FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 10 No. 6, March, 2026, pp 103 – 110
DOI: https://doi.org/10.33003/fjs-2026-1006-4756

IN Vivo BIOACCESSIBILTY AND BIODISTRIBUTION ANALYSIS OF HEAVY METALS IN LETTUCE (*Lactuca sativa*) OBTAINED FROM IRRIGATED DUMPSITES IN MUBI NORTH, ADAMAWA STATE, NIGERIA

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ABSTRACT

The cultivation and consumption of vegetables (mainly lettuce) from farm-land in Mubi environs is of major public health concern. This study was performed to assess the bio accessibility and biodistribution analysis of some heavy metals in lettuce (*Lactuca sativa*) obtained from the three different irrigated dumpsites (Wuro-Alkali, Wuro-Gude and Mayanka). Using *in vivo* based method, a total of 15 rabbits were used and were randomly assigned to cages with five rabbits per cage. The rabbits were fed with lettuce harvested from the irrigated farm-lands for this experiment. The lungs, kidney and liver of the experimental animals were harvested and analyzed for cadmium (Cd), zinc (Zn), Iron (Fe), magnesium (Mg), chromium (Cr), manganese (Mn), lead (Pb) and copper (Cu) using Atomic Absorption Spectrophotometer (AAS).The values of Fe and Cr in kidney of Wuro-Alkali, Wuro-Gude and Mayanka are above the control values of Fe 3.63±0.04 mg/kg and Cr 0.07±0.01 mg/kg. Similarly, the value of Cr in lungs at the three dumpsites are below the control value of 0.16±0.01 mg/kg. Also, the value of Fe and Cr in liver at the three dumpsites are above the control value of Fe 0.71±0.03 mg/kg and the value of Cr in lungs at the three dumpsites are below the control value of 0.03±0.01 mg/kg. Heavy metals concentration in these dumpsites may not appear to pose very serious environmental problems at the moment. However, accumulation of these metals after several years may pose a threat to human health and the environment.

Keywords: In vivo, Lettuce (lactuca Sativa), Metals; Bioaccessibilty, Biodistribution

INTRODUCTION

The use of dumpsite soils as organic manure is widely spread in Mubi, Adamawa state, Nigeria. This practice has been shown to improve soil properties such as organic matter, nutrients, porosity, aggregate stability, bulk density and water retention, and as a result, increase plant productivity (Ogunyemi *et al*., 2003, Oluyemi *et al*., 2008). It is however a known fact that some of the waste products contain hazardous metals such as Ni, Pb, Cd, Zn, and Hg (Olarinoye *et al*.,2010), which perturb the distribution and concentration of these metals in the environment.

Heavy metals pollution of agricultural soil and vegetables is one of the most severe ecological problems on a world scale (Ahmad and Goni 2010) because of their toxicity for plant, animal and human beings, and their lack of biodegradability (Li *et al*., 2006; Jang *et al*., 2006; Zhuang *et al*., 2009). Heavy metals are ubiquitous in environment and it has been exceeded due to both natural and anthropogenic sources (Harmanescu *et al*., 2011). It poses potential threats to the environment and can damage human health through various absorption pathways such as direct ingestion, dermal contact, diet through the soil-food chain, inhalation and oral intake (Park *et al*., 2004; Al-Saleh *et al*., 2004; Komárek *et al*., 2008; Lu *et al*., 2011).

Lettuce is the common term for any plants of the genus *Lactuca* of the flowering plant family Asteraceae (or alternatively, compositae), and especially refers to plants of the commercially important species *Lactuca sativa.* The term lettuce also is used to refer to the edible, succulent leaves of *L. sativa*, also may be eaten cooked. The word *sativa* means "common "(Katz and Weaver 2003). Lettuce is low in calorie and is a moderate source of fiber; it has essentially no fat or protein, and is considered a relatively low to moderate source of vitamins and minerals (Katz and Weaver, 2003).

