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Research Article
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Assessment of Trace Elements in Bissap (*Hibiscus sabdariffa*) Sold in the City of Abidjan in Côte d'Ivoire

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Abstract

Pollutants such as lead, cadmium and mercury are harmful to living things at low concentrations. In Côte d'Ivoire, the drink of Hibiscus sabdariffa Linn generally called "Bissap juice is widely consumed by the population. However, recent studies have revealed a significant presence of heavy metals in the cultivated soils as well as in the plants taken from these soils in the city of Abidjan. Our study aimed to assess the health risks associated with the consumption of Bissap marketed in three (3) communes of Abidjan. Thus, evaluation of trace metallic elements (Pb, Cd, Cu, Zn) in *Hibiscus sabdariffa* L. calyces given as average value: Cd (23,142 ± 13,332 mg/kg), Pb (77,949 ± 15,620 mg/kg) Zn (78.96 ± 15.584 mg/kg) and Cu (42.894 ± 28.394 mg/kg). Bissap juice showed average concentrations Zn (0.139 ± 0.076 mg/L), Pb (0.544 ± 0.143 mg/L) and Cd (0.221 ± 0.064 mg/L). These data compared to Codex Alimentarus and European Commission standards revealed a significant risk of lead and cadmium poisoning in calyxes and Bissap juice and a lower value for metal copper compared to zinc. Our study requires further investigation in order to evaluate all the factors involved in the chain of contamination and to assess possibly the toxicological and pathophysiological risks incurred by the population due to abuse.

Keywords: *Hibiscus sabdariffa*; Trace elements; Bissap; Chronic poisoning

Introduction

Awareness of the dangers of metal trace elements (ETM) is unequivocal for scientists and leaders around the world. The issues raised by the ETM or heavy metals are mainly environmental but above all health [1]. Trace metals (Copper, Zinc, Iron, Arsenic, etc.) are all toxic or toxic at a certain threshold [2]. Some of these pollutants such as lead, cadmium and mercury are harmful to living things at low concentrations [3]. One of the main sources of human intoxication to heavy metals is diet.

The use of chemical fertilizers, food additives, environmental pollutants and others related, promote the presence of heavy metals (HMT) in plants, water, fish and other products used for human consumption [4]. HMTs are a real public health problem with metabolic disorders and chronic diseases (cancer, high blood pressure, neurological diseases, infertility, etc.) and the high costs of managing these diseases [5].

Zinc (Zn) is essential for normal immune function [6] and has been shown to reduce the incidence of diarrhea and pneumonia [7] but also involved with Cu as cofactors of superoxide dismutase enzyme to fight against oxydative stress. The need for Cu also derives from its involvement in a myriad of biological processes, including antioxidant defense, neuropeptide synthesis and immune function [8,9]. Cu deficiency may result in impaired development of the cardiovascular system, bone malformation and ongoing neurologic and immunologic abnormalities into infancy and beyond [10,11].

In terms of health, the level of morbidity and mortality remains high in Côte d'Ivoire, mainly affecting women and children. General morbidity remains characterized by communicable diseases (Tuberculosis) and by no communicable diseases (NCDs) in a health context of concern [12]. According to the WHO, NCDs show a marked increase and are responsible for 31% of deaths in Côte d'Ivoire in recent years [13]. Thus, no communicable diseases are a national health concern and remain dominated by metabolic diseases, cardiovascular diseases and cancers [14].

Hibiscus sabdariffa Linnaeus var. Sabdariffa, known in Côte d'Ivoire under its vernacular name of Bissap, Dah or Dâ foura [15], is sold in almost all public spaces, making Ivorian eating habits (feast, accompaniment of snacks, refreshing-toning, etc.).

