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Original Research Article

Assessment of Lead and Cadmium Concentration in Liver and Kidney of Sprague Dawley Rats Exposed to Cultured *Vernonia amygdalina* Leaves

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Abstract

Lead and cadmium are among the heavy metals whose presence is feared because of their toxicity. They enter the food chain by consuming plants harvested from contaminated sites. In this study, we subjected Sprague Dwaley rats to a 14-day chronic exposure to extracts of *Vernonia amygdalina* leaves contaminated with different concentrations of lead and cadmium solutions. The rats were sacrificed and the liver and kidneys organs were removed. All the samples were determined by flame atomic absorption spectrophotometry. The results showed that the concentrations contained in the administered extracts are 0.26ppm; 3.662ppm; 4.878ppm; 5.47ppm for lead on the one hand and 0.046ppm; 1.773ppm; 6.26ppm; 30.55ppm for cadmium on the other hand. Rats in the control lot received distilled water. The different bioaccumulated concentrations in the kidneys are in the order of 0.02ppm; 0.06ppm; 0.17ppm; 0.28ppm; 0.59ppm for lead and 0.06ppm; 0.06ppm; 0.51ppm; 0.64ppm; 0.78ppm for cadmium. In the liver the following concentrations were accumulated 0.02ppm; 0.06ppm; 0.22ppm; 0.24ppm; 0.42ppm for lead and 0.04ppm; 0.10ppm; 0.15ppm; 0.35ppm; 0.45ppm for cadmium. Considering a longer exposure period, the bioaccumulated concentrations will be higher because circulating concentrations have not been measured. The bioaccumulative nature of these metals in the liver and kidney is important in assessing the risks associated with the consumption of products contaminated with lead and cadmium.

Keywords: Bioaccumulation, Plomb, Cadmium

INTRODUCTION

Environmental pollution has become inevitable with the increase of human activity. Thus, anthropogenic activities have led to an increase in the levels of heavy metals in the environment, particularly lead and cadmium. These elements are potentially dangerous and play no physiological role in the body (Chaouali *et al.,* 2022). This contamination of the environment by heavy metals is a challenge faced by developing countries (Adeyinka *et al.,* 2023). In addition, environmental pollution by heavy metals even at low concentrations with long-term cumulative health effects is a major health concern worldwide (Opaluwa *et al.,* 2012; Diaz *et al.,* 2021). Due to their accumulation, their non-biodegradability, their translocation in the food chain and their toxicity at certain concentrations, the presence of heavy metals in ecosystem compartments is a real concern (Aekola *et al.,*

Table 1. Treatment of *Vernonia amygdalina* plant with different concentrations of Pb and Cd

Lots of vegetation vases	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5	Lot6
Treatment	Control	5,06ppm Pb+1,53ppm Cd	40,48ppm Pb +12,24ppm Cd	647,68ppm Pb +195,84ppmCd	5181,44ppm Pb+1566,72 ppmCd	10362,88ppm Pb+3133,44 ppmCd

Legend: Vase= vegetation vase, Pb=lead, Cd= cadmium

2008). The translocation in the food chain by plants is all the more worrying because fresh vegetables are more and more used in nutrition rather than red meat which is the source of various kinds of diseases like diabetes, cardiovascular diseases, cancer and others (Volpe *et al.,* 2019). Vegetables are very important for human health with their contribution in vitamin C, folic acid, minerals as well as for their biochemical role and antioxidant properties (Ofoedu *et al.,* 2021). However, vegetables contaminated by heavy metals do not only serve as a nutritional resource but also as a source of chemical pollutants that are not essential to human physiology (Ruzaidy *et al.,* 2020). However, vegetables contaminated by heavy metals do not only serve as a nutritional resource but also as a source of chemical pollutants that are not essential to human physiology (Mukherjee *et al.,* 2023).

