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# The Effect of Heavy Metals in Mining Emissions on Worker's Blood Lead Levels: A Case Study of The Gumushane Zn-Pb Mine, Turkey

**Highlights:**

- Analysis of heavy metals in underground mine water,
- Determination of the amount of Pb in the blood of mine workers,
- Water pollution

**Keywords:**

- Blood lead levels (BLLs)
- Heavy metals,
- Mining
- Wastewater
- ICP-MS

## ABSTRACT:

The wastewater from mining facilities contains toxic elements such as arsenic (As), cadmium (Cd), chromium (Cr), manganese (Mn), zinc (Zn), and lead (Pb). If not disposed of correctly, it can lead to environmental pollution in case of accidents or overflow. Lead-zinc mining activities pose a significant risk of high blood lead levels (BLLs) among workers. Therefore, monitoring the blood lead levels of workers exposed to these heavy metals, especially lead, is crucial.

This study aims to determine the levels of potentially harmful elements in the wastewater dam of the largest Zn-Pb mine in the Black Sea Region. It also seeks to assess the environmental and human health risks comprehensively. Additionally, the study aims to establish a relationship between the levels of lead in the mining wastewater in Gumushane and the lead levels accumulated in the blood of workers exposed to this metal.

The study analyzed heavy metals such as As, Zn, Cu, Sb, and Pb in mining wastewater using Inductively Coupled Plasma – Mass Spectrometer (ICP-MS). The results indicated high levels of Pb in the studied wastewater. Furthermore, the blood lead levels of workers in the mining area (n=30; mean: 7.42 µg/dL) exceeded background levels (>40 µg/dL). These results suggest a significant relationship between the presence of lead in wastewater and high lead levels in the blood of individuals with high exposure to concentrated lead.

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**Ethics Committee Approval:** ''The Effect of Heavy Metals in Mining Emissions on Worker's Blood Lead Levels: A Case Study of Zn-Pb Mine, Turkey'' the ethics committee application on the subject was discussed at the meeting of the Gümüşhane University Scientific Research and Publication Ethics Committee dated 22/04/2024 and numbered 2024/4; it was unanimously decided that the project complies with the current legislation.

## INTRODUCTION

Heavy metals are a group of metals and metalloids that can harm human health when exposed in excess. Some of these metals, such as Ag, As, Be, Cd(II), Cr(VI), Cu(II), Hg, Ni(II), Pb(II), Sb, and Zn, are toxic and lack any known beneficial or biological functions (Granero & Domingo, 2002). Certain essential micronutrients such as Zn(II), Cr(III), Fe(II), and Co(II) are present in the metal active sites of enzymes (Virág et.al,2016). These micronutrients play crucial roles in biological systems, including the human body. However, at high levels, they can be toxic despite retaining their biological function.

Lead (Pb) is a group 4A element with atomic number 82 and an atomic mass of 207.2 g/mol. It is a silver-bluish white metal found in small amounts in the earth's crust. It is highly malleable, ductile, and a relatively poor conductor of electricity. Lead's main oxidation states are +2 and +4, with +2 being the prevalent form in the environment. The solubility of lead compounds in water depends on factors such as pKa, hardness, salinity, and the presence of humic material (U.S. ATSDR, 2007). Industrially synthesised organic lead compounds, such as alkyl-lead compounds, are fuel additives to prevent engine knocking. Human exposure to these compounds occurs through inhalation of leaded petrol vapours, dermal exposure to leaded petrol, mining, and ingesting lead-contaminated soil, food, or water. These compounds can convert to divalent lead ions in the body. Alkyl-lead compounds are part of the EPA's program to reduce their use and develop safer alternatives. Pb is commonly found in acidic water and exists in various forms, such as $PbSO_4$, $PbCl_2$, and ionic lead. In water, tetraalkyl-lead compounds undergo breakdown into inorganic lead oxides (Hill, 2005; UNEP, 2008). Depending on the presence of other ligands, Pb speciation differs in freshwater and seawater (UNEP, 2008). Some Pb settles in bottom sediment as a long-term sink for the metal (OECD,1993). The average background content of Pb in bottom sediments is around 30 to 45 mg/kg, but in polluted rivers, the lead content ranges from 700 to 2.600 mg/kg due to industrial and mine discharges (Kabata-Pendias,1999). Pb adsorption decreases with water hardness, and under specific pH conditions, lead either precipitates into bed sediments or repels from the adsorbent surface.

