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Estimation of some heavy metals and biochemical variables in blood and soil samples from Kirkuk city, Iraq

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ABSTRACT

This study is focused on the determination of some heavy metals levels (Se, Pb and Cd) with the study of biochemical parameters in blood samples, such as superoxide dismutases (SOD), catalases (CAT), glutathione peroxidases (GPX), glutathione reductases (GRX), vitamin C and vitamin. The samples were collected from 50 individuals at four distinct fuel stations in Kirkuk city in Iraq. To obtain the practical data, atomic absorption spectrometer, UV spectrophotometer and high performance liquid chromatography were used. The results indicated remarkable differences in the concentrations of Se, Pb and Cd in the analyzed samples compared to the control group. These Levels were lower in fuel station workers, whereas and the Pb and Cd concentrations were higher in their blood. Additionally, the results of chemical variables showed a decline in the antioxidant concentrations (SOD, GPx, Vit C, Vit E). In terms of oxidative stress (MDA), there was a significant increase in its concentration among the fuel station workers. In addition, the concentration of toxic heavy metals (Pb, Cd) in the soil of fuel stations was measured using the graphite furnace atomic absorption spectrometer. The Pb and Cd levels in the soil were within the permissible limits, indicating no adverse impact of on the soil.

Key words: Heavy metals, Fuel stations, Antioxidant, Oxidative stress, Biochemical parameters.

Introduction

The environmental pollution with heavy metals has become a significant global concern due to its detrimental impact. Heavy metals exhibit toxic behavior when its level passed the permission ran. The impacts on humans become apparent cumulatively, leading to various diseases such as cancer, mutagenesis, and chronic inflammation caused by lead and cadmium (Bose-O'Reilly et al., 2010; Mohammed et al., 2022). Workers at fuel stations expose to multiple heavy metals like lead, mercury, and cadmium, resulting in severe health issues. These metals can diffuse in the environment and accumulate in the human body to be toxic at elevated levels (Georgieva, 2002). For instance, lead and cadmium, induce oxidative stress by generating free radicals and the presence of malondialdehyde (MDA) serves as an indicator for determining oxidative stress (Mortada et al., 2001; Husien et al., 2020). Antioxidants play a vital role as the primary defense against free radicals. The human antioxidant defense system comprises enzymatic and non-enzymatic systems, including superoxide dismutases (SOD), catalases (CAT), glutathione peroxidases (GPX), glutathione reductases (GRX), vitamin C, and vitamin E. These mechanisms constitute the internal defense systems of the body, safeguarding against cell damage induced by free radicals (Zainal, 2022; Yadav et al., 2016). Furthermore, these enzymes rely on cofactors such as copper, zinc, and selenium to activate the maintaining of cellular functions and preventing oxidation. The significance of antioxidants has become paramount with heigh exposure to free radicals (Pais & Jones, 1997). Several studies have been conducted within fuel station in various Iraqi provinces referring to an increase in lead and cadmium concentrations (Azize, 2018), as well as a decrease in selenium (Al-Helaly & Ahmed, 2014).

Additionally, a decline in both enzymatic and non-enzymatic antioxidant concentrations have been reported. This is attributed to environmental damage within the polluted work environment caused by emissions from gasoline vapor (Azize, 2018; Al-Helaly & Ahmed, 2014; Al-Fartosy et al., 2017). Global ecosystems have experienced widespread contamination with heavy metals due to various human activities, including the transfer of these metals in the food chain, posing a threat to human health. Environmental problems, notably escalating over the past 25 years (Singh & Kalamdhad, 2011).

Soil, whether in urban or agricultural areas, serves as a fundamental repository for metals released into the environment from diverse human sources. When these metals enter the soil. A proportion of these metals remain relatively stable due to their nature, while others are more variable, migrating either to groundwater or plants (biotic availability). There is a broad consensus within the scientific community about the hypothesis to the risks associated with the presence

RESEARCH ARTICLE

of heavy metals for living organisms (Selim, 2013; Alamgir, 2016). It is reported that toxic metal levels in soil near fuel stations increase due to emissions from the stations (Arya & Geetha, 2019).

The main goal of this work is to determine the concentration of some heavy elements (Se, Pb and Cd) in the blood of workers and concentration of (Pb and Cd) in the soil at the same fuel stations in addition to analyses of some biochemical parameters, such as SOD, CAT, GPX, GRX, Vit C, and Vit E. This is the first research aiming to present data on the blood of workers and the soil collected from the same fuel stations in Kirkuk city in Iraq.