Vernonia amygdalina is a vegetable widely used in African households in nutrition and phytotherapy. Planted on polluted soil, it is very quickly contaminated by heavy metals such as lead and cadmium (Déguénon *et al.,* 2020). The accumulation and amplification of heavy metals in human tissues through the consumption of vegetables and medicinal plants can have dangerous consequences for health (Hlihor *et al.,* 2022). The risk assessments do not take into account the bio-accumulated fractions and those eliminated by the purification systems of the exposed organism.

The present study aims to evaluate the level of exposure of plants on a soil contaminated by the heavy metals lead and cadmium and their transfer to the target organs liver and kidneys of the consumer. The consumer chosen here for the experiment is the Sprague Dawley rat.

MATERIAL AND METHODS

The *Vernonia amygdalina* plants used are grown on natural soil in order to eliminate any hypothesis about the effect of other contaminants due to anthropogenic activities. The soil is taken from an area of one square meter by 10 cm depth in a classified natural forest (Ahozon natural forest). The soil is dried and sieved on the experimental site and then weighed. The seedlings are put on the ground after having wetted them with 100mL of water from the site. Six (6)lots of vegetation vases are made up with 10 vases per lot. Table 1

After ten weeks, the plants in the vegetation vases of lots 1, 2, 3, 4 were harvested and then wrapped in aluminum foil for drying in the laboratory. The plants of the vases of batches 5 and 6 were burned. The harvested *Vernonia amygdalina* leaves are air dried at laboratory temperature for about 2 weeks to obtain a constant weight. They were then ground into powder. A quantity of the powder obtained was determined spectro-photometrically to determine the actual concentration of lead and cadmium absorbed by the plant in the leaves.

100g of this powder was macerated in 500mL of distilled water in a refrigerator at 4°C for 3 days under constant stirring. The aqueous extract was filtered with Whatman paper n°1.

The rats weigh between 200-250 grams. They are acclimatized in an animal house, randomized and divided into 6 groups of 10 animals, 5 males and 5 females. The animals were fed with kibble and water. Rats were chronically exposed, i.e. orally administered at a dose of 100 mg/kg animal weight for 14 consecutive days. The extracts of *Vernonia amygdalina* administered contained different concentrations of lead and cadmium (see table 2). Rats in the control group received only distilled water. Table 2

Animals are deprived of food for a minimum of 12 hours to stabilize their metabolism. The mortar, pestle and dissection equipment are carefully cleaned with a 10% nitric acid (HNO_3) solution and rinsed with distilled water before and after each sample. The animals were sacrificed, the liver and kidneys were carefully removed, weighed using a BEL L303i electronic balance (Accuracy: 0.001g) and stored in a 10% formalin solution installed in a dry place until analyses. The collected and preserved organs are dried in an oven at 65°C and then finely ground in an agate mortar.

For mineralization, the biological materials were reweighed and then introduced into borosilicate vials. Variable volumes of 4mL to 16 mL of a mixture of 30% hydrogen peroxide (H_2O_2) and 67% nitric acid (HNO_3) were added in the proportions 1 H_2O_2 : 3 HNO_3(Ouro-Sama, 2019). H_2O_2 was first added and then allowed to act at room temperature for 18 hours before adding the nitric acid. After about 2 hours of reaction, the samples were heated on a hot plate at 90°C until almost complete

Table 2. Treatment of the rat lot

Lots	Treatments
Lot1''	Control (distilled water)
Lot2''	*Vernonia amygdalina* (0,046ppm Cd+0,26ppm Pb)
Lot3''	*Vernonia amygdalina* (1,773ppm Cd+3,662ppm Pb)
Lot4''	*Vernonia amygdalina* (6,26ppm Cd +4,878ppm Pb)
Lot5''	*Vernonia amygdalina* (30,55ppmCd +5,47ppm Pb)

Cd= cadmium ; Pb= lead

Table 3. The concentration of Pb and Cd measured in the leaves after the treatment of the lots

Lots of vegetation vases	Lot 1'	Lot 2'	Lot 3'	Lot 4'
Pb, Cddose administered	Control	5,06ppm Pb+1,53ppm Cd	40,48ppm Pb + 12,24ppm Cd	647,68ppm Pb +195,84ppmCd
Response obtained in the leaves	0,046ppm Cd + 0,26ppm Pb	1,773ppm Cd + 3,662ppm Pb	6,26ppm Cd + 4,878ppm Pb	30,55ppmCd + 5,47ppm Pb

