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Bio-availability and Bio-accessibility of Lead and Zinc in Contaminated Soil from Cwymystwyth Lead Mine Site

Y. Abdullahi [a], A. S. Aska [b], C. Roberts [c], M. S. Abdu [d] and J. Gambo [d*]

[a] Department of Biology, Aminu Saleh College of Education, Azare- Bauchi State, Nigeria.
[b] Department of Chemistry, Aminu Saleh College of Education, Azare-Bauchi State, Nigeria.
[c] Department of Environmental Science, University of Wolverhampton, U.K.
[d] Department of Forestry Technology Binyaminu Usman Polytechnic, Hadejia, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Cwmystwyth Lead Mine was an abundant mine site with pugh's and kingside water drainages shows contaminated water in the research area with no much scientific evidence to ascertain the level of the pollution. Hence this research was designed to study level of lead and zinc in contaminated soil in which the bio-availability and bio-accessibility were measured. Sixteen (16) soil samples were taken at random using soil auger and a hand trowel. The samples were dried using an oven set at a constant temperature of 400^{o}C for 72 hours. Wire mesh (250 microns) was used to sift the samples. The Unified (BARGE) method was used. The mimics mixtures of saliva, gastric, duodenal, and bile fluids. Three-stage mimic processes were performed, in the mouth, the stomach and intestinal cavities. All mimic digestive fluids were placed in the rotator water bath for 1hr at 37^{o}C. The bioaccessibility of the soil Samples were analyzed by inductively coupled plasma-optical emission spectrophotometer (ICP-OES) method. The results were obtained using XRF and ICP methods. The percentage concentration of lead in the topsoil was 0.64% and in the bottom soil was 1.47%, with a total mean concentration of 1.06% in combined top and bottom soil. Zinc

**Corresponding author: E-mail: jagfati4real@gmail.com;*

concentrations in the top and bottom soils were 0.22 and 0.45%, respectively, with a computed total mean of 0.34%. The findings revealed a highly significant difference between lead and zinc in both the top and bottom soil samples (LSD = P0.05). The average concentrations of lead and zinc extracted in both the stomach and intestinal stages were 15.98% and 1.23%, respectively

Keywords: Bio-availability; bio-accessibility; lead; zinc.

1. INTRODUCTION

Soil from contaminated sites, particularly mining areas where Lead, Zinc, and other toxic or harmful elements have been explored for a long time, may have a higher availability of toxic metals pollutants because the soil has larger soil particles, allowing these elements to sink to ground water levels. Pb and Zn could be ingested by humans and other animals if they drink or consume plants that have these metals deposited on them.

Ingestion of dust is the most significant route of exposure to environmental toxins. Children, in particular, can inhale soil and dust through willful hand-to-mouth motions or accidentally by consuming food that has fallen on a polluted floor [1]. In addition to inhalation of suspended soil particles, Pb exposure to children living near pb polluted areas includes related soil ingestion via hand-to-mouth actions [2].

Although children may be exposed to Pb as a result of their childlike behaviour of putting their hands in their mouths and eating Pb-polluted toys and other items, it is unlikely that they will ingest it (ATSD,1999C). Due to the abundance of pollutants floating freely in the environment, breathing is the most common route of contamination.

Many inorganic compounds have the potential to be dangerous, thus it's important to understand/comprehend their sources, transport, and fate in nature, as well as the paths through which they might be transferred to people. Understanding the principal sources of Pb is crucial to risk assessment. Pb is transported via air, water, soil, and food channels, as well as contaminant fate consolidated with exposure pathway focus on the concentration of chemicals with which individuals may come into contact through oral, inhalation, or skin/eye contact [3].