Inorganic Pb compounds are not metabolized and accumulate in bones at a rate of 90%. Organic Pb compounds have a high affinity for fat and nerve tissue. Triethyl Pb is metabolized to trimethyl Pb by the cytochrome P450 enzyme system within 24 hours following exposure. The second product of organic Pb compounds is the conversion to inorganic Pb (Goyer, 2001; Ellenhorn, 1997). Pb accumulation in the human body causes increased oxidative stress, lipid peroxidation, and damage to cell membranes, resulting in overall cell damage. Pb inhibits the activity of 5-aminolevulinic acid dehydratase, leading to haemoglobin oxidation and potential red cell hemolysis (Pourrut et al., 2011). Additionally, increased Pb in blood (B-Pb) concentration alters the balance between reactive oxygen species (ROS) and antioxidants, leading to oxidative stress and cell damage (Figure 1).

Lead disrupts the function of necessary antioxidant enzymes such as superoxide dismutase and catalase (Violante et al., 2010). However, it also interferes with glutathione, a key player in maintaining the body's balance and defence against reactive oxygen species (ROS). When lead attaches to glutathione, it is ineffective, leading to increased oxidative stress (Batool et al., 2017). Even a tiny amount of lead deposition in the human body can result in cellular malfunction and severely damage an individual's health.

The region of Gumushane, located east of the Black Sea, is significant in terms of metallic mines. Gold, copper, lead, and zinc are the main metallic mines that form important deposits in the region. In this study area (Figure 2), grades 2.27-7.18% Cu, 1.38-9.98% Pb, and 4.98-18.57% Zn were determined. The mining reserve is 198,000 tons of processed and 400,000 tons of possible reserves.