It's very affordable cost makes it one of the street foods, the most consumed by the population especially by the younger who are fond of sweets. The vegetable growing of Bissap (*Hibiscus sabdariffa L.*) occurs in the urban and peri-urban areas of the cities of Abidjan and Yamoussoukro [16]. However, in recent years, several studies have highlighted a worrying presence of heavy metals in these vegetable soils in Abidjan and in the crops grown there [17-19]. Moreover, the presence of these contaminants was also noted in the plants sold on the Abidjan markets [20]. The present study generally aims to determine the levels of four (4) trace metals, namely cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn), in *Hibiscus sabdariffa L.* (Dried chalices and Bissap juice) sold in the city of Abidjan. It is part of a contribution to the assessment of the level of exposure of populations to MTEs and therefore to the reduction of the risks of proliferation of metabolic and chronic diseases.

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Materials and Methods

Study area

Abobo, Yopougon and Adjamé were the three communes of the District of Abidjan (Côte d'Ivoire) which served as a framework for our research. These communes are distinguished by their demographics and their fluctuations (human, commercial, etc.) Abobo covers an area of about 9,000 hectares, with a population estimated at 1,030,658 inhabitants. According to the latest census, it is the second most populous commune in Abidjan (and Côte d'Ivoire) after Yopougon [21]; first Ivorian commune by its demography with a population estimated at 1,071,543 habitants. Unlike the two previous municipalities, Adjamé has an estimated population of 372,978 habitants and constitutes only the 5th most populated municipality [21]. However, this commune is distinguished by the external affluence it records every day. Indeed, Adjamé represents a very important trade and human transit hub for the District of Abidjan.

Sampling

The biological material was composed of dry calyces of *Hibiscus sabdariffa Linn.* and Bissap beverages or "Bissap juice".

Our study sites were selected on the basis of the demographic factor [21] and previous studies carried out [20]. In the three communes, the calyces were bought in the main supply markets, while beverages came from markets as well as other busy public spaces (bus stations, crossroads, sidewalks, etc.). Only traders with a high daily customer base were included in this study. The goal is to have an excellent representativeness of the consumer population of Bissap.

Samples kept in their original containers were transported in coolers chilled by carboglaces; Calyces were packed in hermetically sealed plastic bags. Each sample was labeled to facilitate future identification.

Samples preparation

Concerning the calyces, it consisted in passing the dry flowers to the machine for a crude fragmentation. This step is essential to optimize the extraction of Bissap juice; traders ensured this phase of fragmentation for their customers.

Concerning the drink, the dry calyces of Hibiscus sabdariffa were then soaked in water at room temperature for an average of 1 to 2 days or boiled at least 100°C in water between thirty (30) minutes and one (1) hour. Then, after filtration, sugar was added for the formulation of the final beverage. However, other ingredients (fruits, mint leaves, chemical flavors, etc.) could be added.

Determination of trace elements

The determination of the metallic trace elements (ETM) was carried out by atomic absorption spectrophotometry with an air-acetylene flame (Varian AA20 Pattern®, France). The different concentrations were read in triplet by ranges of standard elements of copper, zinc, cadmium and lead at the following wavelengths: 229.6 nm for Cd, 217.8 nm for Pb, 325.5 nm for Cu and 214.6 nm for Zn. Concerning calyces, a mass of 0.3 g of dried calyxes milled and placed in a porcelain crucible was placed in an oven at 600°C for 5 hours. After cooling, 5 mL of nitric acid (1N) were added to the ash obtained and then brought to total evaporation on a sand bath previously heated. To the residue are added 5 ml of hydrochloric acid (0.1N). The solution was then re-baked at 400 °C. for 30 min. The final residue was recovered in 10 ml hydrochloric acid (0.1N) and then transferred to a 50-ml flask. The procedure was repeated three (3) times and then the contents of the completed flask were diluted with hydrochloric acid to 50 ml. The trace elements contained in the solution were then assessed after blank tests [22,23]. Concerning the Drink (Bissap juice), a volume of 6 mL of each sample was taken and then placed in dry tubes before assaying the ETMs contained in the solution.

Statistical analysis

The GraphPad Prism 7 software was used as a means of statistical data processing. The ANOVA test and multiple comparison (Tukey) of the variation of means were applied to the data. The differences were considered significant for $p<0.05$ to $\alpha=0.05$.