Materials and Methods

Blood analyses

Blood samples were collected from 50 individuals exposed to heavy metals from four different fuel stations in Kirkuk province for this study. The control group was consisted of 20 individuals not exposed to pollution who do not work in the industrial sector.

A 10 mL blood sample was taken from each individual and placed in a sealed plastic tube free from any anti-coagulant materials (plain tube). The samples were left at room temperature until clotting occurred, then placed into centrifuge (model, country) for 10 minutes at a speed of 3000 revolutions per minute. Serum was drawn using a fine pipette and used for further chemical and biochemical analyses

The studied metals (Se, Pb and Cd) were determined using atomic absorption spectrophotometer (AAS) (Nova-350, Germany) (Parsons, 1997) and some biochemical variables (SOD, GPx and MDA) were assessed using UV spectrophotometer (HANNA Photometer, Romania) (Karatas et al., 2003; Günzler et al., 1974; Guidet & Shah, 1989). The vitamin (Vit C, Vit E) levels were measured using high performance liquid chromatography (HPLC) (Shimadzu, Japan) (Romero Rodriguez et al., 1992; Driskell et al., 1982).

Soil analyses

Soil samples for the study were collected near the four fuel stations. The samples with 5-15 cm depth were taken with a plastic spoon to avoid cross contamination and labeled. Each sample was dried under sun light and sieved (0.05 mm). The samples were kept in labeled polyethylene bags and then used for heavy metals determination.

0.5 g of each sample was transferred to a beaker 150 mL and washed with few drops of deionized water and 10 mL of concentrated HNO_3, then left for 30 minutes. The excess of acid was evaporated until 15 mL of a mixture acid solution ($HCl:HNO_3$) (1:2) after a good mixing with 35 mL of deionized water was added. 5 mL of the previous eluent was added to 5 mL of hydrazine solution (4%), then 5 µL from this sample injected to run the analysis.

The metals (Pb and Cd) in the soil were assessed using the graphite furnace atomic absorption spectrometer (GFAAS) (NOVA-350, Germany) (Ahmad & Ansari, 2022).

Statistical analysis

The experimental data was statistically analyzed using analysis of variance (ANOVA) and the results were summarized and presented in means and standard deviations.

Results and Discussion

Blood analyses

The results indicated various concentrations of Se. They were (52.211 ± 0.912, 63.033 ± 1.369, 49.362 ± 0.867 and 48.138 ± 2.110) mg/mL at station 1, 2, 3 and 4 respectively as shown in Table 1. However, the Se concentration was (71.361 ± 1.680) mg/mL for the control group. This reveals that the Se level in workers from the fuel stations was lower compared to the control group referring to be a source of threat on the health of the workers by its impact on enzymes deficiency. We consider that this result can be attributed to the polluted environment. Se is considered a fundamental component for many enzymes, such as glutathione peroxidase, which has the ability to prevent chronic diseases (Kucharzewski et al., 2003). It is well known that Se deficiency affects negatively on the immune system (Ferenčík & Ebringer, 2003). Our result is in agreement with a research conducted in Turkey (Kömüroğlu et al., 2017), which reports a decrease in the Se concentration in the blood

Table 1. *Concentrations of (Se, Pb, Cd) in the blood serum of fuel station workers compared to the control group*.

Element	Mean±SD				
	Station 1	Station 2	Station 3	Station 4	Control
$Se_{(mg/mL)}$	52.211±0.912	63.033 ± 1.369	71.361 ± 1.680	48.138 ± 2.110	49.362 ± 0.867
$Pb_{(mg/mL)}$	0.813±0.019	0.682 ± 0.024	0.197 ± 0.016	1.062 ± 0.020	0.710 ± 0.035
$Cd_{(mg/mL)}$	0.0731±0.0018	0.0821 ± 0.028	0.01838 ± 0.018	0.0693 ± 0.081	0.0743 ± 0.036

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serum of fuel station workers. However, our result differs from a study in Mosul, Northern Iraq, where no significant differences were found in the Se concentration among workers at a petrol station (Al-Fartosy et al., 2017).

The results of Pb concentrations in the blood serum were 0.813 ± 0.019, 0.682 ± 0.024, 0.710 ± 0.035 and1.062 ± 0.020 mg/mL at stations 1, 2, 3 and 4 respectively, while for the control group the Pb concentration was 0.197 ± 0.016 mg/mL (Table 1). These results indicate that the Pb levels in the fuel station workers was once again higher compared to the control group. Pb is known as a toxic element leading to functional impairment in various human organs in addition to damaging the central nervous system and blood formation (Ara & Usmani, 2015). Furthermore, the high level of Pb causes an oxidative damaging by generating free radicals (Reactive Oxygen Species-ROS) (Carocci et al., 2016). These results are in line with other research conducted on fuel station workers (Al-Rudainy, 2010; Pranjić et al., 2002) indicating that the Pb levels are higher in workers compared to control groups.