All ingestion routes pass through the nasal and vocal cavities before reaching the systemic circulation. Because food is essential for the growth and development of the human body, the link between creatures in the food chain may result in the transfer of pb detrimental effects to people for those who live outside of mining zones. However, because pb impacts the neurological and circulatory systems, it may influence the growth rate of both sexes. It may also impair growth hormone-producing glands. Without proper protection measures, abandoned mine waste would quickly spread through the air, water, and precipitation, contaminating nearby agricultural lands [4]. Chemicals released into the air, water, or farming land may also find their way into human bodies via a route sequence. Although various human activities, including fuel burning, agriculture, mining, and municipal waste incineration, have contributed to the increased Pb level in the environment [5]. Pb in the soil, on the other hand, comes predominantly from mining, as well as from the refinement of parent rocks and, in general, from leaded petroleum additives. Because of its poor solubility, Pb can collect and become accessible in the soil for several years, and it is resistant to microbial destruction [6]. As a result, Pb soil is important due to its toxicity to humans.

Cardiovascular, neurological, gastrointestinal, reproductive, and haematological disorders have all been linked to pb toxicity in humans [7], and as a dangerous trace element, pb has been the topic of substantial human research [5]. Bioaccessibility studies have long been used as a means of determining the possibility for human exposure to ingested contaminants. It also denotes the maximum concentration of pollutant that is specifically accessible for absorption in the intestine [8]. Bioavailability, on the other hand, could refer to the proportion of pollutants in the small intestine after ingestion of soil to the gastro intestinal phase [9]. However, Ruby et al. [10] defined bio accessibility as the portion of a contaminant that is absorbed by the body through the gastro intestinal system and is assessable to the body by eating, oral inhalation, and skin contact.

Zinc is a vital trace element with a wide range of physiological and biochemical functions. It is found in essential metallo-enzymes, as well as

major metabolic pathways and the creation of deoxyribonucleic acid (DNA). Furthermore, the human body requires a particular amount of zinc for growth and development [11]. Zinc is also said to be a common component of soil and is found in abundance in nature (Adriano, 2001), in [12]. Zinc levels in the soil continue to rise as a result of quarrying, agricultural activities, sewage slime application, fossil fuel burning, and industrial activities (Basta et al 2005) in [12]. In comparison to other PHES, zinc is readily soluble in soil and is both accessible and movable. Zinc associated to Fe, Mn oxides is the most available phase to plants in acidic low-concentration mineral soils [13]. As a result, zinc absorption can also be obtained from cereals and legumes, which are the primary sources of food in underdeveloped nations, and are thus the primary sources of zinc for the majority of the population [14]. Zinc is an essential nutrient for humans, with a recommended daily allowance (DRA) of 15mg/d for men and 12mg/d for women (USEPA, 2009). In humans, intense inhalation of anomalous zinc has resulted in dryness of the throat, coughing, and chest pain, whereas intense oral absorption of high zinc concentrations has resulted in gastrointestinal disturbance and pancreatic damage. Additional chronic oral intake has had negative effects on the blood, resulting in a drop in haemoglobin levels [15]. However, unlike prior literatures that focused solely on the phytotoxicity of zinc to plants, an excess percentage of absorbed Zn in the human body has substantial impacts [16].

2. MATERIALS AND METHODS

2.1 Sample and Sampling

Sixteen (16) soil samples were taken at random sites around the mining regions with a soil auger and a hand trowel, with the results being analysed. The samples were labelled with the numbers 1 through 16. The moist samples were transported to a laboratory for further examination and analysis.

2.2 Methodology

The methodology in this research work comprises the sample preparation, digestive fluids preparation, and the Unified Barge Method that will be used for the mimicking of the digestive organs that includes stomach and intestine.