In many developing countries it is a common practice to grow vegetables along banks of rivers passing through urban centers. Water of such rivers has often been reported to be polluted by heavy metals (Mashauri and Mayo, 1990; Kashem and Singh, 1999; Othman, 2001). The uptake and bioaccumulation of heavy metals in vegetables is influenced by many factors such as climate, atmospheric depositions, the concentrations of heavy metals in soils, the nature of soil and the degree of maturity of the plants at the time of the harvest (Scott *et al*., 1996; Voutsa *et al*., 1996). Air pollution may pose a threat to post-harvest vegetables during transportation and marketing, causing elevated levels of heavy metals in the vegetables, (Sharma *et al*., 2008a). Elevated levels of heavy metals in vegetables are reported such as long term uses of treated and untreated waste water (Adeniyi, 1996; Sinha *et al*., 2005; Sharma *et al*., 2006). Other anthropogenic sources of heavy metals include the addition of manures, sewage sludge, fertilizers and pesticides which may affect the uptake of heavy metals by modifying the physico-chemical properties of the soil such as pH, organic matter, bioavailability of heavy metals in the soil (Yusuf and Osibanjo, 2006). Cultivation areas near highways are also exposed to atmospheric pollution in the form of metal containing aerosols. These aerosols can be deposited on soil and absorbed by the vegetables or alternatively deposited on the leaves and fruits and then absorbed (Edem *et al*., 2009).

Prolonged consumption of unsafe concentrations of heavy metals through foodstuffs may lead to the chronic accumulation of heavy metals in the kidney and liver of humans causing disruption of numerous biochemical processes, leading to cardiovascular, nervous, kidney and bone diseases (WHO, 1992; Jarup, 2003).

The intake of heavy metal can lead to altering of humans and animals healthy state. Thus, the carcinogenic effects generated by continuous consumption of fruits and vegetables loaded with heavy metals such as Cd, Pb or even Cu and Zn are known. These heavy metals may lead to gastrointestinal cancer (Trichopoulos *et al*., 1997; Turkdogan *et al*., 2002) and cancer of the pancreas, urinary bladder or prostate (Waalkes and Rehm, 1994). The sad thing about the pollution of the environment with heavy metals is that they can only be transformed from one oxidation state or organic complex to another (Jing *et al*., 2007; Lone *et al*., 2008). Once the environment becomes polluted with heavy metals, it begins its journey to man's body (Okoronkwo *et al*., 2005; Islam *et al*., 2007) by been readily absorbed by plants which are subsequently consumed by man.

Lettuce and radish were found to be more responsible than other vegetables for the accumulation of heavy metals in humans through the edible portion (Itanna, 2002). The consumption of lettuce has become very popular in most urban centers in Mubi, Adamawa State, Nigeria.

Heavy metal contamination of vegetables cannot be underestimated as these foodstuffs are important components of human diet. However, intake of heavy metal contaminated vegetables may pose a risk to the human health. For these reasons, the *in vivo* bioaccesibility and biodistribution analysis of heavy metals and their potential health impact to the communities was investigated and presented in this study. The aim of this research is to assess the *in vivo* bioaccessibility and absorption of the extractable heavy metals using *in vivo* based method and also the biodistribution of heavy metals in the liver, kidney and lungs of rabbit's model.

MATERIALS AND METHOD

Study Area

Mubi North is among the 21 Local Government Areas of Adamawa State and is located on latitude $11^{o}5^{1}$N and longitude $13^{o}5^{1}$E. It has altitude of 696 m above sea level with an annual mean rainfall of 1,220mm and a mean temperature of 15.2^{o}C during hamattan periods from November to February and temperature of 39.7^{o}C. The town is essentially a mountainous landscape transverse by river Yedzaram and many tributaries. The Mandara and Adamawa Mountains formed part of this undulating landscape (Mansir, 2006).

