finally leads to the production of more RBC, in turn, rises in Hb and HCT levels (Shnaa, 2021). Gasoline caused a significant increase in total white blood cell (WBC) count for the workers was greater than the control group (7.1 and 6 $\times 10^3$ cell/mm^3 respectively) which could be a result of the body's defense mechanism trying to protect the body from being vulnerable to infections having been poisoned by a foreign body (gasoline).

The study showed significant differences in the lymphocyte percentage in the workers and for the control groups (37.4 and 35 % respectively). The results showed that the lymphocyte was greater in the workers' blood than in the control group, it could be because of the defensive role of the lymphocyte against diseases and inflammations that could happen in the workers' bodies because of exposure to gasoline always (Muhamad and Omer, 2022).

The study showed significant differences in the platelets **(**Thrombocytes) percentage, the mean of the platelets for the workers and the control groups was (190 and 181respectively). The platelet number for the workers was greater than the control group, it could be because the gasoline has a stimulation effect on the bone marrow which causes increasing in megakaryocyte formation. There are significant differences in RDW for the workers and the control groups (41 and 30). A high
RDW means that there's variation in the size of your red blood cells beyond what's considered normal. A high RDW may be a sign of anemia (Teklu, 2021)

Table 3. Effect of gasoline exposure on some hematological parameters among workers.

Parameters	Workers	Control	P values
RBC ($\times 10^6$)	5.6	4.9	0.01
Hb(mmHg)	14.9	13.8	0.05
Hematocrit (%)	38	34	0.05
Total WBC ($\times 10^3$)	7.1	6	0.05
Lymphocytes (%)	37.4	35	0.05
Thrombocytes ($\times 10^3$)	190	181	0.05
RDW	41	30	0.01

3.4. Correlations of the duration of Years of working with hematological parameters

Table 4 shows The hematological parameters like HCT levels, RBC count, hemoglobin, platelet count, lymphocyte percent, and WBC decreased as years of exposure increased. Therefore, chronic exposure to benzene may lead to permanent suppression of bone marrow functioning, accompanied by a reduction in the formation of new blood cells causing aplastic anemia (Teklu et al., 2021).

Table 4. Effect of years of working in a gasoline station on some hematological parameters.

Hematological parameters	Years of working		
	15-20 (n=5)	**21-25(n=5)**	**≥26 (n=5)**
RBC ($\times 10^6$)	5.5 [a]	5.3 [a]	4.9 [b]
Hb(mmHg)	14.8 [a]	14 [b]	13.7 [c]
Hematocrit (%)	38 [a]	37.4 [b]	36 [c]
Total WBC ($\times 10^3$)	7.4 [a]	6.8 [b]	6 [c]
Lymphocytes (%)	37.4 [a]	36.9 [b]	35 [c]
Thrombocytes ($\times 10^3$)	194 [a]	189 [b]	187 [b]
RDW	40.8 [a]	40.5 [a]	41.3 [a]

4. Conclusions

Higher concentrations of the Fe, Co, Ni, Pd, Cu, Se, Hg, and Zn metals were seen in the blood serum of workers who work in gasoline stations. By increasing the years working in gasoline stations the concentrations of the serum Fe, Co, Ni, Pd, Se, and Hg were increased but Cu and Zn were decreased. The concentration of RBC, Hb, hematocrit, Total white blood cells WBC, and lymphocytes in the gasoline was decreased. The workers who work ≥26 years in gasoline stations have lower

concentrations of the RBC, Hb, hematocrit, Total white blood cells WBC, and lymphocytes when compared with the workers who work 15-20 and 21-25 years

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