Results

The majority of calyce sold in the market was found in Adjamé market and the Bissap juice performed in majority following a hot extraction (Figure 1).

Distribution of trace metal in Bissap calyces

The results showed the presence of cadmium, lead, zinc and copper at varying concentrations in the calyces of Hibiscus sabdariffa. Bissap calyces sold in the city of Abidjan (Figure 2) had relatively high Cd contents (23.14 ± 13.33 mg/kg), Pb (77.95 ± 15.62 mg/kg), Zn (78.96 ± 15.58 mg/kg) and Cu (42.90 ± 28.39 mg/kg) with substantially equal lead and zinc.

The trace metal content (Table 1) was lower in Adjame than in the other two communes. Yopougon calyces were richer in Zn and Cu, while Abobo's calyces were richer in Cd and Pb. However, average concentrations between study areas showed significant differences ($P<0.001$), except Cd in the calyxes at Adjamé and Yopougon with ($P=0.144$).

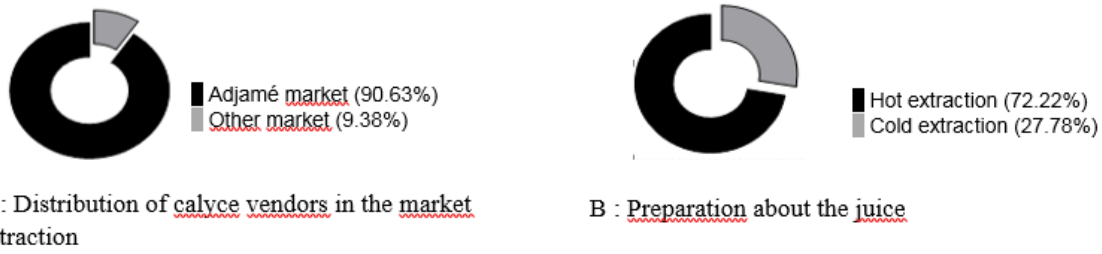

Figure 1: Distribution of calyce in the market (A); Preparation about the juice extraction (B).

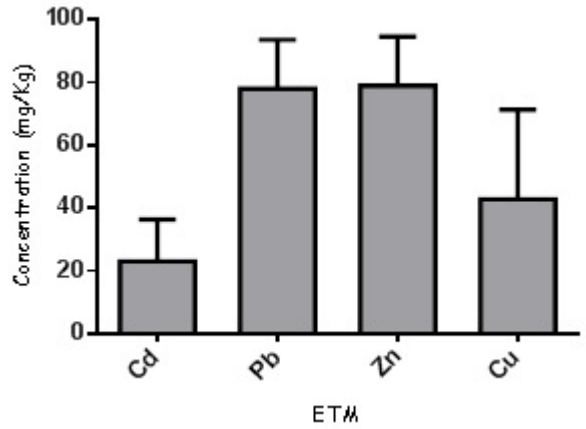

Figure 2: Mean rate of trace metallic elements in calyces.

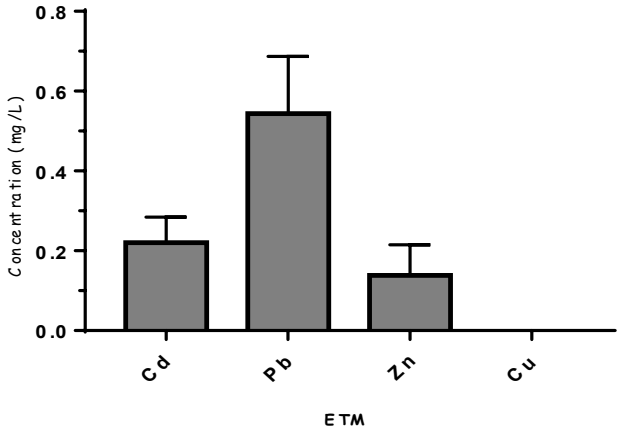

Figure 3: Mean rate of trace metallic elements in the Bissap juice.