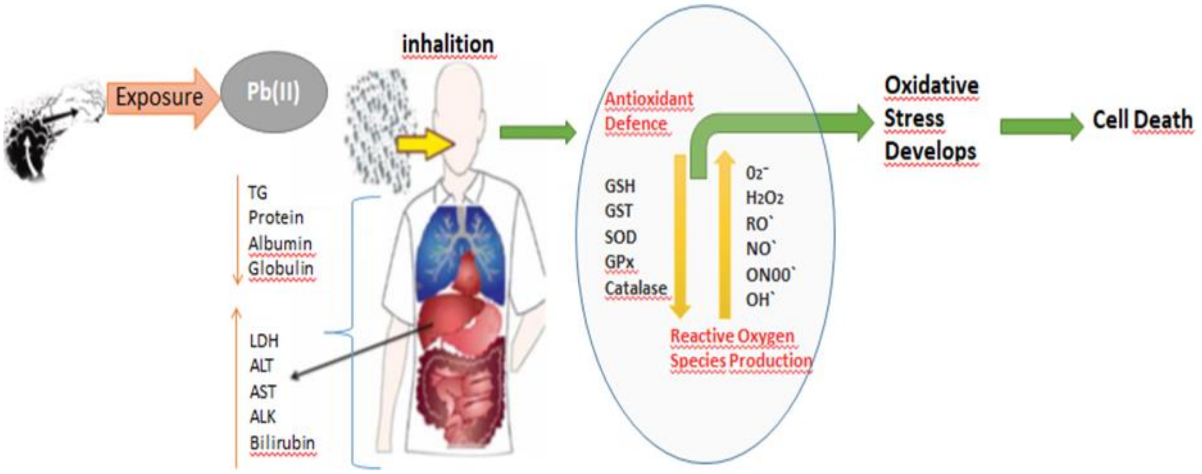

**Figure 1**. Schematic Presentation of Lead Distribution in Humans

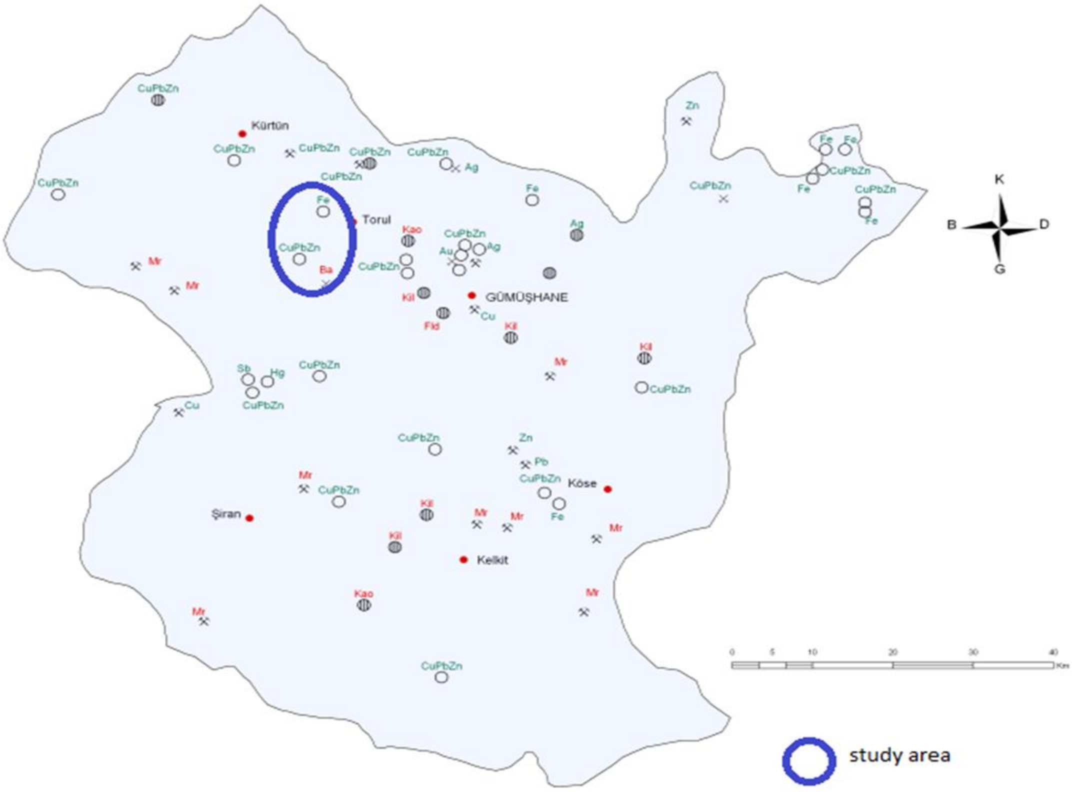

**Figure 2.** Mineral Map of Gumushane and Sampling Study Area (MTA, 2023)

The basic ores in lead production are galena (PbS), anglesite ($PbSO_4$) and cerussite ($PbCO_3$). Most ores contain less than 10% lead. Ores containing more than 3% lead are economically suitable for producing lead. These ores are first crushed and ground. Then, they are subjected to a froth flotation process to increase the lead content to over 70%. Sulfide ores are subjected to a roasting process. As a result of this process, the ore is separated into lead-oxide, lead-sulphate and silicates. The lead-oxide obtained is melted in the blast furnace and reduced. At the end of this process, a large portion of the lead in the ore becomes metallic. At the end of the process, 3 separate layers are formed on top of the molten metallic lead layer. These plates contain slag (silicates containing around 1.5% lead), sulphides containing 15% lead and iron or copper arsenite. These plates, which are by-products of the process, contain economically usable copper, zinc, cadmium and bismuth. The main product obtained is molten metallic lead, which still contains significant amounts of arsenic, antimony, bismuth, zinc, copper, silver, and gold. The molten mixture is passed through a sulfuric furnace with air and vapor, causing impurities other than silver, gold, and bismuth to oxidize. The oxidized metals rise to the top of the mixture and are separated from the lead in this way (Alakabak, 1993; Uysal, 1987).

Exposure to Pb and its resulting toxicity can have detrimental effects on nearly every system in the body, making it a critical environmental health and safety issue. The most obvious and common way lead enters the body is through inhalation. Lead vapor and dust reach the lungs through the respiratory tract and pass from the lungs into the blood. Some large particles that cannot reach the alveoli melt in the mucosa, and some are thrown back. The remaining particles enter the digestive system by being swallowed. Absorption from the gastrointestinal tract varies depending on the body's calcium, iron, fat, and protein levels. The mining industry involves many risks (dust, gas, noise, vibration, ventilation, etc.) in production processes (Özbolat & Tuli, 2016). Underground mine waters are one of these risks. In underground mines, water accumulates in the pit for various reasons. Surface waters entering the pit interact with heavy metals from ore and side stones. This situation often poses a serious risk to employee health (URL, 2024). Prolonged exposure to these metals, particularly in enclosed spaces like the mining industry, poses health risks. Pb poisoning is a significant public health concern, accounting for 0.6% of the Global Burden of Disease (WHO, 2010) and 540,000 deaths worldwide. It is well-documented that lead, a toxic heavy metal in the environment, causes various health problems in the human body when individuals are exposed to dust, water, and contaminated food through ingestion and inhalation. Lead has many negative effects, from neurological and cognitive disorders to kidney and cardiovascular system damage. In addition, it has been reported in various studies that low blood lead levels increase the risk of death due to circulatory diseases and cancer (Byun et al., 2020). Pb enters the human body and primarily binds to hemoglobin, which is efficiently distributed. Excretion occurs through urine and feces, with a half-life of about 30 days in the blood. Approximately 76-94% of lead in the body accumulates in bones and teeth. The binding limit value is 70 µg Pb/100 ml blood, and individuals above 40 µg Pb/100 ml blood should be kept under surveillance (Bertram et al., 2022). Occupational exposure limit values are provided in Table 1.