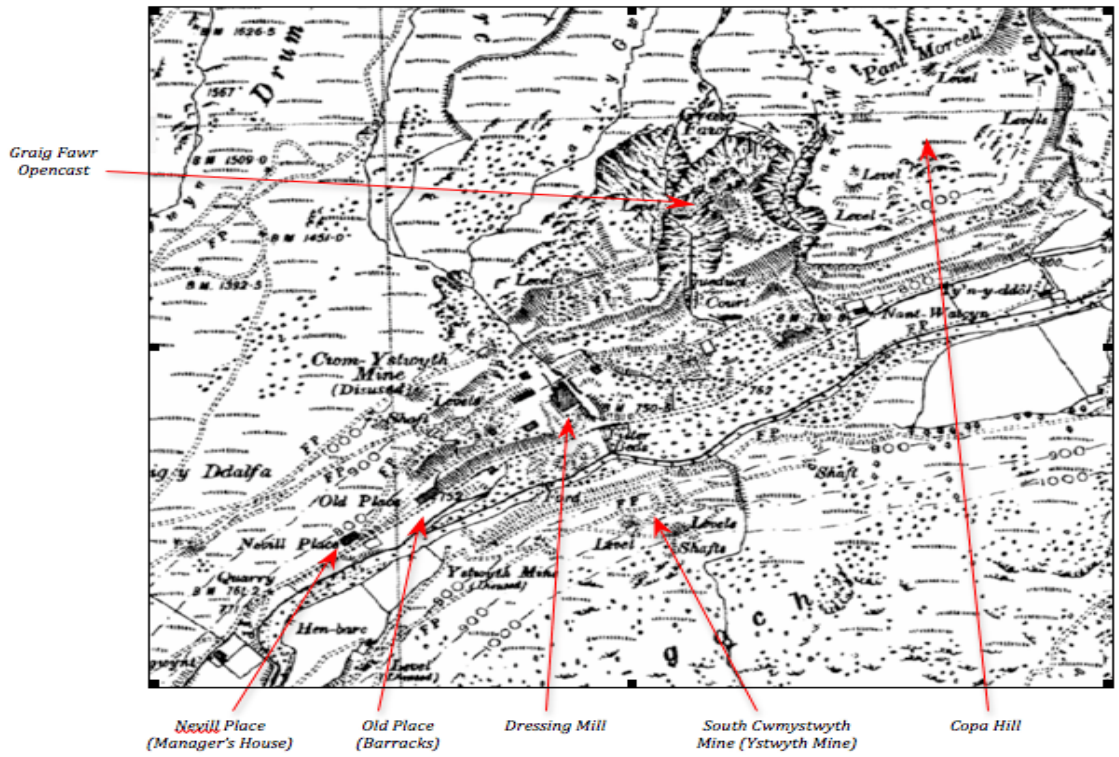

Fig. 1. Cwmystwyth lead mine
Source: http://welshminestrust.org/cwmystwyth-mine-photo-archive-2/

2.3 Sample Preparation

Using an oven set at a constant temperature of 400 degrees Celsius for 72 hours, the samples were dried to prevent any modification of the mineralogical properties of the samples [17]. Wire mesh (250 microns) was used to sift the samples. To prepare the samples for examination, they were mashed in a pestle and mortar, then sieved and used as is.

2.3.1 Digestive fluids preparation

Wragg et al. 2011, Cave and Wragg 2012, and Wragg et al. 2012 developed a standard process for the preparation of digestive fluids, which was endorsed by the Bioaccessibility Research Group of Europe (BARGE). To conduct the unified barge method (UBM) test, a mixture of saliva, gastric, duodenal, and bile fluids was prepared. Four sets of solutions were prepared, with each fluid consisting of a combination of one inorganic solution, one organic solution, and specific enzymes. Each fluid is formed from the combination of one inorganic solution, one organic solution, and unique enzymes. 3 hours were spent agitating the solutions of each fluid on the magnetic agitation system. Checking the PH values of each fluid and adjusting the values to the correct tolerance using NaOH was done (1M). All fluids were placed in the rotator water bath for one hour at 37 degrees Celsius.

