



Levels of Heavy Metals and Their Risk Assessment in Kolanuts (*Cola nitida* Schott & Amp; Endl.) Collected from Cote d'Ivoire, West Africa

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Authors' contributions

This work was carried out in collaboration among all authors. Author KR designed the study, wrote the protocol, fitted the data and wrote the first draft of the manuscript. Author NY checked the first draft of the manuscript and achieved the submitted manuscript. Authors DMV, NY, AA, CA, SD and BH performed the statistical analysis, managed the literature and assisted the experiments implementation. Author BH expertized the results interpretations. All authors managed the literature, read and approved the submitted manuscript.

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ABSTRACT

Background: Kolanut represents a significant economic interest for this country as well as many African households and public authorities. Despite its obvious importance, the sector of the kolanut is facing a delicate sanitary quality of the marketed product. The majority of the production (90%) of kola is consumed daily fresh by people and could cause a serious health problem for consumers if the toxicity due to heavy metals were proven.

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Aims: This study aimed to determine the heavy metals levels in kolanuts and estimate the risks of nuts consumption on population health in Côte d'Ivoire.

Study Design: Samples were collected from farmers, rural collectors, urban stores in districts (Mountains, Comoe, Lagoons, Down-Sassandra) and big storage centers of Anyama and Bouake.

Methodology: Concentrations of 3 heavy metals were measured using Atomic Absorption Spectrophotometry. The analysis of lead and cadmium was performed in flame mode (Air / nitrogen), with an AAS type VARIAN SPECTRAA 110 provided the furnace GTA 110. While, the analysis of mercury was carried out in hydride mode with a SAA system equipped with a vaporization unit (VGA 77).

Results: Results showed the presence of the 3 heavy metals in kolanuts samples, with concentrations ranging from 5.37 µg/kg to 11.21 µg/kg, 17.49 µg/kg to 51.01 µg/kg and 19.99 µg/kg to 40.35 µg/kg for lead, cadmium and mercury, respectively. Based on the concentrations and the daily consumption of kolanuts estimated at 0.6 g/person in Côte d'Ivoire, the intakes values estimated by heavy metals were $4.8.10^{-3} \pm 4.9.10^{-4}$ µg/j, $1.3.10^{-2} \pm 9.07.10^{-4}$ µg/j and $1.7.10^{-2} \pm 1.99.10^{-3}$ µg/j for lead, cadmium and mercury, respectively. The exposure daily doses (EDD) are all lower than the toxicological reference values. Thus, the occurrence of a toxic effect from Pb (HQ = $1.94.10^{-5} < 1$), Cd (HQ = $1.9.10^{-4} < 1$) and Hg (HQ = $3.4.10^{-4} < 1$) after Kolanuts consumption is very unlikely since the HQ are all less than 1.

Conclusion: Consumption of kolanuts from Côte d'Ivoire would not present any health risk for the consumer.

Keywords: *Cola nitida*; heavy metals; cadmium; lead; mercury; Intake; consumption; Côte d'Ivoire.

1. INTRODUCTION

Kolanut (*Cola nitida*) belongs to the plant family of Malvaceae, having about 140 species of trees native to the tropical rainforests of Africa [1,2,3]. In Côte d'Ivoire, the kolanut production area corresponds to that of cocoa (*Theobroma cacao*) with a cultivated area ranging from 1 to 15 ha and nearly 2 thousand producers involved in the sector [4,5]. The post-harvest process business is mainly supported by smallholder farmers, rural hawkers, rural storage points, communal storage sites, and wholesale stores [4,6]. Côte d'Ivoire is the top country in production and exportation of kola, with about 260,000 tons of fresh kolanuts per year [4,7]. This reflects a significant economic interest for this country as well as many African households and public authorities. For example, they are of great economic interests in exchanges between Côte d'Ivoire and Sahelian countries, with annual exportation revenue close to 130 billion CFA francs [8,6,9]. According to Dibi [5], Côte d'Ivoire has two wholesale stores represented by Bouake market and the city of Anyama, even if production follows a distribution which generally goes from producers to large storage, processing and export centers, via rural collectors and urban stores [4].

The importance of kolanuts is relative to its chemical composition. Indeed, it is source of bioactive and functional compounds such as

polyphenols and caffeine which reflects an increasingly growing interest for industries [10,3]. Also, it is consumed for these stimulating and energizing properties, promoting the physical and psychic endurance of manual workers [11,12]. Thus, they are used in food industry for beverages production [13]. The pharmaceutical industry also exploits its wealth in alkaloids (including caffeine and theobromine) to develop analeptic and analgesics products [14,4,3]. In addition, the kolanut has a social importance for various cultural and customary rituals such as births, marriages and funerals [15,16].