**Table 1.** Occupational Exposure Limit Values of Pb (Mevzuat, 2013)

| **EINECS (1)** | **CAS (2)** | **Name of chemical stsubances** | **Limits Long Time TWA (3) (8 hour)** | | **Limits Short Time STEL (4) (15 min.)** | | **CEILING (5) High limit** | | **Trans mission of disease** |
|---|---|---|---|---|---|---|---|---|---|
| | | | **mg/m$^3$** | **ppm** | **mg/m$^3$** | **ppm** | **mg/m$^3$** | **ppm** | |
| 233-046-7 | 75-74-1 | Tetramethyl lead | 0.075 | - | - | - | - | - | skin |
| 233-046-7 | 78-00-2 | Tetraethyl lead | 0.075 | - | - | - | - | - | skin |
| 201-159-0 | 7439-92-1 | Inorganic lead and its compounds | 0.15 | - | - | - | - | - | skin |

(1) EINECS: European Inventory of Existing Commercial Chemical Substances. (2) CAS: Service registration number of chemical substances. (3) TWA: Time-weighted average measured or calculated for a specified reference period of 8 hours. (4) STEL: The upper limit value for exposure that should not be exceeded for a period of 15 minutes unless another period is specified. (5) CEILING-Ceiling value: Exposure limit value that must not be exceeded during any part of the working period.

In mining operations involving lead and ionic lead compounds, it is necessary to measure the blood lead level using biological monitoring, absorption spectrometry, or an equivalent method as specified in ANNEX-2 of the Regulation on Health and Safety, in order to comply with Biological Limit Values and Health Surveillance Measures.

When working with chemical substances, it is important to measure lead (Pb) in ionic lead compounds and lead itself. This can be done using biological monitoring, absorption spectrometry, or an equivalent method. It's important to note that lead can also be absorbed through the skin. Once in the body, about 85-90% of the lead is absorbed into the membranes of red blood cells, 1% is transported freely, and the rest is bound to albumin. When lead particles are inhaled, 90% of them are absorbed into the body. Lead remains in the blood for about 30 days and is excreted from the bones over 27 years (Özbolat & Tuli, 2016). Lead has significant negative metabolic effects on the human body. Some of these effects include disrupting the activity of glucose-6-phosphate dehydrogenase, leading to the loss of red blood cells. Lead also inhibits the ferrochelatase enzyme, reducing iron (Fe) incorporation into haemoglobin and causing anaemia (Charkiewicz et al., 2020; Zhushan, 2020). This imbalance also leads to oxidative damage due to glutathione (GSH) and glutathione disulfide (GSSG). Additionally, it depletes antioxidants rapidly, leading to an increase in reactive oxygen species in the body. While liver enzymes such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT), as well as total bilirubin values, increased in lead-exposed rats, these values decreased with flavonoid treatment, alleviating the liver damage caused by lead (Wafa et al., 2019). The half-life of lead is 20-30 years in the skeleton and one month in the blood. Consequently, lead exposure may lead to various disorders, such as irritability, headache, central nervous system issues, and abdominal pain. Moreover, it has been scientifically proven that an increase in lead levels causes a decrease in IQ (Buchet et al., 1990; Lars, 2003).

## MATERIALS AND METHODS

This study was carried out between January 2, 2023, and July 31, 2023, in a mine in the Torul-Köstere district of Gümüşhane (Figure 2), among twelve different occupational groups at risk and mine workers not involved in any at-risk occupation. The study comprised 30 male mine workers exposed to lead in their profession, carefully selected from the 20-55 age group. The study population comprised individuals from among twelve at-risk occupations: fortifier, operator, firer, loader, underground truck operator, truck driver, geological engineer, electrician, mining engineer, concrete transmixer operator, and technician. The study aimed to investigate their exposure to lead present in both the mine's wastewater and its reserves, considering the proximity of their work areas to these sources.