2.4 Unified Barge Method (UBM)

1. (9.0 ml) of saliva fluid were added to 0.6 g of soil using pipette for the 'Gastric' and 'intestinal' extractions.
2. The extract vessels were covered, and vibrate manually for thirty seconds.
3. (13.5 ml) of gastric fluid was added to each experiment equally.
4. The pH was adjusted to 1.2 ± 0.05 using pH meters.
5. Extraction containers were remained covered and placed in the extractor, incubated in end-over-end turning, at 37 ^{0}C for one hour.
6. 'Gastric' as well as 'intestinal' extract was removed from the incubation after an hour, and the suspensions' pH was calculated.
7. At this point, if the pH of the experiment is below or above required (i.e. 1.2-1.5) then the experiment should start again from the beginning for the UBM stomach extract, until the accepted pH achieved.
8. The pH was adjusted between 1.2 and 1.5 by use of 1.0 ml of concentrated HCl.
9. The extract from the 'Gastric' phase was then centrifuged at 3600 rpm for 15 minutes.
10. Extract was orderly kept after correct addition of 0.5 ml of NHO_3.
11. For the 'intestinal' phase, 27.0 ml of duodenal fluid and 9.0 ml of bile fluid was added to mixture using pipette.
12. Then shake by hand for 30 second; and the pH was taken to confirm that it is 6.3 ± 0.5
13. If the pH is above or below required, adjustment has to be made via the dropping of 1.0 ml of (37%) of conc. HCl using pipette, or 1 ml or 10 ml of NaOH base on the requirement.
14. The mixture was taken back to incubator at 37 ^{0}C and rotate for an additional 4 hours.
15. Then centrifuged at 3600 rpm for 15 minutes.
16. (1.0 ml) of $NH0_3$ was added, and the pH was recorded at the end of the intestinal incubation time.

2.5 Bioavailability Extracts method using BARGE Method (UBM)

The soil samples were subjected to a bioavailability extraction test using the unified barge method (ubm) at 37 degrees Celsius. A three-stage mimic process was performed, starting with the mouth cavity and progressing to include the stomach and intestinal cavities, with a stomach PH of 1.20.5 and an intestine PH of 6.30.5. Using a pipette, 9.0 ml saliva fluid was added to each 0.6g of soil sample to obtain the results. The vessels containing the extracts were covered and vibrated for thirty seconds. Each experiment received 13.5 mL of stomach fluid, which was distributed evenly. It took one hour for the extraction vessels to be inserted in the extractor and incubated in the extractor with the ends turned over. After one hour of incubation, the gastric and intestinal extracts were removed from the incubation chamber and the pH was measured. The pH of the solution was adjusted using 1.0 mL of concentrated HCl. The extract from the "gastric phase" was centrifuged at 3600 rpm for 15 minutes, after which it was stored after being treated with 0.5 ml of HNO3 for 15 minutes. The intestinal phase was formed by adding 27.0 ml of duodenal fluid and 9.0 ml of bile fluids to the mixture using a pipette to create the intestinal phase. The content was shaken for 30 seconds, after which the pH was measured. To modify the pH, one milliliter of 37 percent concentrated HCl or one millilitre and ten

millilitres of NaOH were added, depending on the necessity. The combination was incubated at 370°C and rotated for a further 4 hours, after which the contents were centrifuged at 3600rpm for 15 minutes to extract the protein. The pH was measured at the end of the intestinal incubation time after the addition of one millilitre of HNO3.

2.6 Sample Data

2.6.1 Integrated XRF and ICP data

Table 1. Pb extraction in gastric (G) and intestine (INT) phase

Sample No.	XRF Pb (%)	XRF Pb (mg/kg)	G raw	G (mg/l)	Int raw	Int (mg/l)	% Pb extraction in G	% Pb extraction in INT
YA- 01B	0.879	8790	160000	1600	8300	83	18.20	0.94
YA- 02B	0.196	1960	39000	390	3200	32	19.90	1.63
YA- 03B	1.846	18460	270000	2700	17000	170	14.63	0.92
YA- 04B	2.05	20500	290000	2900	17000	170	14.15	0.83
YA- 05B	2.674	26740	400000	4000	24000	240	14.96	0.90
YA- 06B	1.112	11120	210000	2100	16000	160	18.88	1.44
YA- 07B	5.79	57900	210000	2100	18000	180	3.63	0.31
YA- 08B	2.196	21960	190000	1900	14000	140	8.65	0.64
YA- 09B	0.399	3990	84000	840	4400	44	21.05	1.10
YA- 10B	0.241	2410	42000	420	2700	27	17.43	1.12
YA- 11B	0.159	1590	55000	550	3400	34	34.59	2.14
YA- 12B	2.074	20740	840000	8400	27000	270	40.50	1.30
YA- 13B	1.419	14190	780000	7800	23000	230	54.97	1.62
YA- 14B	0.354	3540	180000	1800	12000	120	50.85	3.39
YA- 15B	0.538	5380	88000	880	1800	18	16.36	0.33
YA- 16B	0.348	3480	94000	940	5200	52	27.01	1.49
					Average		23.48	1.26
					Median		18.54	1.11
					Minimum		3.63	0.31
					Maximum		54.97	3.39
					St. Dev.		14.54	0.75