Despite its obvious socio-economic importance, the sector of the kolanut is facing a delicate sanitary quality of the marketed product. Indeed, the misuse of chemical fertilizers and pesticides in production and technological post-harvest treatments present disadvantages for the kolanuts quality [17,18].

For example, several studies have revealed the presence of heavy metals in Kolanut [19,20,21]. These contaminants have also been detected in certain foods such as corn, onion leaves, cocoa, bread, honey and fish [22,23,24,25].

According to Daugey *et al.* [26] and Itumoh *et al.* [27], the heavy metals are toxic contaminants for living beings, even at very lower concentrations.

They have no known beneficial effect on the cell while their toxicity develops by bioaccumulation along the food tract [28]. This is the case of lead, cadmium and mercury which attract attention in the food industry, due to their clearly established toxicity on human health. Indeed, they are recognized as toxic at the hematological, neurological and renal level [29,30]. They have carcinogenic, osteoarticular and teratogenic effects [31,32,33].

The majority of the production (90%) of kola is consumed daily fresh by people [16] and could cause a serious health problem for consumers if the toxicity due to heavy metals were proven. The presence of heavy metals could also slow down the export of this raw material to new markets, which would constitute a significant shortfall for all actors in the kola sector.

The aim of this study was to determine heavy metals (Pb, Cd and Hg) levels in kolanuts produced in Côte d'Ivoire in order to estimate the health risk for consumer.

2 MATERIALS AND METHODS

2.1 Investigation Site

The study was conducted in the main areas of kolanuts production, big storage and distribution centers in Côte d'Ivoire. The investigated regions are located between 2°30' and 8°30' of West longitude and between 4°30' and 10°30' of North latitude. Thus, the mountain district (pole 1), the districts of Comoe and lagoons (pole 2) and the district of Bas-Sassandra (pole 3) were selected as production areas while the cities of Anyama and Bouake represent the storage and distribution centers (Fig. 1).