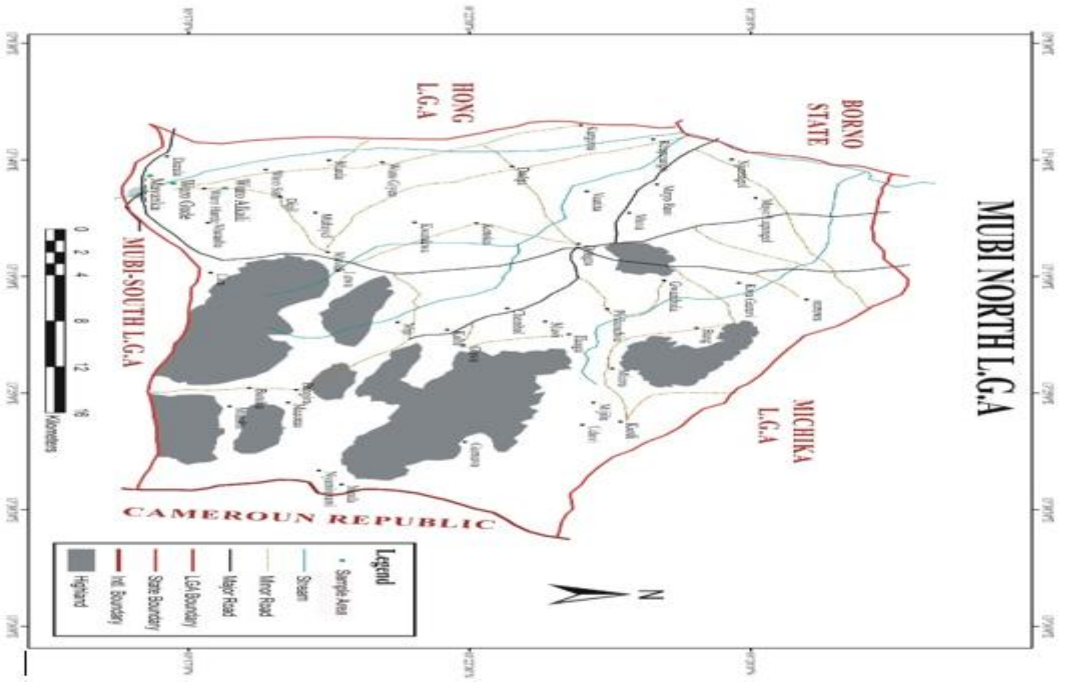

Figure 1: Map Showing the Three Different Sample Locations

Sample Collection

Vegetable samples were collected from Wuro-Gude (WG), Wuro-Alkali (WA) and Mayanka (MY) dumpsites. The sites were commercial vegetable farms and are considered one of the most important sites that supply vegetable to markets within the town and beyond.

Ashing of Vegetable Samples

Vegetable samples were digested using the dry ashing method (Alam *et al.*, 2013). Then 0.5 g of grounded powder samples was weighed and transfer to a clean crucible, which was label according to the sample number, and dry-ashing process was carried out in a muffle furnace by stepwise increase of the temperature up to 550 °C and was then left to ash at this temperature for 6hrs (Kalagbor and Diri, 2014). The samples were removed from the furnace and allowed to cool in the hood carefully. The ash was wet with 1ml distilled water and 2.5ml conc. HCl was added. It was then cooled and filtered using Whatman No.1 filter paper. The filtered samples were diluted up to mark of 100 ml.

In Vivo Animal Study and Treatment

A total of 15 rabbits weighing about 1000-3000g were used for this study. The rabbits were randomly selected and distribute into three study groups, and the rabbits were accommodated under standard condition. All the animals were under 12-hours dark-light cycle with regulated temperature. Clean tap-water and lettuce from different dumpsite were provided throughout the study period (Bwatanglang *et al*., 2017)

In Vivo Experiment of Heavy Metals Exposure

Rabbits were used for *in vivo* study to determine the bioaccessibility of heavy metals. The research problem that leads to this research is that the cultivation and consumption of vegetables (mainly lettuce) from farm in Mubi environs is of major public health concern and the major source of irrigation water is from untreated source and lettuce is one of the commonest vegetables that are cultivated within this suburb of Mubi community. The rabbits were randomly assigned to cages with five rabbits per cage. Following fasting overnight, the rabbits were weighed and three were used as control while the others were feed with lettuce for two weeks and allowed to drink tap water. At the end of the 14-days period, the rabbits were fasted overnight, weighed and slaughtered. Kidney, lungs and liver samples were collected, which were stored in a plastic bag. All wet samples were immediately store at 80 ^{0}C prior to digestion. (Shuo *et al*., 2019)

Statistical Analysis

Statistical analysis used for the data analysis include application of descriptive statistic for deducing minimum, maximum and mean concentration of detected metals and corresponding standard deviation, data were expressed as Mean $\pm SD$. One-way analysis of variance was performed and the significance were set at $p < 0.05$.