Table 2. Zn extraction in gastric (G) and intestine (INT) in percentages

Sample No.	XRF Zn (%)	XRF Zn mg/kg	G raw	G (mg/l)	Int raw	Int (mg/l)	% Zn extraction in G	% Zn extraction in INT
YA- 01B	0.302	3020	20000	200	2200	22	6.62	0.73
YA- 02B	0.019	192.7	1100	11	290	2.9	5.71	1.50
YA- 03B	0.226	2260	20000	200	2100	21	8.85	0.93
YA- 04B	0.234	2340	26000	260	2200	22	11.11	0.94
YA- 05B	0.309	3090	29000	290	3100	31	9.39	1.00
YA- 06B	0.052	524.4	1200	12	220	2.2	2.29	0.42
YA- 07B	0.117	1170	2800	28	330	3.3	2.39	0.28
YA- 08B	0.066	661.4	1500	15	170	1.7	2.27	0.26
YA- 09B	0.087	870.8	3900	39	800	8	4.48	0.92
YA- 10B	0.049	487.8	2400	24	620	6.2	4.92	1.27
YA- 11B	0.028	279.4	3100	31	590	5.9	11.10	2.11
YA- 12B	3.548	35480	520000	5200	65000	650	14.66	1.83
YA- 13B	1.557	15570	250000	2500	34000	340	16.06	2.18
YA- 14B	0.039	386.7	7000	70	1200	12	18.10	3.10
YA- 15B	0.135	1350	11000	110	1300	13	8.15	0.96
YA- 16B	0.034	344.5	3300	33	580	5.8	9.58	1.68

Sample No.	XRF Zn (%)	XRF Zn mg/kg	G raw	G (mg/l)	Int raw	Int (mg/l)	% Zn extraction in G	% Zn extraction in INT
					Average		8.48	1.26
					Median		8.50	0.98
					Minimum		2.27	0.26
					Maximum		18.10	3.10
					St. Deviation		4.89	0.77

2.7 Sample Analysis

Determine the bioavailability and bio accessibility of lead (Pb) and zinc (Zn) in humans (Zn) for the measurement of bioavailability and bio accessibility of the soil samples, the inductively coupled plasma-optical emission spectrophotometer (ICP-OES) method published by Wragg et al [8] was used. We extracted two bioavailable extracts from each sample, one at the end of the gastric phase and another at the end of the gastrointestinal phase, and analysed them separately. Every batch of sixteen (16) samples was subjected to a reference material extraction process in both the gastric and gastrointestinal phases.

3. RESULTS AND DISCUSSION

3.1 Results

The results of XRF analysis shows the concentrations of lead and zinc from the top and bottom samples collected from the soil.

Table 3. Concentration of heavy metals (Pb and Zn) in top and bottom sample of contaminated soil

Heavy metals	Concentration in soil profiles (%)		Mean (Heavy metals)
	Top soil	**Sub soil**	
Lead (Pb)	0.64	1.47	1.06
Zinc (Zn)	0.22	0.45	0.34
Mean (Soil profile)	**0.43**	**0.96**	
LSD (P ≤ 0.05)			
Heavy metals	0.19**		
Soil profile	0.19**		
Heavy metals x Soil profile	Ns		

