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Determination of Total Heavy Metals Content in Local Antidote Manufacturing Resources

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Abstract

The present work was initiated for searching and evaluating the total heavy metal content in some selected local resources used by traditional healers in locally made antidotes to search for their toxicity. Elekat public slaughterhouse in the city of Bukavu, the central market of Kadutu and in the Ruzizi plain, precisely in Sange city, have been the purchased centers. The samples of bull and cow horns, oil palm nut shells, cow bones, ginger, black pepper and Mwenga salt were used. The spectrometric method has been used to carry out heavy metal content in each local resource using the atomic absorption spectrometer (VARIAN 1275). The heavy metals found and their average content are as follows: copper (Cu: 124.79 mg/kg), lead (Pb: 76.41 mg/kg), chromium (Cr: 33.9 mg/kg), zinc (Zn: 190.86 mg/kg), Arsenic (As: 107.41 mg/kg) and cadmium (Cd: 0.05 mg/kg). The averages of total heavy metal contents for all samples are below the standard of each one. The determination of the heavy metal contents in these samples allows evaluating human intoxication risk. These results show that antidote technology can be run without any toxicity risk and showed statistically significant differences compared to the controls ($p < 0.05$) for arsenic, lead and copper and no statistically significant differences ($p > 0.05$) for chromium, zinc and cadmium have been recorded. However, most of the heavy metals molecules being thioloprives and then bioccumulatives, antidotes made from them shall not be consumed during a long period.

Keywords

Heavy Metals, Local Resources, Contamination, Antidote

1. Introduction

In the worldwide, the crimes committed from poisonings are innumerable. The lethal effects of some drugs have been known since ancient times. The Greeks and Romans used plant extracts or mineral compounds for criminal purposes and this use continued through the Middle Ages and the Renaissance, to the present day [1]. The discovery in the 8th century by the Arab alchemist Geber of arsenic powder, odorless and tasteless, had the most dramatic consequences. The toxicity of arsenic was so dreadful that it was referred to as the "powder of succession" at the courts of French kings and princes in the 14th century and at the Vatican in Italy during the Renaissance. Several people have been guilty of the harmful effects of arsenic [2].

In African countries, particularly in the Gulf of Guinea, various studies have revealed pollution of surface sediments in the coastal marine environment or in lakes and rivers by heavy metals under strong industrial and urban pressure. Among these heavy metals, we can cite: copper (Cu), zinc (Zn), lead (Pb), chromium (Cr), manganese (Mn), arsenic (As), cadmium (Cd) etc., that once in the plant by bioaccumulation from industrial wastewater, are absorbed through the roots of the plant and are transferred to all parts: stems and leaves of the plant and thus becoming harmful to human beings [3].

In the Democratic Republic of Congo (DRC), modern medicine faces a major challenge; that of finding effective products to treat the increasing number of cases of poisoning that have continued to be recorded in recent years. The Congolese state does not have appropriate structures for handling cases of poisoning. There is no toxicological information center or poison treatment center [4]. Faced with increasingly numerous and frequent cases of poisoning and the therapeutic failures of modern medicine, these populations prefer to consult traditional medicine where they are treated and sometimes find satisfaction for their health problems. A person who suspects that he/she was poisoned, did a test before getting any kind of treatment. Once the results prove positive, she searches for antidote prescribed by traditional healers [4] [5]. Indeed, several heavy metals can be very dangerous for human health and for other living beings when they are present in the environment at high concentrations [5].

In Bukavu city, animal waste such as horns, bones and hooves of livestock are not spared from this contamination and would constitute a potential source of contamination in the sick person who would be treated with an antidote made from this waste. We assumed that the waste is exposed to heavy metal contamination because most of them are collected around the public slaughterhouse in the city of Bukavu located downstream from the Essence quarter where anthropogenic activities are conducted such as welding, servicing vehicle batteries, changing engine oil, etc. Like bull horn; cow horns and cow bones used in this study were bought at the public slaughterhouse of Bukavu city; we have assumed that they can be contaminated and then the need for heavy metals analysis is so important. It is important to highlight here that no one has done similar work and this fact demonstrates the innovation aspect of our research. We would like to highlight that this is an innovative work because there is no one who has done similar work. The fact that several heavy metals molecules are supposed to contain sulfur and then become more exposed to the bioaccumulation, it is preferable to avoid a longue period of consumption of antidotes made from these local resources.