The results of Cd concentrations clearly show a significant increase compared to the control group as summarized in Table 1. This change in statistical results indicates on contaminated studied area with the Cd. The elevated Cd concentrations among industrial workers can be linked also to industrial polluted sites (Nordberg et al., 2004). Additionally, Cd occurs naturally with zinc and lead in sulfide ores. Cd has a direct connection with certain chronic diseases, such as hypertension. High blood pressure can be taken as an excellent indicator of Cd exposure for individuals working in the fuel stations, leading to increase systolic and diastolic blood pressure (Caciari et al., 2012). These results are consistent with other research (Al-Terehi et al., 2021; Azize, 2018) indicating higher Cd levels in fuel station workers.

The SOD concentrations in the blood serum of workers from the fuel stations were 1.426 ± 0.04, 1.522 ± 0.10, 1.442 ± 0.02 and 1.62 ± 0.036 mg/mL at station 1, 2, 3 and 4, respectively as illustrated in Table 2. In contrast, the enzyme concentration in the control group was 2.03 ± 0.232 mg/mL. These results indicate that the SOD enzyme level for the workers at the fuel stations is lower than the results of the control group. This decrease of the SOD enzyme level is related to the polluted environment. This enzyme has a significant effect on human essentially to protect the body's cells from free radicals that promote aging or cell death (Krishnamurthy & Wadhwani, 2012). Additionally, trace elements (Zn, Cu, Mg) are components of SOD and are associated with antioxidant functions. The enzyme deficiency may make the control over free radicals to be vulnerable. The replacement of these elements with Pb diminishes the activity of SOD (Reena et al., 2012). This result is comparable with other research (Azize, 2018; Al-Fartosy et al., 2017) referring to a decrease in the enzyme concentration in fuel station workers.

The GPx concentrations in the blood serum of workers from the fuel stations workers were lower compared to the GPx concentrations in the control group due to the enzyme concentration levels were (6.372 ±0.921, 6.760 ±0.921, 4.316 ± 0.413 and 5.326 ± 0.168) µ/mg at stations 1, 2, 3 and 4 the enzyme concentration in the control group was 8.328 ± 0.362 µ/mg as summarized in Table 2. This decline corresponded with the pollution effects present in fuel stations, where the levels of heavy metals, such as Pb and Cd increase because of the inhalation from petroleum products. Consequently, these toxic metals reduce the antioxidant capacity leading to increase oxidative stress and inflammation (Vidal-Liñán et al., 2010). This results agree with the literature (Al-Fartosy et al., 2017, Rizk et al., 2020) confirming that the GPx enzyme levels tend to be lower in fuel station workers.

The MDA concentrations in the blood serum of workers at the fuels stations increase compared to the MDA concentrations analyzed in the control group as the concentrations for fuel station workers were 6.02 ± 0.89, 5.76 ± 0.123, 7.02 ± 0.63, 5.39 ± 1.02 mmol/l. In contrast, the concentration for the control group workers was 3.432 ± 0.1426 (mmol/l). These results indicate that this rise is ascribed to the previously mentioned reason representing to

Table 2. *Concentrations of SOD, GPx, MDA in the blood serum of fuel station workers compared to the control group.*

Parameter	Mean±SD				
	Station 1	Station 2	Station 3	Station 4	Control
$SOD_{(mmol/l)}$	1.426 ± 0.04	1.522 ± 0.10	1.442 ± 0.02	1.62 ± 0.036	2.03 ± 0.232
$GPx_{(\mu/mg)}$	6.372 ± 0.921	6.760 ± 0.921	4.316 ± 0.413	5.326 ± 0.168	8.328 ± 0.362
$MDA_{(mmol/l)}$	6.02 ± 0.89	5.76 ± 0.123	7.02 ± 0.63	5.39 ± 1.02	3.432 ± 0.1426

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the heavy metal levels. This leads to enhanced production of ROS and decreases the antioxidant defense system in cells and rises the concentration of MDA (Hussain et al., 1999; Whaley-Connell et al., 2011). Our result is consistent with other research (Azize, 2018; Al-Fartosy et al., 2017) conducted on fuel station workers, indicating a significant increase in the MDA concentrations in similar environment.