Biodistribution/Bioaccesibility of Heavy Metals in Liver, Kidney and Lungs

Biodistribution is a method of tracking where compounds of interest travel in an experimental animal or human subject. Before running the digestion, the organs were weighed and immersed in a solution containing 7 ml of 70% HNO_3 acid and 1 ml of 30% H_2O_2. (JHD-Guangdong Guanghua Chemical Factory Co. Ltd. Shantou, Guangdong, China, 515000). The following day, the organs were then digested by heating at temperature of ~100 ^oC. After the digestion, 3 ml of two percent HNO_3 solution was added and the level of the heavy metals were quantified using Atomic Absorption Spectrophotometer (AAS) (Bwatanglang *et al*., 2017)

The bioaccessibility (%) of heavy metals was calculated by dividing the extractable heavy metals in the organs of the *in vivo* method by total heavy metals in the lettuce.

$$\text{Bioaccessibility (\%)} = \frac{extractable\ metal}{total\ metal} \times 100 \qquad (1)$$

RESULTS AND DISCUSSION

Biodistribution of Heavy Metals in Organs of Rabbits Fed with Lettuce from Wuro-Alkali

The graph in Figure 2 shows the results for the mean concentration of elements in lungs, kidney and liver of rabbits fed with lettuce obtained from Wuro-Alkali dumpsite. It can be seen from the result that the element with the highest value in liver is Fe with 4.70 ± 1.78 mg/kg and the element with lowest value is Cd with value 0.21±0.02 mg/kg. The control value has Cd 0.05±0.01 mg/kg and Fe 0.71±0.03 mg/kg. The difference in concentration of these elements may be as a result of heavy metals obtained from the dumpsite.

In kidney, the highest element is Fe with the value of 6.88±3.06 mg/kg and the element with the lowest value is Pb with value of 0.15±0.09 mg/kg. The control value has Cr 0.07±0.01 mg/kg as the lowest value and Fe as the element with the highest value of 3.63±0.04 mg/kg. The difference in these values may be as a result of the accumulation of elements in the dumpsite.

In lungs, the element with the lowest value is Cr with value 0.13±0.00 mg/kg and the element with the highest value is Fe with 5.35±1.91 mg/kg. The control value has Cr with the lowest value 0.16±0.01 mg/kg and Fe with the highest 3.07±0.01 mg/kg. The difference in these values may be as a result of heavy metal accumulation in the dumpsites.

From the results, it might be concluded that the liver is the primary organ for the bioaccumulation of heavy metals. The levels of Fe in the different tissues in rabbits are mainly due to the presence and metabolism of haemoglobin. The liver has a vast vascular network where blood passes through. Iron released from the breakdown of haemoglobin, as well as excess Fe found in the body is stored and detoxified (Avenant-Oldewage and Marx, 2000). Rabbits fed with lettuce from dumpsite soils accumulated elevated Fe, Cr, and Cd in liver, kidney, and lungs compared to controls. The liver showed the highest metal loads, confirming it as the primary organ for heavy metal bioaccumulation due to its vascular network and storage functions. These findings align with recent studies (Naveenkumar *et al*., 2026; 2025 Pakistani rat model) showing similar organ-specific accumulation patterns from contaminated feed.