### Heavy Metal Analysis of Mining Wastewater

The research involved three sampling stations, with three samples taken at each station. Wastewater samples from mining were analyzed for heavy metals using the ICP-MS device (Agilent Technologies 7700, Japan). This analysis was conducted due to the potential impact of mine operation wastewater (Bertram et al., 2022). The water samples were filtered with a 0.22 μm pore size filters and acidified with 0.5% v/v $HNO_3$, and stored in polyethylene bottles. All chemicals used were of analytical-reagent grade. Standard solutions for the elements were prepared by diluting a multi-element standard (ICP standard Multi IV) of 1000 mg/L Al, As, Cd, Co, Cr, Cu, Ni, Pb, and Zn obtained from Merck (Darmstadt, Germany). The $HNO_3$ 65% and $H_2O_2$ 30% were also from Merck. ICP–MS operating conditions are given in Table 2.

**Table 2.** Operating Conditions for ICP-MS

| | | | |
|---|---|---|---|
| **Gas** | Argon | Water temperature | 15-45 °C |
| **Argon gas pressure** | 500-700 | TMP RPM | 95-100 % |
| **Helium gas pressure** | kPa | Nebulizer pump speed | 0.3 rps |
| **Helium gas flow rate** | 90-130 kPa | Sampling | 15/42 sec |
| **Plasma gas flow rate** | 4.5 mL/min | Integration time | 0.1-1 sec/Point |
| **Plasma Power** | 15 L/min | Software | Mass Hunter(Version B.01.03) |
| | 1550 W | | |

### Determination of blood lead levels of workers

The research involved individuals aged 20-55 with a blood lead level below 40 µg/ml who did not have cancer or ischemic heart disease. Blood samples were collected from workers exposed to the environment by the workplace doctor and the state hospital. Lead levels in the blood were analyzed in the biochemistry laboratory of an accredited institution. Blood samples (9–10 mL) were taken from all workers, centrifuged, and stored at −40 °C until further analysis. The samples were collected in standard commercial evacuated tubes containing sodium heparin (13x100 mm/6ml Fıratmed heparin tubes). Thirty blood samples from miners were analyzed using the Rank Hilger H. 1550 model, graphite-containing tube 500 µL heparinized blood samples were diluted 1: 4 with 2 ml % 0.1 Triton X-100 and vortexed.

A calibration line was drawn with 100, 200, 400, and 600 ppb standard solutions using AAS, and Pb levels in blood samples were measured accordingly. All experiments were conducted three times at each concentration, resulting in a linear regression with a coefficient of determination ($R^2$) of 0.9989. Table 3 shows the linear dynamic range (LDR), coefficient of determination ($R^2$), limit of detection (LOD), limit of quantification (LOQ), and relative standard deviation (RSD %).

**Table 3.** Analytical Performance Data of the Method for the Determination of Lead

| **Element** | **Linear range, µg/L** | **Coefficientof determination, $R^2$** | **LOD, µg/L** | **LOQ, µg/L** | **RSD, % (n=3)** |
|---|---|---|---|---|---|
| Pb | 4-10 | 0.99894 | 0.99 | 3.31 | 2.6 |

The detection of heavy metals can be achieved using various methods and techniques such as fluorescence, UV-VIS spectrometry, and electrochemical detection. In this study, AAS was used for the determination of Pb content in blood samples and ICP-MS for wastewater samples due to their accuracy and precision. The process of sample preparations and analysis is outlined in Figure 3.