Cadmium=Cd ; Plomb=Pb

evaporation of the reagents without letting the residues calcine. They were left at room temperature for cooling. The blanks were prepared and processed under the same respective conditions for both sets of samples with the same reagents. Each mineralization was filtered, made up to 20 mL with distilled water and kept at room temperature for their analysis. The trace elements were determined in the obtained solutions, by the Atomic Absorption Spectrophotometer (AAS) flame for Cd and Pb.

Statistical analysis

The XLSTAT software allowed us to perform the descriptive statistics of the lead and cadmium levels in the liver and kidneys. The z-test for one sample/bilateral test for the comparison of the mean Pb and Cd levels in kidneys and liver of treated animals in comparison to controls. P-values less than 0.05 were considered statistically significant. Then the data were processed in Excel version 2013 and saved in CSV2 format and finally imported into R.4.3.0 software. Using the C function, combinations were performed to obtain vectors. The PLOT function was used to construct point clouds and the LINERS function was used to join the corresponding points with line segments.

RESULTS

The leaves of *Vernonia amygdalina* used for the treatment of animals

The *Vernonia amygdalina* leaves used to treat the animals are grown on natural soil. The Ahozon forest soil contains 2.84% organic matter compared to 2.98% for the same soil that was used for crops. Therefore, the threshold of acceptability of the vegetable for lead and cadmium is reached at 2048ppm which corresponds to 3.45g/kg soil for Pb and 1.05g/kg soil for Cd. At this concentration on natural soil, the plants are burned. The same thing is revealed for the concentration of 1.72g/kg soil for Pb and 0.52g/kg soil for Cd. This shows that the uptake of metals varies with the organic matter content of the soil. Due to this, the plants of lots 5 and 6 in table 1 were burned. The unburned leaves, when assayed, showed a concentration of lead and cadmium as presented in Table 3. The control plants contain traces of lead and cadmium, but they are not treated with the crop. From lot 2' to lot 4' the response obtained in relation to the dose is very weak and is more precisely observed in lot 4'. Indeed, the adsorption of the heavy metals lead and cadmium does not only depend on the concentration available in the medium but on other factors such as cationic bridges which are energy dependent. Table 3

Evaluation of lead and cadmium concentration in the liver and kidney of rats

Flame SAA analysis of the liver and kidneys of animals treated with lead- and cadmium-contaminated extracts shows the presence of these two toxic metals in the organs indicated. The livers of control laboratory animals contain some traces of lead and cadmium. The traces of lead and cadmium observed probably come from the kibble used for their food. The concentrations observed are significantly different from those of the control animals (lot 1'') in the liver. Tables 4 and 5 allow us to make a statistical analysis of the results obtained.

The theoretical mean for lead is 0.02 and 0.04 for

Table 4. Descriptive statistics of Pb, Cd/liver observations

Variable	Observations	Obs. with missing data	Obs. without missing data	Minimum	Maximum	Mean	Standard deviation
Pb/liver	4	0	4	0,055	0,421	0,233	0,150
Cd/liver	4	0	4	0,096	0,448	0,261	0,165

Table 5. One-sample/two-way z-test for Pb and Cd/liver concentrations

Difference	0,213	0,221
z (Observed value)	2,847	2,680
|z| (Critical value)	1,960	1,960
p-value (bilateral)	0,004	0,007
alpha	0,05	0,05