***: Highly significant, ns: Non-significant*

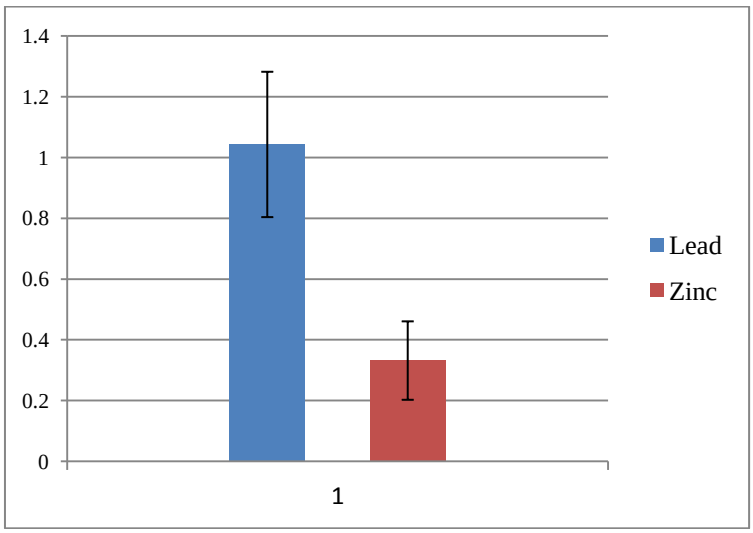

Fig. 2. Concentration of Lead (Pb) and Zinc (Zn) in the contaminated soil

Table 4. Concentration of Lead (Pb) and Zinc (Zn) in gastric and intestinal phase

Organs	Concentration (%)		Mean (Organs)
	Pb	**Zn**	
Gastric	23.48	8.48	15.98
Intestine	1.26	1.19	1.23
Mean (Heavy metals)	**12.37**	4.84	
LSD (P ≤ 0.05)			
Heavy metals	2.58 **		
Organs	2.58 **		
Heavy metals x Organs			1.92 **

***: Highly significant*

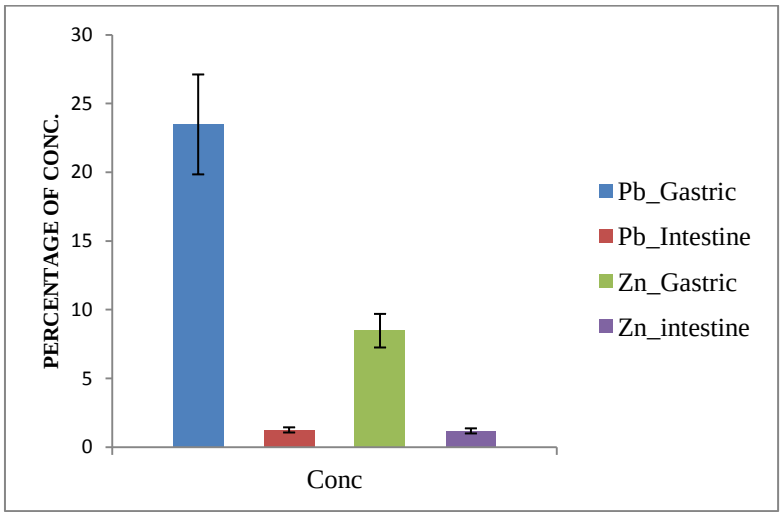

Fig. 3. the mean concentrations of lead and zinc extracted in both gastric and intestine

4. DISCUSSION

Table 1 and table 2 clearly indicates the sample Data during Integrated XRF Test and ICP Test respectively. Table 1 shows Pb extraction in gastric (G) and intestine (INT) phase while table 2 indicate Zn extraction in gastric (G) and intestine (INT) in percentages respectively.

In the results, Table 1 shows the mean percentages of lead and zinc in top soil and subsoil (bottom) samples, respectively. Overall, 1.06 percent of the total mean lead concentration was found in both top and bottom soil, with the top soil holding 0.64 percent and the bottom soil carrying 1.47 percent, respectively, resulting to 1.55 percent of the total lead concentration. However, the top soil had a zinc concentration of 0.22 percent, while the bottom soil had a zinc concentration of 0.45 percent, resulting in a total mean zinc concentration of 0.34 percent. Top soil contained 0.43 percent of the overall Mean concentration of the soil profile, while bottom soil had 0.96 percent of the total Mean concentration. Figure 1 shows the lead and zinc concentrations in the top and bottom soils, which may be found above. The mean concentration of Lead in the sample was clearly higher than the mean concentration of Zinc in the sample.