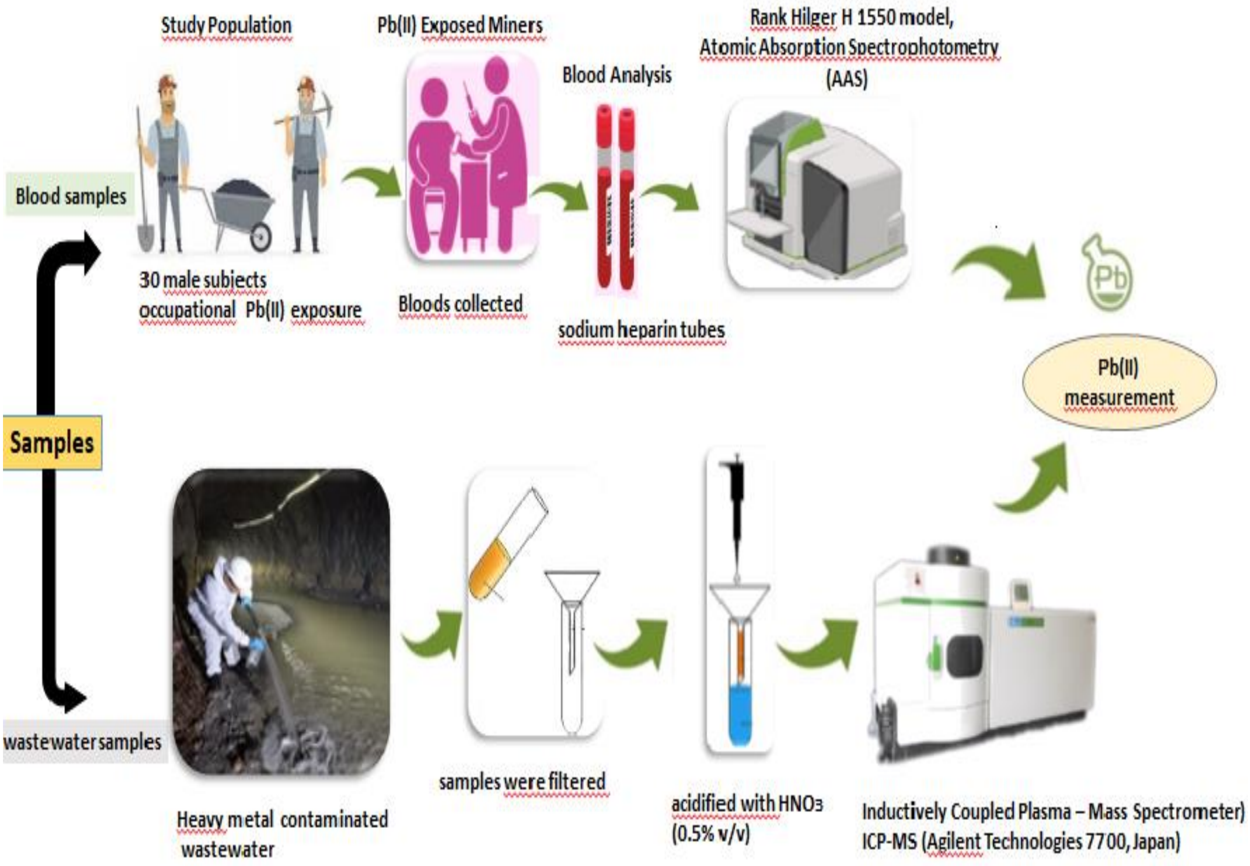

**Figure 3**. Schematic Analytical Procedure of the Sample Preparation and Analysis

## RESULTS AND DISCUSSION

### Results of Heavy Metal Levels in Wastewater Samples

The study obtained the following results by analyzing underground mine water samples from different sites: Three different wastewater settling pools within the mine. The results of three parallel studies and their averages are shown in Table 4.

**Table 4.** The Average Concentration of Heavy Metals in Wastewater Pools (P)**,** (ppb)

| Heavy metals | Test method | P1435 | P1454 | P1470 | Average(µg/L) |
|---|---|---|---|---|---|
| **Pb** | **SM 303/K-EPA 200.7** | **174.2** | **176.74** | **188.01** | **179.65** |
| Zn | SM 303/K-EPA 200.7 | 5.41 | 5.65 | 6.28 | 5.78 |
| As | SM 303/K-EPA 200.7 | 12. 5 | 12.55 | 13.88 | 12.96 |
| Cu | SM 303/K-EPA 200.7 | 46.21 | 46.88 | 48.27 | 47.12 |
| Cr | SM 303/K-EPA 200.7 | 40.12 | 41.56 | 46.63 | 42.77 |
| Cd | SM 303/K-EPA 200.7 | 1.08 | 1.21 | 3.47 | 1.92 |
| Ni | SM 303/K-EPA 200.7 | 50.14 | 51.87 | 56.72 | 52.91 |
| Co | SM 303/K-EPA 200.7 | 4.23 | 4.36 | 4.67 | 4.42 |
| Al | SM 303/K-EPA 200.7 | 798.2 | 801.5 | 825.8 | 808.50 |

SM: Standard methods for the examination of water and wastewater.
EPA: Environmental Protection Agency.