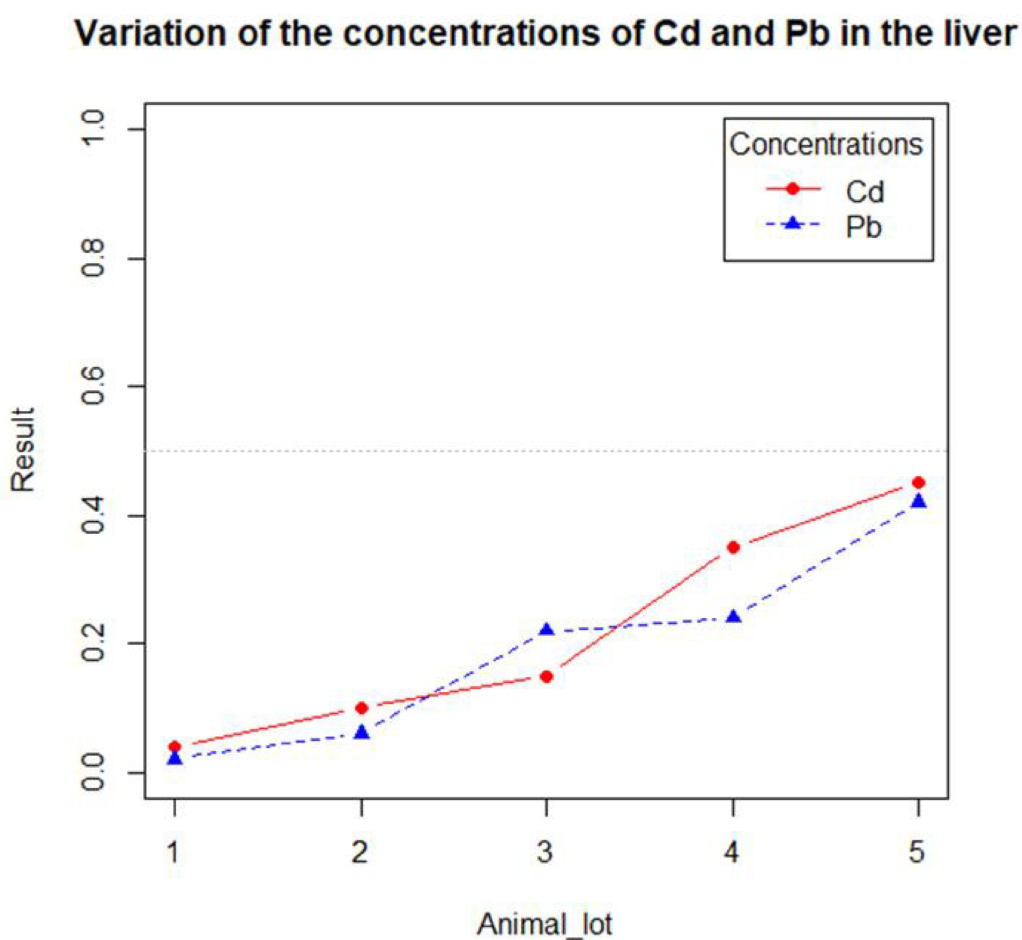

Figure 1. Variation of Pb and Cd concentration in liver.

cadmium with a significance level at 5%. The null hypothesis (H0): the difference between the means is equal to 0. The alternative hypothesis (Ha): the difference between the means is different from 0. The calculated p-value is lower than alpha=0.05 (significance level). The null hypothesis is therefore rejected. The concentration of lead and cadmium in the liver of the animals is statistically different. The figure below allows us to appreciate the level of Pb and Cd contamination in the liver of the animals in each lot. Figure 1

Lead and cadmium are progressively accumulated in the liver of animals. The highest concentration of lead and cadmium observed in the liver is 0.42ppm and 0.45ppm respectively.

Table 6. Descriptive statistics of observations (Pb and Cd) in kidneys

Variable	Observations	Obs. with missing data	Obs. without missing data	Minimum	Maximum	Mean	Standard deviation
Pb/kidneys	4	0	4	0,058	1,593	0,525	0,718
Cd/kidneys	4	0	4	0,056	0,777	0,494	0,313

Table 7. One-sample/two-way z-test for Pb and Cd/reins concentrations

Diference	0,505	0,434
z (Observed value)	1,405	2,778
|z| (Critical value)	1,960	1,960
p-value (bilateral)	0,160	0,005
alpha	0,05	0,05