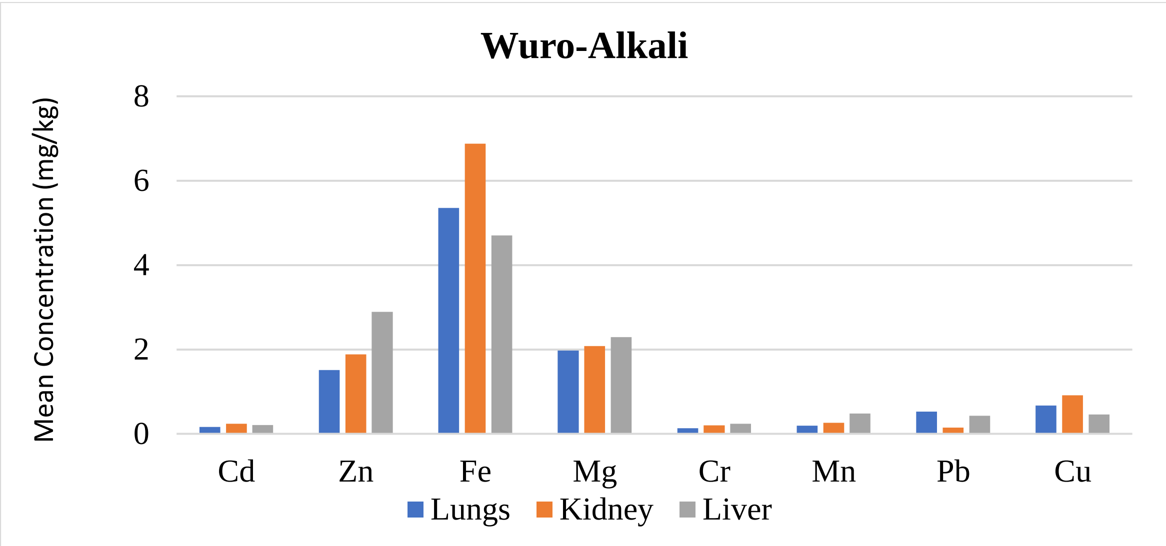

Figure 2: Mean Concentration of Heavy Metals in Lungs, Kidney and Liver

Biodistribution of Heavy Metals in Organs of Rabbits Fed With Lettuce from Wuro-Gude

Figure 3 shows the results for the mean concentration of elements in lungs, kidney and liver of rabbits fed with lettuce obtained from Wuro-gude dumpsite. From the result, it can be seen that Cr have the least value of 0.07 ± 0.05 mg/kg among the elements and Fe has the highest value of 4.11 ± 1.33 mg/kg among the elements. From the control, Cr has the least value of 0.03 ± 0.01 mg/kg and Fe has the highest value of 0.71 ± 0.03 mg/kg. The difference in these values may be as a result of accumulation of heavy metals in the dumpsite.

In kidney, the element with the lowest value is Cr with the value of 0.03 ± 0.00 mg/kg and the element with the highest value is Fe with 9.41 ± 1.26 mg/kg. The control value has Cr with lowest value of 0.07 ± 0.01 mg/kg and Fe with the highest value of 3.63±0.04 mg/kg. The difference in these values may be as a result of accumulation of heavy metals in the dumpsite.

In lungs, Cr is the least of the elements with value of 0.06 ± 0.00 mg/kg and Fe has the highest value of 5.68 ± 4.13 mg/kg. From the control, it can be seen that Cr has the least with 0.16±0.01 mg/kg and Fe has the highest value of 3.07 ± 0.01 mg/kg. The difference in these values may be as a result of accumulation of these metals in the dumpsite.

High concentration of Fe particularly in the kidney may be as a result of the slow elimination rate of Fe by the rabbits once it has accumulated (Avenant-Oldewage and Marx, 2000). Rabbits fed with lettuce from Wuro-Gude dumpsite showed Fe as the highest accumulated metal in liver, kidney, and lungs, with kidney recording the peak (9.41 mg/kg), likely due to slow Fe elimination. Chromium was consistently lowest across organs, falling below control values in lungs. Recent studies (Hao *et al*., 2025**;** El-Maleh *et al.*, 2025) confirm that Cr accumulates in rabbit tissues causing hepatic injury, while Fe dominance reflects physiological storage. The kidney emerges as the primary target organ for heavy metal accumulation, consistent with current literature.