When the results were examined, it was found that many heavy metals, especially lead 179.65 (µg/L) exceeded the permissible limit in wastewater (Table 5). According to the International Agency for Research on Cancer (IARC), Pb is a possible carcinogenic substance in humans (Jarup, 2003). According to the US EPA, the regulatory limit for Pb in drinking water is 15 µg/L (Martin et al., 2009). The WHO recommended safe limits of Pb in wastewater and soils used for agriculture are 0.01 and 0.1 ppm, respectively (Chiroma et al., 2014). The World Health Organization (WHO) and the American Environmental Protection Agency (USEPA) have set the limit values for lead at 0.01 ppm (Table 5) (WHO, 1992; Poyraz, 2014; US EPA, 2014). Upon examination, it was found that the mining wastewater contained elevated levels of several heavy metals, particularly lead, exceeding the permissible limit.

**Table 5.** Some Acceptable Heavy Metal Concentrations in Wastewater By USEPA and WHO (ppm)

| Heavy metals | WHO (mg/L) | USEPA (mg/L) |
|---|---|---|
| Zn | 5.00 | 5.000 |
| Cd | 0.30 | 0.010 |
| Cu | 3.00 | 1.300 |
| Cr | 0.05 | 0.400 |
| **Pb** | **0.01** | **0.006** |
| Ni | 0.02 | 0.200 |

### Results of Pb levels in blood samples

Mine worker’s blood lead levels are categorized into three outcomes. The biological limit value is the maximum concentration of the chemical substance and its metabolite in the appropriate biological environment, serving as an indicator of its effect. Medical surveillance is conducted ‘if the average lead concentration in the air exceeds 0.075 mg/m$^3$ over a 40-hour workweek, and if any employee's blood lead level is over 40 µg Pb/100 ml of blood. According to the Biological Limit Values and Health Surveillance Measures specified in ANNEX-2 of the Regulation on Health and Safety Measures in Working with Chemical Substances; for ionic lead compounds and lead’ the phrase is included and it also includes the measurement of Pb using biological monitoring, absorption spectrometry or a method that can provide equivalent results. According to (Table 6) BLLs was defined as an elevated blood lead level (EBLL) of ≥40 µg/dL

**Table 6.** Blood Lead Levels (mean ± SD) for Working Groups (µg/dL)

| Sample | Mining workers | Blood lead levels (*µ*g/dL) |
|---|---|---|
| 1 | Fortifier | 10.01±2.52 |
| 2 | Bucket operator | 6.74±1.14 |
| 3 | Jumbo operator | 6.30±2.74 |
| 4 | Firer | 7.71±3.33 |
| 5 | Loader | 8.00±4.66 |
| 6 | Underground truck operator | 7.73±2.12 |
| 7 | Operator | 4.23±2.81 |
| 8 | Operator | 4.50±3.21 |
| 9 | Operator | 4.30±2.14 |
| 10 | Fortifier | 9.66±3.45 |
| 11 | Truck driver | 4.70±1.22 |
| 12 | Geological engineer | 6.71±2.33 |
| 13 | Electrician | 6.93±5.14 |
| 14 | Bucket operator | 7.76±1.44 |
| 15 | Firer | 8.41±2.21 |
| 16 | Fortifier | 8.93±3.47 |
| 17 | Electrician | 5.91±6.98 |
| 18 | Bucket operator | 7.70±4.45 |
| 19 | Fortifier | 8.72 ±3.33 |
| 20 | Bucket operator | 7.83±2.11 |
| 21 | Mining engineer | 10.51±1.31 |
| 22 | Bucket operator | 7.37±2.78 |
| 23 | Jumbo operator | 6.93±3.55 |
| 24 | Concrete transmixer operator | 10.03±4.77 |
| 25 | Electrician | 6.20±1.36 |
| 26 | Underground truck operator | 7.14±2.77 |
| 27 | Bucket operator | 7.24±3.63 |
| 28 | Fortifier | 9.15±2.85 |
| 29 | Jumbo operator | 7.60±.3.96 |
| 30 | Technician | 8.40±1.78 |
| | **Average** | 7.42±2.55 |
| | **Max** | 10.51±1.31 |
| | **Min** | 4.23±2.81 |