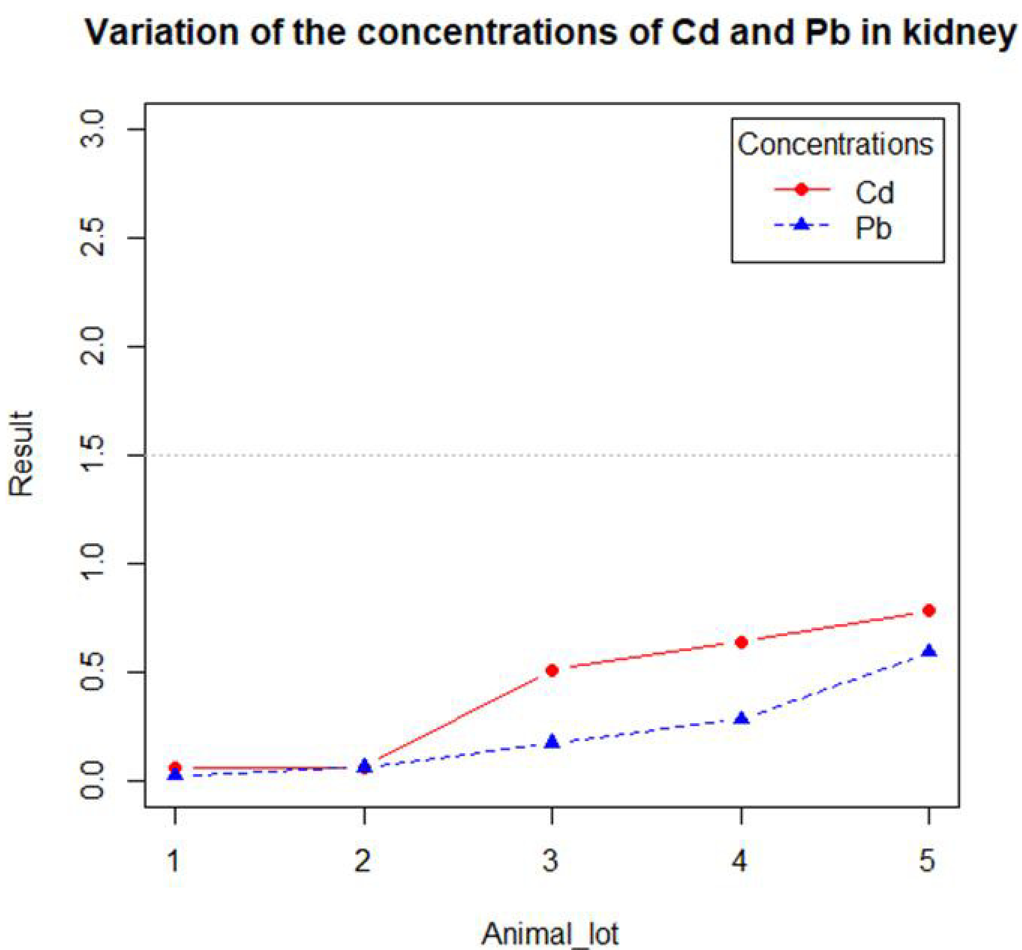

Figure 2. The evolution of Cd and Pb concentration in the kidneys

In contrast to the liver, there is not much variation in lead and cadmium concentrations in the kidneys of animals in lot 1" which are the controls and those in lot 2" which received the untreated extracts which contain very low concentrations of both metals. The variation in the concentration of cadmium and lead is progressive. Tables 6 and 7 allow us to make a statistical analysis of the results obtained. Table 6, 7

The theoretical mean is 0.02 for lead and 0.06 for cadmium. The level of significance is 5%. H0: The difference between the means is equal to 0. Ha: The difference between the means is different from 0. Since the calculated p-value is higher than the threshold significance level alpha=0.05; the null hypothesis H0 cannot be rejected. Lead and cadmium concentrations in the kidneys of rats treated with contaminated *Vernonia amygdalina* are not different from those of rats treated with uncontaminated *Vernonia amygdalina*. Although there is no statistically significant difference, it should be noted that there is a slight increase in the concentrations in the target organs as shown in Figure 2. The highest concentration of lead observed in the kidneys is 0.59ppm and for cadmium is 0.78ppm.