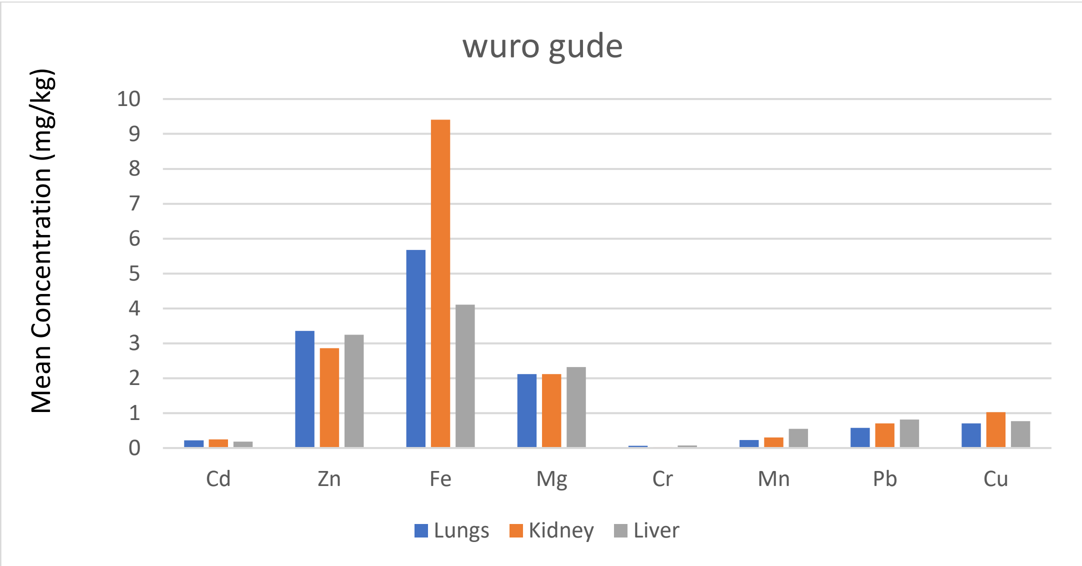

Figure 3: Mean Concentration of Heavy Metals in Lungs, Kidney and Liver

Biodistribution of Heavy Metals in Organs of Rabbits Fed with Lettuce from Mayanka

The graph in Figure 3.3 shows the result of the Bioaccessibility and biodistribution of heavy metals in lungs, kidney and liver of rabbits fed with lettuce obtained from mayanka dumpsite. It can be seen from the graph that the value of Cr is the least in liver with the value of 0.09±0.06 mg/kg and the one with the highest value is Fe with the value 5.64±2.43 mg/kg. Comparing with the control value 0.03±0.01 mg/kg, it can be concluded that the value of Cr and Fe in the liver of the rabbits fed with lettuce from mayanka dumpsite is higher than the control value.

In kidney, it can be seen that Cr has the lowest value of 0.11±0.01 mg/kg and Fe has the highest mean concentration of 6.82±2.35 mg/kg. The control value has Cr 0.07±0.01 mg/kg as the lowest value and Fe has the highest value of 3.68±0.04 mg/kg. It can be seen that, the value of Cr and Fe in the kidney of rabbits fed with lettuce from mayanka dumpsite is higher than the control value. The reason may be that, the values of these elements are high in the dump site.

In lungs, it can be seen that Cr has the lowest value of 0.11±0.08 mg/kg and Fe with the highest value of 3.39±1.28mg/kg. The control has Fe with the highest value of 3.07±0.01 mg/kg and lowest with Cr with the value of 0.16±0.01 mg/kg. It can be concluded that, in lungs the concentration of Fe is higher than the one of control and Cr is less than the value in control. This may be as results of accumulation of heavy metals in dumpsite.

However, the accumulation of Fe and Cr in the liver reinforces the view that the liver plays a protective role against chronic heavy metal exposures by producing metallothioneins acting as a storage site, and being a vital organ in the regulation of heavy metals (Avenant-Oldewage and Marx, 2000). At the Mayanka dumpsite, Fe accumulated highest in rabbit liver, kidney, and lungs (peak 6.82 mg/kg in kidney), while Cr was lowest, confirming the liver as a storage organ and kidney as a major target. Recent studies (Hao *et al*., 2025; El-Maleh *et al*., 2025; Capcarova *et al*., 2024) confirm that dumpsite-derived Cr and Fe accumulate in rabbit tissues, inducing hepatorenal toxicity even at low levels.