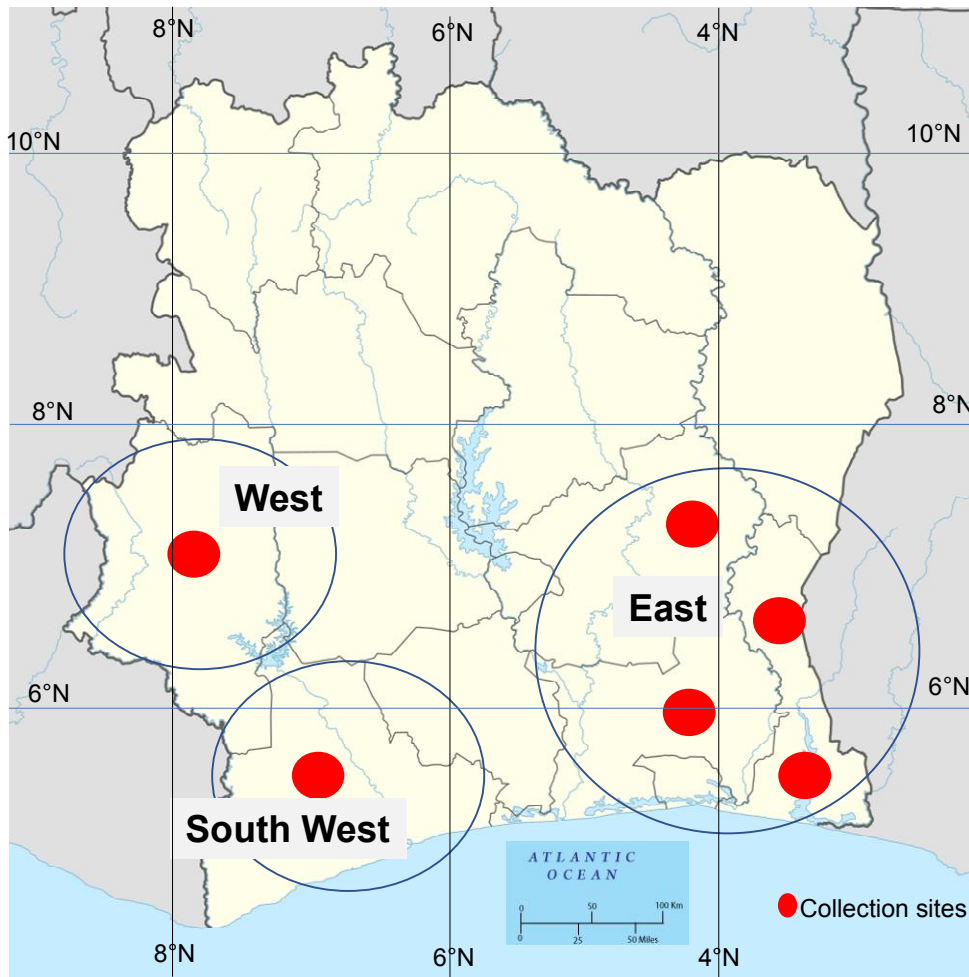


Fig. 1. Production areas and storage centers of Kolanuts in Côte d'Ivoire

2.2 Plant Material and Sampling

The biological material of this study consists of fresh nuts of *Cola nitida* Vent. (Schott & Endl) collected from farmers, rural collectors, urban stores and big storage centers, in accordance with the Regulation No 333/2007 of the European Commission [34]. So, 27 Samples were collected by storage centers (Anyama and Bouake) and by production pole namely 9 samples per type of actors. In total, 135 fresh kolanuts samples, weighing 2 kg each, were used for this study. Kolanuts was authenticated by N'Guessan botanist in the National Floristic Center (CNF) in Abidjan, Côte d'Ivoire, Training and Research Unit of Biosciences, Félix HOUPHOUËT-BOIGNY University where a voucher specimen was documented.

2.3 Mineralization and Analysis of Heavy Metals

The analyzes of heavy metals was conducted by using the method perfectly mastered and validated in accordance with the work of Kouadio *et al.* [6]. Kolanuts were treated according to the method described by Nyamien *et al.* [12].

2.4 Estimation of the Risk of Exposure to Heavy Metals from Kolanuts Consumption

The risks considered in this study derived solely from the consumer exposure through ingestion of kolanuts contaminated with heavy metals. The assessment methodology was conducted according to the model of Codex Alimentarius about risk assessment [35]. This procedure follows four main steps [36] including the hazard identification, the hazard characterization, the exposure assessment and risk characterization.

Assessment risk leads to the calculation of the Estimated Daily Intake (EDI) and the Exposure Daily Dose (EDD) from the average amount of 0.6 g per day of kolanuts consumed by an Ivorian adult [37,12]. The exposure scenarios where the individual is the most exposed have been used (maximalist assumption). EDI and EDD of heavy metals linked to the consumption of kola nuts were determined from equations 1 and 2:

$$EDI = C \times Q \quad (1)$$

Where EDI is the estimated daily intake ($\mu\text{g}/\text{d}$); C the Concentration of heavy metals in kolanut

($\mu\text{g}/\text{kg}$) and Q the Daily consumption of kolanut (kg/d).

$$EDD = C \times Q \times F/P \quad (2)$$

Where EDD is the exposure daily dose ($\mu\text{g}/\text{kg}/\text{d}$); C the Concentration of heavy metals in kolanut ($\mu\text{g}/\text{kg}$); Q the Daily consumption kolanut (kg/d); F the Frequency of exposure ($F = 1$) and P the body weight of an Ivorian adult.

*The average body weight of an adult is conventionally equal to 70 kg according to the American Environmental Protection Agency (US EPA) [38].

The risk characterization for threshold effects was expressed by the hazard quotient (HQ). It was calculated for the oral route of exposure from equation 3:

$$HQ = \frac{EDD}{TRV} \quad (3)$$

Where HQ is the hazard quotient; EDD the exposure daily dose ($\mu\text{g}/\text{kg}/\text{d}$); TRV the Toxicity Reference Value fixed by the Codex Alimentarius.

If $HQ < 1$, the occurrence of a toxic effect is very unlikely;

If $HQ > 1$, the appearance of a toxic effect cannot be excluded.

2.5 Statistical Analysis

Data has been captured with Excel Spreadsheet and were statistically treated using Statistical Program for Social Sciences (SPSS 20.0, SPSS for windows, USA) at 5% significance. The statistical test consisted in a one-way analysis of variance (ANOVA) with the origin of kola nuts. The statistical differences have been highlighted by the test of Duncan test at the 5% level of significance.

3. RESULTS

3.1 Trends of Heavy Metals Concentrations in Kolanuts

The concentrations of heavy metals in kolanuts samples are presented in Table 1. Results showed the contents ranging from 5.37 $\mu\text{g}/\text{kg}$ to 11.21 $\mu\text{g}/\text{kg}$, 17.49 $\mu\text{g}/\text{kg}$ to 51.01 $\mu\text{g}/\text{kg}$ and 19.99 $\mu\text{g}/\text{kg}$ to 40.35 $\mu\text{g}/\text{kg}$ for lead (Pb), cadmium (Cd) and mercury (Hg), respectively. The mean concentration of lead (8.03 ± 0.82

µg/kg) was less than cadmium (22.07 ± 1.54 µg/kg) and mercury (27.80 ± 3.26 µg/kg). These concentrations are below the maximum accepted limits by the Codex Alimentarius which are 50 µg/kg for cadmium and of 100 µg/kg for lead and mercury [39].