Table 3 illustrates that the concentration of vitamin C in the blood serum of fuel station workers is lower compared to the vitamin C concentration measured in the control group. This can be explained by the depletion of this vitamin when exposed to heavy metals. Vitamin C has the ability to directly eliminate free radicals such as hydroxyl and superoxide ions (Hafez & Kishk, 2017). These results are in line with the literature (Azize, 2018; Al-Fartosy et al., 2017) indicating that heavy metals emitted from fuel stations reduce the vitamin C levels in blood, consequently increasing oxidative stress and inflammation in the body.

The concentration of vitamin E in the blood serum of workers was low compared to the concentration in the control group at all studied fuel stations (1-4). This decline is ascribed as the increased level of lipid peroxide MDA, as Vitamin E's function is to prevent MDA formation. This plays a crucial role in protecting individuals from oxidative stress (Mocchegiani et al., 2014). These results confirm other results (Azize, 2018; Al-Fartosy et al., 2017), showing a decrease in the vitamin E concentration in the blood of fuel station workers.

Soil analyses

The Pb concentrations in the soil collected from the studied fuel stations were 2.017 ± 0.751, 3.852 ± 0.862, 4.168 ± 0.933 and 3.095 ± 0.896 mg/kg at stations 1, 2, 3, and 4 respectively (Table 4), while the target value for Pb concentration in soil according to the World Health Organization (WHO) is 85 mg/kg. According to our results, the Pb levels are within the permission range of the World Health Organization (WHO) standards (Ernst, 1996). The accumulation of toxic heavy metals poses a risk to human health, especially Pb generated from the combustion of Pb-containing fuels. Tetraethyl-Pb is used in some petroleum products to enhance engine performance, impacting the environment directly (Hussein et al., 2020). This study does not agree with another study (Arya & Geetha, 2019) showing an increase in the Pb levels in soil samples from fuel stations. Therefore, we suggest that further research needs to be performed in order to fully assess if there could be any negative impact on the environment and human health.

The Cd concentrations in the soil were 0.273 ± 0.122, 0.246 ± 0.009, 0.872 ± 0.163 and 0.243 ±0.008 mg/kg as shown in Table 4. However, the permitted concentrations for Cd in soil according to the WHO standards are 0.8 mg/kg (Ernst, 1996). Consequently, the Cd concentrations in the soil from the fuel stations were within the standards set value by WHO. Cd is known as a heavy metal, accumulating in soil and strongly binding to its particles, causing significant environmental damage (Kubier et al., 2019). These results disagree with another study (Arya & Geetha, 2019) that indicated an increase in the Cd concentrations in the soil for fuel stations.

Table 3. *Concentrations of (Vit C, Vit E) in the blood serum of fuel station workers compared to the control group.*

Vitamins	Mean±SD				
	Station 1	Station 2	Station 3	Station 4	Control
Vit C$_{(mg/dl)}$	0.86 ± 032	0.61 ± 0.34	0.77 ± 0.18	0.59 ± 3.40	1.16 ± 0.79
Vit E$_{(mg/dl)}$	0.91 ± 1.20	0.73 ± 3.40	0.78 ± 0.41	0.84 ± 0.25	1.16 ± 0.15

Table 4. *Concentrations of (Pb, Cd) in the soil of fuel stations*

Elements	Mean±SD				
	Station 1	Station 2	Station 3	Station 4	Target value of soil (mg/kg)
Pb$_{(mg/kg)}$	2.017 ± 0.751	3.852 ± 0.862	4.168 ± 0.933	3.095 ± 0.896	85
Cd$_{(mg/kg)}$	0.273 ± 0.122	0.246 ± 0.009	0.872 ± 0.163	0.243 ± 0.008	0.8

*Top soil (0-15) cm

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Conclusions

Determination of some heavy elements was successfully performed in the blood serum of fuel stations workers in Kirkuk city along with analyses of some biochemical parameters. The results indicated that occupational exposure to heavy metals (Pb and Cd) among numerous fuel station workers in Kirkuk city promotes also oxidative stress. In addition, this generates high levels of free radicals and MDA compared to the control group. The decline in antioxidant levels, including GPx, SOD, vitamin E, C, and Se can be related to the oxidative stress effect. However, the soil analyses indicate that there is no effect on the concentrations of studied metals. As a result, we suggest that control tests should be conducted regularly for workers in fuel stations and providing them with antioxidant and vitamin doses depending on the control tests results. This becomes vital for those workers exposed to long-term pollution in order to get protection for the immune system.

Lastly, the Pb and Cd levels in the soil were within the permissible limits, indicating no adverse impact.

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