2. Materials and Methods

2.1. Materials

During this research, we used the following local resources: bull and cow horns, cow bones, oil palm nut shells, ginger, black pepper and Mwenga salt. The first three were purchased from the public slaughterhouse in the town of Bukavu, the fourth from private sources in Sange in the Ruzizi plain and the last three were purchased on the Kadutu market in Bukavu.

The horns and bones were purchased from the Elekat slaughterhouse on October 2, 2019, the oil palm nut shells were purchased from private individuals in Sange on October 25, 2019, the ginger, black pepper as well Mwenga salt were purchased at Kadutu market on November 2, 2019. All samples were packaged in polyethylene bags.

A plastic basin, a pair of gloves and distilled water were used for cleaning the purchased samples. Drying in the sun was done on clean rubber. The mass of each raw material was weighed using an ordinary balance. In the laboratory, after sorting the coarse materials with a diameter greater than 2 mm, 1 g of sample for each raw material was taken three times at random from a certain quantity, then dried in an oven at 90˚C until at constant weight.

They were then crushed using a porcelain mortar and sieved using a sieve with a mesh size of less than 63 μm. After the homogenization of these three dry samples, a composite sample of each raw material was extracted and then stored in 250 ml plastic bottles for analysis [6]. **Figure 1** shows the different samples used in this study.

Figure 1. Testing samples. **Note: CDT:** Bull horns, **CDV:** Cow horns, **ODV:** Cow bones, **CNP:** Oil paln nut shells, **SM**: Mwenga salt, **GGB:** Ginger t, **PVN:** Black pepper.

2.2. Laboratory Analysis Method

A quantity of 250 mg of each sample was weighed, transferred into 20 ml flasks and decomposed hot in the presence of a mixture of hydrofluoric acid (HF 48%) and aqua regia (concentrated HNO_3/HCl, 1:3 v/v) and under outlet pressure of 6 psi for six hours to achieve complete decomposition. Once digested, the samples were transferred to 100 ml flasks containing 5.6 g of boric acid (H_3BO_3) and 20 ml of distilled water. After adjusting to the mark with distilled water, the solutions thus obtained were left to stand for 24 hours before carrying out the analysis. The solutions were analyzed three times with an air-acetylene flame and at the required wavelengths for each metallic element according to the methods described using a VARIAN 1275 AA brand atomic absorption spectrophotometer [7] [8]. The samples of local resources used during this study were analyzed in Kigali, Rwanda in the laboratory called LuNa Smelter Laboratory located 3 km from the main station of Nyabugogo on the road to Byumba.

For the analysis and interpretation of the data, we used the Statistical Package for Social Sciences (SPSS) software using the "t-student" statistical test and a comparison of statistical means were made for the different concentrations of each heavy metal in all local resources used.

Ethical considerations were taken into account during this work in the sense that neither money nor any other form of influence peddling was used before obtaining the analysis results of our samples. Everything was done in strict compliance with the administrative and technical regulations of the aforementioned laboratory.

3. Results

The results of analysis of heavy metals in each local resource to be used in the manufacture of antidote are shown in **Table 1**.

Table 1. Heavy metals content (mg/kg) in samples.

Biomass	As	Pb	Cr	Cu	Zn	Cd
CDT	146.05	98.15	18.06	151.94	356.48	0.00
CDV	150.45	113.64	20.24	187.72	570.06	0.00
ODV	263.55	193.84	87.85	332.31	287.42	0.16
CNP	81.69	31.65	100.3	59.57	34.90	0.19
SM	53.52	58.84	1.12	85.18	2.24	0.00
GGB	33.99	18.76	3.61	36.82	43.88	0.00
PVN	22.93	19.99	6.13	19.99	41.08	0.00
Mean	**107.45**	**76.41**	**33.9**	**124.79**	**190.86**	**0.05**

Source: LuNa Smelter Laboratory of Kigali, Rwanda.

Table 2 shows the different values of heavy metal contents which represent the maximum contamination thresholds for cattle.

Table 2. Maximum average content (mg/kg) to diagnose contamination in cattle [9].

N°	Element	Content	Source
1	Zn	1261	NRC, 2001, Blais (2005)
2	Cu	498	NRC, 2001, Blais (2005)

Continued

3	As	299	WHO (1993), Bertin *et al.* (2013)
4	Pb	230	NRC, 2001, Blais (2005)
5	Cr	110	NRC, 2001, Blais (2005)
6	Cd	6.99	NRC, 2001, Blais (2005)

Note: **NRC**: National Research Council; **WHO**: World Health Organization.