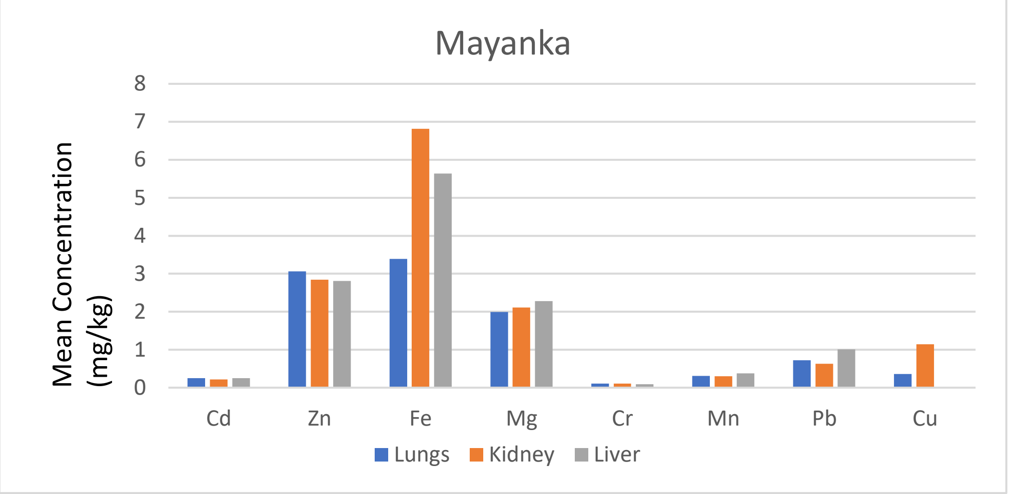

Figure 4: Mean Concentration of Heavy Metals in Lungs Kidney and Liver

Bioaccessibility Studies of Heavy Metals in Organs

Bioaccessibility is defined as the proportion of a pollutant that can be dissolved in the gastrointestinal environment, indicating the relative amount of pollutant in the matrix that can be absorbed by the body, and higher Bioaccessibility indicate greater potential for absorbing pollutant. The heavy metals in the vegetables enter the body through gastrointestinal tract, and cannot be 100% absorbed by the digestive system. Therefore, the health risk of heavy metals could be overestimated if the total content of the heavy metals is used to carry out risk assessment instead of the total absorption content. Thus, Bioaccessibility may be more accurate in assessing risk of heavy metals in vegetables. Common method for determining Bioaccessibility of heavy metals in in vivo experiment in this case, animal model was used to mimic the gradual transfer of ingested compound through the digestive tract, this method has been used to assess the risk of heavy metals in food. (Huang *et al.*, 2016) Studies have shown that the biochemical prosperities of heavy metals at various digestive stages are related to the characteristics of the heavy metals. Figure 3.4 shows the graph of Bioaccessibility of heavy metals in the organs. From the graph of *in vivo* experiment, the Bioaccessibility of heavy metals for wuro-Alkali ranges from 0.25% - 51.08%, Wuro-Gude ranges from 0.45% - 54.75% and for Mayanka it ranges from 0.53% - 57.88%. Magnesium is having the highest %, indicating that the vegetables could not dissolved by the digestive fluid.

If metals have high percentage (%) of bioaccessibility, it means that they are highly accessible in the stomach to be absorbed. The absorbed metals can be transported to organs tissues and can be accumulated causing toxic effect. On the other hand, low % of Bioaccessibility indicates that the metals are not readily accessible in the human digestive system. A study reported by Barsby *et al*., 2012 shows that the bioaccessibility of Cd was the lowest compared to other toxic metals. The high Mg % bioaccessibility in the three dumpsites in this study may be influenced by the sewage irrigation and fertilization of the dumpsites and also the sewage effluents are considered not only a rich source of organic matter and some nutrients, but these also elevate the level of heavy metals (Singh *et al*., 2010). Bioaccessibility measures the fraction of a pollutant dissolved in the gastrointestinal tract, with higher values indicating greater absorption potential. Using total metal concentrations overestimates health risk, making bioaccessibility-corrected assessment more accurate. In this in vivo rabbit study, bioaccessibility ranged from 0.25%–57.88% across dumpsites, with magnesium (Mg) showing the highest percentage, indicating poor dissolution by digestive fluids. High Mg bioaccessibility may be influenced by sewage irrigation and fertilization (Singh *et al*., 2010). Conversely, low Cd bioaccessibility has been reported (Barsby *et al*., 2012), suggesting metal-specific digestive behavior.