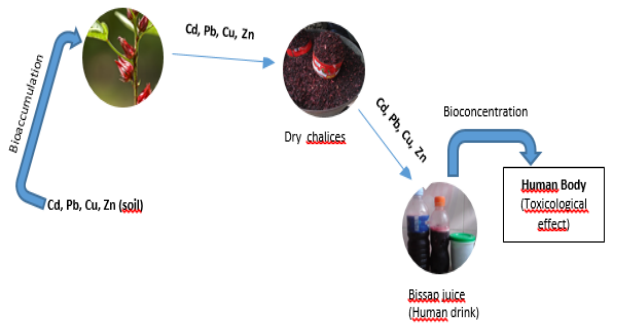

Figure 4: TOC graphic.

Distribution of trace metal in Bissap juice

The presence of trace copper was found in the beverages of Hibiscus Sabdariffa. Zinc (0.14 ± 0.07 mg/L) was present in Bissap juices at very low levels compared to toxic metals (Cadmium and Lead). However, Pb (0.54 ± 0.14 mg /L) was twice as high as Cd (0.22 ± 0.06 mg /L) in Bissap juice sold in Abidjan (Figures 3 and 4).

Mean Pb and Zn levels showed significant differences between the three study areas. On the other hand, those of the Cd were statistically identical except between Abobo and Adjame (P=0.09). Bissap dosed juice showed higher levels of Cd to Yopougon, Pb to Abobo and Zinc to Adjamé (Table 2).

Area market	Trace elements (mg/Kg)			
	Cadmium	Lead	Zinc	Copper
Abobo	40.64 ± 2.34*	93.26 ± 3.04*	75.74 ± 4.03	32.24 ± 2.82
Adjame	12.33 ± 1.42	58.34 ± 2.45	63.13 ± 3.95	16.83 ± 1.09
Yopougon	16.45 ± 1.24	82.25 ± 2.12	98.01 ± 1.93*	79.60 ± 2.42*

*p<0.001

Table 1: Repartition of trace metallic elements in calyces according to area market.

Area market	Trace elements (mg/L)			
	Cadmium	Lead	Zinc	Copper
Abobo	0.16 ± 0.03	0.72 ± 0.03*	0.14 ± 0.04	ND
Adjame	0.21 ± 0.03	0.50 ± 0.04	0.22 ± 0.03*	ND
Yopougon	0.29 ± 0.05*	0.41 ± 0.03	0.06 ± 0.02	ND

*p<0.001

Table 2: Repartition of trace metallic elements in the Bissap juice according to area market.

Discussion

In this study, the concentrations of metals (Cd, Pb, Zn and Cu) recorded in the dry calyces of Bissap were compared with those observed by other researchers. Thus, in their work on the synthesis of Hibiscus sabdariffa, [24] reported concentrations of 6 mg/kg of Cu; 1.8 mg/kg in Pb and up to 65 mg/kg in Zn in the dry calyces of Bissap from Mali and Nigeria. These Cu and Pb data are much lower than those obtained in the calyces sold on the markets of Abidjan (Côte d'Ivoire) but identical for the Zn. However, the Zn levels (814 mg/Kg) presented by [25] for purple red calyxes were much larger than ours. Hibiscus sabdariffa can be used for soil phytoremediation [26]. The presence of trace metals in calyxes could be explained by bioaccumulation resulting from soil contamination in crop soils [27], but also by exposure to environmental dust, Engine exhaust etc. [20].

Adjamé was the main calyce supply area and the least populated; this may justify the lower grades recorded in this study area. Thus, the levels of TME in the calyces of Adjame would be an indicator to better understand the level of initial contamination and by extrapolation the level of exposure to metals for each of the study areas. However, by referring to the maximum permitted Cd (0.2 mg/kg) and Pb (0.3 mg/kg) limits for edible plants by [28]; in Zn (20 mg/kg) and Cu (100 mg/kg) reported in the publication by [17], there would be a high risk of Cd, Pb and Zn contamination for consumers in various forms of dried calyces from Abobo, Adjamé and Yopougon. On the other hand, the Cu content had been found to be below the recommended limit value for this metal. In the study by [29], the testicular cytotoxicity of rats with water extracts of Hibiscus sabdariffa could be justified by the presence of lead [30] in the Bissap calyces. The presence of Cd, Pb, Cu and Zn in the Bissap juice sold in Abidjan would be likely due to the presumed existence of these metals in the calyces of Hibiscus sabdariffa. However, the copper contents were below the detection limit and could not be evaluated.