DISCUSSION

It was found that the kidneys accumulated both metals to a much greater extent than the liver. These results were confirmed by those of Wang *et al.,* 2021 who showed an accumulation of cadmium in the kidney and liver after consumption of industrial foods contaminated with both metals. Taking into account the concentrations contained in the administered extracts and the responses obtained, it is thus deduced that the adsorption of lead and cadmium is subordinated to transmembrane mechanisms which can be protein pores or ATP-dependent enzymatic pumps. The circulating dose was not taken into account, but the transit is done before the bioaccumulation. The results of Córdoba-Gamboa *et al.,* 2023 showed this after measuring lead in the blood of vulnerable children in a Mexican population. An affinity of the kidneys in the accumulation of cadmium is noticed. This result is confirmed by the work of Swaileh *et al.,* 2002 who showed an increase in the concentration of cadmium in snails as the concentration of the environment increases. These two heavy metals lead and cadmium have no physiological interest for the two organs liver and kidneys. Bioconcentration and even their biomagnification present health risks. El-Gendy *et al.,* 2010 address in the same sense and confirm these results by showing a regression of the snail population subjected to chronic lead exposure at increasing concentration. In the same way Ibraheem *et al.,* 2022; Bakr *et al.,* 2022; Liu *et al.,* 2019 showed that cadmium has a negative activity on glutation S-transferase, catalase and malondialdehyde after exposure of Drosophila melanogaster and Bellamya aeruginosa. The dose response obtained after exposure to the vegetable on the one hand, and that obtained with the administration of extracts of the contaminated vegetable on the other hand, shows the perfect integration of metals in the food chain. Vegetables have antioxidant potentialities that prevent metals from acting but do not prevent the bioaccumulation of metals that can be expressed later and create damages for the target organism. The work of Ibraheem *et al,* 2022, which presents a correction by plants of the pathologies created by lead and cadmium intoxication, does not make it possible to know if the plant has the potential to eliminate the metals. The inhibition of the action of metals can be explained in two ways. The first can be explained by the fact that the element is made non-bioavailable by speciation which makes it inactive. The second explanation concerns the elimination by protein complexes such as metalloproteins, Nuclear factor erythoid-related factor 2 (Lian *et al.,* 2023). In the latter case, the work should not stop at correcting the effects created, but also at quantifying the toxic elements in the urine, feces, and other waste disposal systems of the exposed organism (Hu *et al.,* 2023). In the former case, the change in speciation may be challenged as soon as conditions permit in the host organism. This would result in the release of the elements into storage organs for further damage without re-exposing the organism. The best option to choose is to prevent the integration of toxic elements including lead and cadmium which play no physiological role in the food chain. According to the work of Bhattacharya *et al.,* 2020 ;no clinical studies using microorganisms that mitigate cadmium-induced effects have shown significant results in the literature. The most plausible solution would be to direct research towards the remediation of lead and cadmium contaminated soils prior to their use in the cultivation of various consumable products such as fruits and vegetables. This proposal is in line with the work of Wang *et al.,* 2023 who showed a decrease in oxidative stress caused by lead and cadmium after the use of an organic silicon fertilizer. Yao *et al.,* 2023 in their work not only used Prunus persica extracts to mitigate the effects created by lead but the extract was used to remove lead assimilated in the faeces. This is more appreciable.

CONCLUSION

All in all, heavy metals lead and cadmium enter the food chain from plants when they are bioavailable in the crop soil. They accumulate in the plant organs and enter the food chain after chronic exposure. After consumption of vegetables contaminated by these heavy metals, they are bioaccumulated in the liver and kidneys where they play no physiological role. However, their presence in the liver and kidneys of the consumer is the cause of several pathologies and metabolic damages. In order to avoid the accumulation of heavy metals lead and cadmium in the liver and kidneys, it is preferable to harvest vegetables on uncontaminated soil rather than using mitigation measures related to their presence in the host organisms

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