Table 3 shows some heavy metal standards to compare with the total content found in the GGB, PVN and SM.

Table 3. Maximun average content (mg/kg) to diagnose of plants and soils [10] [11].

N°	Plants (Case of GGB & PVN fruits)			Soils (Case of SDM)	
	Symbol	Content	Reference	Content	Reference
1	Zn	150	WHO (1993), Barkouch (2007)	3820	French standard: January 8th 1998, Baize (2000)
2	Cu	78.1	CIQUAL (2013), Foine (2017)	107	Idem
3	As	97.03	Codex France Standard (1999), Barkouch (2007)	200	Idem
4	Pb	63.07	Idem	3088	Idem
5	Cr	107	Idem	57	Idem
6	Cd	1	Idem	0.42	Idem

Table 4 shows the different values of heavy metal contents which represent the maximum contamination thresholds for human being.

Table 4. Recommended thresholds for heavy metals for human consumption [12].

Symbol	Average content (mg/kg)	Assimilable value (15% of average: mg/kg)	Maximum threshold (mg/kg/day adult person weight of 60 kg)	Reference
Zn	190.8	28.62	40	NASFNB 2001, Pia *et al.* (2007)
Cu	124.7	18.7	10	Idem
As	107.4	16.11	1	FAO/WHO (2004)
Pb	76.41	11.46	0.1	Idem
Cr	33.9	5.08	1.3	Idem
Cd	0.05	0.0075	0.76	IARC 2001, Pia *et al.* (2007)

NASFNB: National Academy of Sciences Food and Nutrition Board; **I.A.R.C.:** International Agency for Research on Cancer.

Statistical analysis

The statistical analysis of the results using t-student t test at the significance level (α: 0.05) on a single sample led to results such that for the case of arsenic, lead and copper, the content averages are statistically and significantly different ($p < 0.05$) for As, Pb and Cu while for Cr, Zn and Cd: $p > 0.05$. The figure below shows how the variation in the heavy metal content in each sample is established.

4. Discussion

In general, results from **Table 1** show that local resources of animal origin, i.e. cow horn, bull horn as well as cow bones, showed high results in heavy metal levels. compared to the remaining. Furthermore, zinc is the heavy metal which was characterized by a highest total content (570.06 mg/kg) and this in cow horn, while cadmium was relatively absent in almost all resources used although recorded with very low levels (0.157 mg/kg) and (0.1966 mg/kg) respectively in cow bones and oil palm nut shells. The high value of zinc content (570.06 mg/kg) in cow horn compared to that of zinc in bull horn (356.48 mg/kg) could be explained by the genetics of the cow which is more favorable to this element compared to that of the bull according to the results found during a study carried out in Canada on the presence of certain heavy metals in the ration of cattle [8].

Concerning the results obtained for copper content in all local resources used, it was noted that cow bones were characterized by the highest value of this metal (332.31 mg/kg) when black pepper was the local resource with the lowest copper content value (19.9 mg/kg). This high copper value comes from the concentration of this element in the ration given to cows. Similar results were found by some researchers from DRC, who carried out investigations in the presence of some trace elements in the leafy vegetables sold in Lubumbashi markets. They concluded that copper and zinc were the two heavy metals with high concentration compared to other elements. due to ($ZnSO_4$ and $CuSO_4$) which are also added to the cattle ration [10]. For the case of: ginger, black pepper and oil palm nut shells, the low content of zinc and copper recorded especially in the two first samples, would be justified by the use at more than 70% of organic manure for soil fertilization that is so poor in copper and other trace elements compared to mineral manure [11]. Similar results were found by some researchers in Burkina Faso in 2015, who, have been carrying out investigations into the characterization of the ginger (Zingiber officinale Rosc.) production system, found a deficiency in trace elements after analysis of the stems and leaves of the plant [12]. The high concentration of zinc and copper found in oil palm nut shells sample compared to ginger and black pepper would be due either to contamination during the carbonization process of these oil palm nut shells [13] or to the export of trace elements from phosphate fertilizers used in fields located in the hills compared to oil palm trees grown in the Ruzizi plain where samples for oil palm nut shells used during this study were purchased [14]. Similar results were found by some researchers in Ivory Coast in 2009 who evaluated the export of certain heavy metals (Cu, Cr, Mn and Zn) in the surface sediments of a tropical African lagoon. Indeed, these researchers concluded that the sediments loaded with these heavy metals were the source of the increasing in the content of trace elements in the fruits of certain plants cultivated in Ivory Coast [3]. To reinforce the result of preponderance of zinc and copper compared to other heavy metals, research conducted in India during the 2017 year proved these two heavy metals most accumulated in both species of fish Cyprinus carpio and Labeo rohita for which they had compared the rate of bioaccumulation of certain heavy metals [15] [16]. The lower lead levels recorded in CNP, GGB and PVN respectively (31.65 mg/kg, 18.76 mg/kg and 19.99 mg/kg) compared to local resources of animal origin would be justified by its very slow export speed from the soil to the plant compared to that of zinc and copper whose bioaccumulation export speeds are very rapid [7]-[9].