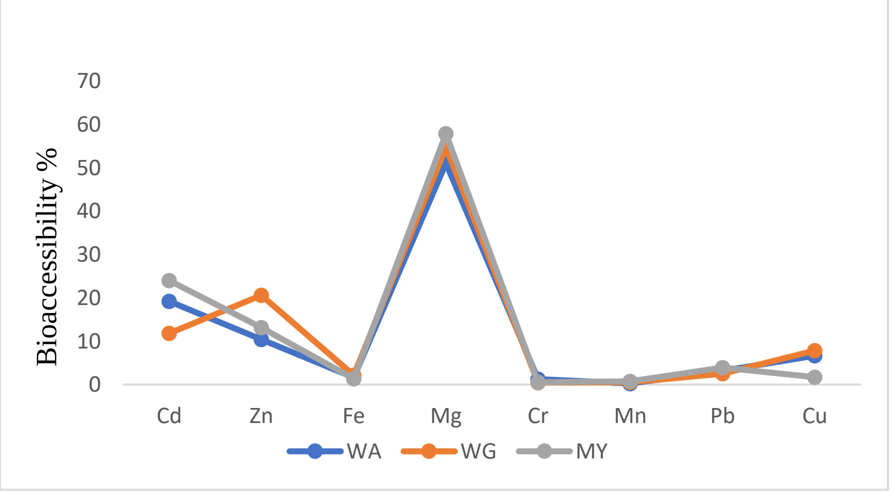

Figure 5: Bioaccessibility Studies of Heavy Metals in the Organ

Organ Weight and Organ-to-Body Weight

Table 1: Organ Weight and Organ-to-Body Weight at Endpoint (Day 14)

Body Weight (g)		**Organ weight (g)**			**Organ weight/Body**		
		Liver	**Lungs**	**Kidney**	**Liver**	**Lungs**	**Kidney**
Control	3036.6±450.14	60.00±43.58	60.00±10.00	63.33±23.09	0.019	0.019	0.020
GA	2077.5±411.93	50.00±32.65	27.50±15.00	30.00±24.49	0.024	0.013	0.014
GB	2875.0±457.34	50.00±29.43	25.00±17.32	35.00±12.90	0.017	0.008	0.012
GC	2425.0±320.15	35.00±37.85	15.00±5.77	25.00±17.32	0.014	0.006	0.010

GA represent rabbits fed with lettuce from Wuro-Alkali, GB; Wuro-Gude and GC: Mayanka

From Table 1, the average body weight of group A feed with lettuce from Wuro-Alkali was found to be 2077±411.93 g after 14 days and the average body weight of group B that was fed with lettuce from Wuro-Gude was found to be 2875.0 ± 457.34 g and also the average body weight of group C fed with lettuce from Mayanka was found to be 2425.0 ± 320.14 g. It can be seen that when compared with the control group body weight 3036.6 ± 450.14 g, all the body weight of the groups were found to be less than the control value. Among the three groups, group B has the highest body weight and group A with the least body weight. The reason for the differences in the body weight may be due to the lettuce eaten from the different dumpsites and also consistency in same diet.

From the table, the control values of the organ - to - body weight of liver, lungs and kidney were found to be (0.019, 0.019, 0.020) respectively. For group A , the organ-to-body weight were found to be (0.024, 0.013, 0.014) respectively and for group B , the organ-to- body weight were found to be (0.017, 0.008, 0.012) respectively and also for group C, the organ-to-body weight were found to be (0.014, 0.006, 0.010) respectively. It can be seen from the table that the value of organ-to-body weight of liver in group A was found to be above the control group and group B and C were below the control value. The reason why the organ-to-body weight of liver in group A is high, may be due to the accumulation of heavy metals from the dumpsite and also from the table, it can be seen that the organ-to-body weight value of lungs in the three groups were found to be less than the one obtained in the control value. Likewise for the kidney, the value of organ-to-body weight within the three groups was found to be less than the one obtained in the controlled. The reason for this may be due to the less accumulation of heavy metals in the dumpsite compared to the controlled value. Rabbits fed dumpsite-grown lettuce (Groups A, B, C) showed significantly lower final body weights (2077–2875 g) compared to controls (3036.6 g), consistent with studies reporting reduced growth performance following heavy metal exposure (Hao *et al*., 2025). Group A exhibited an increased liver-to-body weight ratio (0.024 vs. control 0.019), indicating possible hepatotoxicity, while lung and kidney ratios decreased across all exposed groups findings that align with organ weight alterations observed in rabbits exposed to environmental metal mixtures (Naveenkumar *et al*., 2026).

CONCLUSION

From the studies, all the three dumpsites contained virtually all the heavy metals studied. It was also observed that some dumpsites had higher concentration of these metals than others; this could be attributed to the presence of waste materials carrying higher amounts of these heavy metals. Heavy metals concentration in these dumpsites may not appear to pose very serious environmental problems at the moment. However, accumulation of these metals after several years may pose a threat to human health and the environment.

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