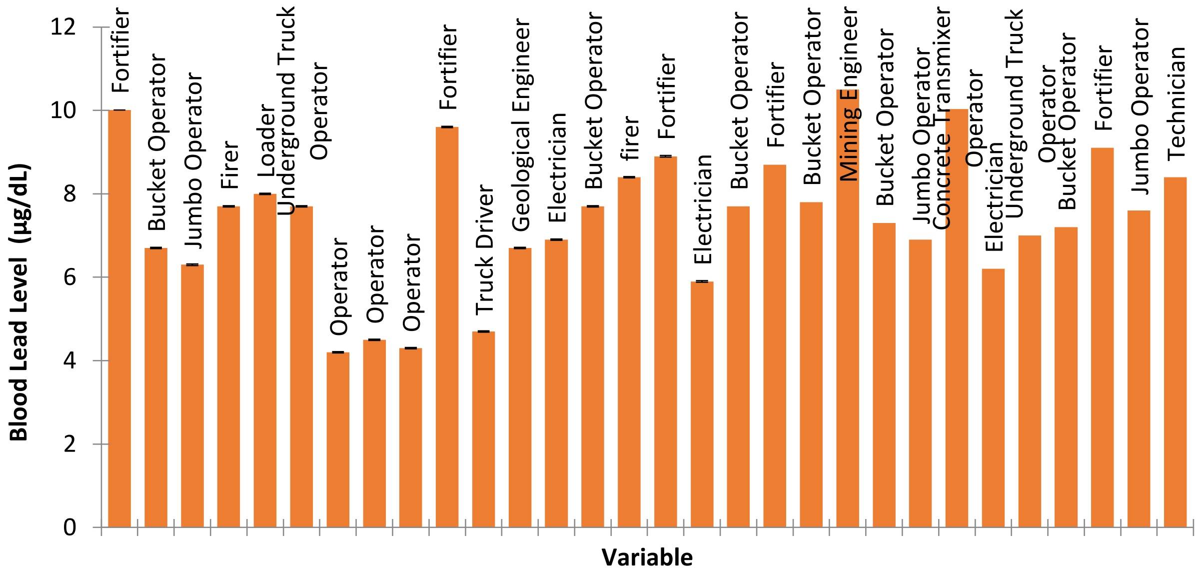

**Figure 4.** Graph of Lead Levels in the Blood of Miners Exposed to Lead

The average blood lead detected in the 30 blood samples included in the study was 7.42 µg/dL. The blood lead values of the miners measured with AAS were found lower than permission limit. No significant difference was found in the distribution of blood lead levels when looking at proximity to the wastewater sample. If we look at the distribution according to professions that are more related to mineral reserves (Figure 4) fortifier and mining engineer constitute the group with the highest blood lead levels.

## CONCLUSION

In this study, lead concentrations in mine wastewater and workers' blood were determined, and these results were evaluated by comparing them with exposure limit values. Study demonstrates that the determination of lead in blood can be achieved using the Triton X-100 and hemolysis method with Atomic Absorption Spectrophotometry (AAS), and ICP-MS provides a simple and fast method for determining lead in wastewater.

The average blood Pb level of the workers was 7.42±2.55 µg/dL (min: 4.23±2.81µg/dL; max: 10.51±1.31 µg/dL), which did not exceed the limit. However, according to the analysis of the mine wastewater, the lead exceeded the allowed limit of 179.65 (µg/L). This study highlights the need for strong policies to reduce lead exposure among workers in the mines of Gümüşhane province. It also emphasizes the importance of using appropriate methods to discharge water from wastewater dams. Depending on the industry's structure and the receiving environment, it is essential to remove specific substances from wastewater before discharge. Melting lead, producing and using lead oxides must be in a completely closed system. As long as there is no fault or leakage in the system, it is the most effective method for protection from lead. Literature research, workplace air measurements, and blood lead levels have shown that the sections with the highest exposure to lead are lead melting, ore enrichment, and laboratory sections. Industrial facilities must establish treatment plants to prevent environmental pollution. In addition to initiating and enforcing safe mining practices, miners in this province must regularly screen for lead toxicity. Making the arrangements required to process, use, transport, and store lead waste, a hazardous chemical substance, in the workplace is essential. To ensure the health and safety of employees, a non-hazardous or less hazardous chemical substance should be used instead of the hazardous chemical substance by applying the substitution method. If the substitution method cannot be used due to the nature of the work performed, the risk should be reduced by taking some measures in order of priority, according to the risk assessment results. Employees who work with wastewater should undergo regular health check-ups. They should also be provided with gloves, full face masks, and liquid-proof overalls to ensure their safety. If an employee's blood test shows heavy metal levels above the legal limits, they should be temporarily assigned to a different role within the company. If they return to their original position after treatment, their exposure time should be reduced.

### Conflict of Interest Statement

The article authors declare that there is no conflict of interest between them.

### Author Contributions

The authors declare that they have contributed equally to the article.

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