According to Fig. 1, lead and zinc had mean concentrations of 1.06 percent and 0.34 percent, respectively. Lead concentrations averaged 1.06 percent and 0.34 percent, respectively, while zinc concentrations averaged 0.34 percent. The results demonstrated a statistically significant difference between lead and zinc in both top and bottom soil samples, with LSD = P 0.05 indicating a statistically significant difference.

The average concentrations of heavy metals eliminated during the stomach and intestinal stages, as well as the extraction results, are shown in Table 2. The average concentration of heavy metals (Pb and Zn) in the stomach phase was 15.98%, while the average percentage of lead and zinc retrieved from the intestine was 1.23 percent, according to the findings.

According to the data, the total mean concentration of lead extracted in both the gastric and intestinal phases was 12.37 percent, while the total mean concentration of zinc removed in both phases was 4.84 percent. The data demonstrated that in both the gastric and intestinal phases, there is a statistically significant difference between lead and zinc removed in the stomach and intestinal stages, respectively. Another conclusion is that there is a considerable difference in the bioavailability of heavy metals in the organs compared to the extracted heavy metals. Figure 2 provides a graphical representation of the mean lead and zinc concentrations collected from stomach and intestinal tissues. The mean value of lead extracted during the gastrointestinal phase was found to be 23.48 percent higher than the mean value of zinc extracted during the gastric phase, which was 8.48 percent in this study. The mean value of Lead extracted in the digestive tract, on the other hand, was marginally higher at 1.26 percent, compared to a slightly lower mean value of Zinc extracted in the intestinal tract of 1.19 percent.

According to Palmer et al. (2015), the presence of lead in the environment can be attributed to a number of human activities, including fuel combustion, agriculture, mining, and waste incineration at municipal waste facilities, in addition to industrial processes. The majority of lead (Pb) in the soil, on the other hand, comes from mining, which supports the findings of this study. Davies and colleagues' studies show that Pb can build up in soil and become bioavailable (1995). It was determined that the soil samples and organs under scrutiny contained a significant level of lead. As a result, lead toxicity to the gastrointestinal system in humans is a significant risk, as indicated in the ATSDR (2005). This is in line with the findings of a large human investigation on harmful trace metals (Palmer, et al 2015). The results of a study on the bio-accessibility of lead (Pb) and zinc (Zn) indicated that ingested pollutants can cause human exposure. It also found that the amount of heavy metals analyzed had increased significantly, indicating that they were potentially accessible for intestinal absorption, which was in line with Wragg et al's findings (2011). According to Palmer et al. [5], the presence of lead in the environment can be attributed to a number of human activities, including fuel combustion, agriculture, mining, and waste incineration at municipal waste facilities, in addition to industrial processes. The majority of lead (Pb) in the soil, on the other hand, comes from mining, which supports the findings of this study. Davies and colleagues' studies show that Pb can build up in soil and become bioavailable (1995). It was determined that the soil samples and organs under scrutiny contained a significant level of lead. As a result, lead toxicity to the gastrointestinal system in humans is a significant risk, as indicated in the ATSDR (2005). This is in line with the findings of a large human investigation on harmful trace metals [5]. The results of a study on the bio-accessibility of lead (Pb) and zinc (Zn) indicated that ingested pollutants can cause human exposure. It also found that the number of heavy metals analysed had increased significantly, indicating that they were potentially accessible for intestinal absorption, which was in line with Wragg et al [8] findings.

5. CONCLUSION

In conclusion, the hazardous heavy metals found in the contaminated soil samples analyzed were shown to be present, and they cause severe toxicity in humans if they are inhaled. According to the findings of the research, lead and zinc are abundant in the Cwmystwyth Lead Mine, and the considerable quantities of lead and zinc present are relatively damaging to the human body. Because the concentrations of lead and zinc in Cwmystwyth Lead Mines were highly significant, this demonstrated the high level of danger to human health posed by the mine.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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