In the case of arsenic, it was found that its content was higher in animal waste compared to other resources (see **Table 1**). The treatment of animals as well as the processing of cowhides carried out around the Slaughterhouse would contribute to the increase of arsenic content. Regarding chromium, the CNP sample recorded a high content compared to that of the others (100.3 mg/kg) while for CDT and CDV we obtained respectively (18.06 mg/kg and 20.24 mg/kg). This high chromium content could be explained by the quality of the ferro-clay soil because during the year 2000, the researcher Baize carried out a study of bi-variable relationships in France where he clearly demonstrated that the more iron there is in a soil, the higher the chromium content will be [11]. The soil of the Ruzizi plain could also be rich in iron and this could explain the high concentration of chromium found in the CNP resource [17] compared to resources from animal and mineral origin. Moreover, cadmium was the heavy metal that was almost absent in all the local resources used except in ODV and CNP where we obtained (0.157 mg/kg and 0.1966 mg/kg) respectively. This difference would come from intense fertilization, pasture plants and/or oil palm plant where the nuts sheets were extracted [18] [19]. These results are similar to those found at Lubumbashi, in the Democratic republic of congo by certain researchers in 2013 who concluded that of all the heavy metals found in leafy vegetables sold in the different markets of the mining area of Lubumbashi: Cu, Co, Pb, Cd and Zn, cadmium recorded low levels compared to others with 1.20 mg/kg Dry Matter (DM) in amaranths and 1.49 mg/kg DM in pepper chard [12]. The purpose for analyzing these heavy metals in the different samples was to compare their contents with the standards used, in order to be sure that the antidote that we are going to make based on these samples will not be toxic to the patients to whom it will be administered. The researcher Baize established in 2000 that the total content of a certain element gives information on the stock of given element in the plant, unfortunately it does not avail information on its mobility in the soil nor its availability in the plant. However, it is desirable to make a clearly distinction between what is natural and what is contaminating, because the metal from anthropogenic contributions certainly presents greater risks than natural metal, often immobilized in relatively inert forms [7]-[10]. This thesis is not excluded for this study especially since the heavy metals present in the samples analyzed could come either from anthropogenic activities or from the nature (case of zinc for horn). The quantity that exists naturally in one of the animal's organs would come from the cow's diet that normally contains zinc and other metals such as copper. Bertin and other researchers were able to demonstrate in 2013 that the average absorption efficiency of heavy metals is estimated at 15%. Thus; the zinc requirements for dairy cattle depending on growth, pregnancy, milk production and stress level of the animal [8]. These authors illustrated a 650 kg cow producing 40 kg of milk, whose zinc requirement is established at 1261 mg/kg/day, taking into account the average efficiency of zinc absorption. which is estimated at 15%. From this quantity, there are only 189.6 mg/kg/day which are absorbable and the 1071.4 mg/kg/day remaining are immobile. Taking into account soil; plant and human risk contamination, the results of our study (see **Tables 2-4**) show no risk. The using of these resources for antidote make to treat poisonings has no risk contamination for poisoned patients. However, we suggest that this antidote shall be thioloprive and then bioccumulative. It could not be consumed during a long period to avoid respiratory problems with the presence of sulfur.

5. Conclusion

The heavy metal contents in the samples analyzed are relatively low compared to those given in the literature. They are therefore below the authorized limit values. The risks of contamination of local resources are not predictable because all the average values in total content for all the heavy metals analyzed were lower than the thresholds recommended by the literature. These results show that the antidote technology to combat poisoning in the city of Bukavu can be implemented without risk of toxicity. However, most of these heavy metals are thioloprive molecules and therefore bioaccumulative, antidotes made from these elements should not be taken for a prolonged period.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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