We have statistically reconciled our data to those of [31] who assessed the Cu and Zn concentrations of two types of Hibiscus sabdariffa beverage marketed in Nigeria: "Sorrel cordial juice" (Cu: 0.056 mg; Zn: 1.46 mg) and the "Black currant cordial juice" (Cu: 0.008 mg; Zn: 0.70 mg). These data as well as those reported by [32] (Zn: 814 mg and Cu: 24.4 mg), were higher compared to our study (Zn: 0.03-0.25 mg; Cu in trace). However, Pb and Zn levels in drinks consumed

in Nigeria based on Hibiscus sabdariffa, showed some results identical to ours [33]. Moreover, when we referred to Codex Alimentarius Codex Stan 179-1991 for fruit and vegetable juices [34] with maximum limits for Pb at 0.1 mg/kg, for Zn at 5 mg/kg and Cu at 5 mg/kg, there was a potential for lead poisoning [0.37-0.75 mg/l] for Bissap juice consumers; this risk would be enhanced by the presence of Cadmium [0.12-0.34 mg/L]. Lead and cadmium are among the top ten (10) most toxic to humans [35].

The low value of Zn and Cu in bissap juice could be justified by the long duration related to the hot cooking of this drink used in majority by the population. The lack of Cu observed in the bissap juice would lead to a bad choice of this drink as a dietary Cu intake; being given that Cu with Zinc are both cofactor of SOD enzyme, important to fight against the oxydative stress in the organism. Other factors could also be considered in assessing the potential hazards to the population: Firstly, the low presence of Zinc, which plays an important role in protecting the organism against cadmium [36,37]; but also in many other biological phenomena such as cell growth and proliferation [38], the fight against free radicals [39], immune responses [40], reproduction [41]. Then, we could also highlight the method of hot extraction which would considerably reduce the anthocyanin content knowing their important role in the antioxidant activity of Bissap [42]. Finally, the ignorance of the actors of the sector of Bissap and the bad manufacturing practices would favor the microbiological risks [43,44].

Prolonged and regular consumption of Bissap which contains Pb and Cd could lead to chronic heavy metal poisoning with disastrous consequences for the health of populations [45]. Works demonstrated the cumulative effect Pb and Cd on rats [46]. This chronic poisoning would have led to an increase in oxidative stress in the heart and liver as well as an alteration of these organs. Researchers have also demonstrated in rats that chronic co-exposure to lead and cadmium, a groundwater pollutant in a city in Algeria, would increase the risk of oxidative stress among the population [47]. Thus, regular and prolonged consumption of Bissap juice could be the cause of chronic diseases [48] such as high blood pressure, renal insufficiency, cancer, neurological disorders, sexual sterility etc.

Bissap juice is a drink of interest for its high consumption but also for its richness in anthocyanin which gives it many therapeutic properties. However, its poverty in essential minerals such as copper and zinc and the inappropriate presence of lead and cadmium beyond accepted recommendations do not militate to its advantage. Thus, given the impact of these heavy metals on the body, regular consumption of this drink by the population does not exclude the risks of prevalence of chronic diseases such as high blood pressure, renal insufficiency, cancer, neurological disorder etc. Our study requires further investigation in order to evaluate all the factors involved in the chain of contamination and to assess possibly the toxicological and pathophysiological risks incurred by the population due to abuse.

Contributors

MGM, AJAAB and DAJ conceived and designed the experiments. MGM and NIGZ performed the experiments. MGM, AJAAB, KKM analyzed the data. MGM and DAJ wrote the paper.

Conflict of Interest

The authors declare that there is no conflict of interest.

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