Statistical analysis revealed no significant difference ($p \geq 0.05$) between different heavy metal levels determined in the kolanuts whatever the actors.

Otherwise, it noted that the relationship between the content of cadmium - lead, and mercury - lead were 2.75 and 3.46, respectively.

Based on the general standard Codex [39] setting maximum levels for certain contaminants in foodstuffs for human consumption, 2.22% of the samples analyzed are higher than the maximum value fixed for cadmium. Unlike lead and mercury where samples analyzed revealed lower levels than the maximum value set (100 µg/kg). Thus, the overall satisfaction of the sanitary quality of kolanut analyzed is 97.78%. Indeed, on 135 samples of kolanuts, only 3 were contaminated (Table 1).

The cumulative mean concentrations of heavy metals for the overall samples studied are displayed in Fig. 2. In general, the concentrations

of heavy metals decrease while passing from farmers, rural hawkers, rural storage points, communal storage sites, and wholesale stores. Thus, the highest content was found from the farmers (67.5 µg/kg); whereas the big storage centers showed the lowest heavy metals cumulative content (52.23 µg/kg).

3.2 Human Health Risk Assessment

The regularly exposed populations are those adults who daily consume kolanut. Table 2 presents the data of the model of quantitative evaluation of the risks related to the consumption of kolanut.

The estimated daily intakes (EDI) were calculated for the levels of heavy metals in kolanuts collected. The results showed that the level of heavy metals evolves according to their concentrations in the matrix. Indeed, the estimated daily intakes of heavy metals were ranged from $3.22 \cdot 10^{-3}$ µg/d to $6.72 \cdot 10^{-3}$ µg/d, $1.05 \cdot 10^{-2}$ µg/d to $3.06 \cdot 10^{-2}$ µg/d and $1.20 \cdot 10^{-2}$ µg/d to $2.42 \cdot 10^{-2}$ µg/d for lead (Pb), mercury (Hg) and cadmium (Cd), respectively. The highest average intake was found with mercury ($1.7 \cdot 10^{-2} \pm 1.99 \cdot 10^{-3}$ µg/d) while lead recorded the lowest average intake ($4.8 \cdot 10^{-3} \pm 4.9 \cdot 10^{-4}$ µg/d).



Fig. 2. Cumulative mean concentrations of heavy metals from selected kolanuts
Values differ statistically at P=5% according to the lowercase letter

Table 1. Mean concentrations of heavy metals in kolanuts samples

ACTORS	Lead ($\mu\text{g}/\text{kg}$)		Cadmium ($\mu\text{g}/\text{kg}$)		Mercury ($\mu\text{g}/\text{kg}$)	
	Average	[Min – Max]	Average	[Min – Max]	Average	Min - Max
FARMERS	10.65 \pm 0.61 ^a	[9.65 – 11.21]	22.97 \pm 0.90 ^a	[21.35 – 23.80]	33.88 \pm 3.16 ^a	[29.76 – 40.35]
COLLECTORS	6.82 \pm 0.73 ^a	[5.95 – 7.71]	25.48 \pm 1.41 ^a	[22.56 – 26.81]	29.23 \pm 3.03 ^a	[25.33 – 37.47]
STORES	8.47 \pm 1.18 ^a	[6.55 – 9.92]	23.03 \pm 2.99 ^a	[21.77 – 51.01]	22.60 \pm 3.14 ^a	[19.99 – 28.25]
CENTERS	6.15 \pm 0.75 ^a	[5.37 – 7.08]	19.44 \pm 1.14 ^a	[17.49 – 26.16]	26.64 \pm 3.68 ^a	[20.16 – 31.42]
Average	8.03 \pm 0.82	[5.37 – 11.21]	22.07 \pm 1.54	[17.49 – 51.01]	27.80 \pm 3.26	[19.99 – 40.35]
FML ($\mu\text{g}.\text{kg}^{-1}$)	100		50		100	
Sample (%) < FML	100		97.78		100	
Sample (%) \geq FML	0		2.22		0	
Overall satisfaction	97.78 %					

FML: Fixed Maximum Level by the regulation Codex [35]; Min: minimum; Max: maximum
Means with the same letters exponentiating in the same column are not different at 5% according to Duncan test

Table 2. Quantitative evaluation of the exposure of heavy metals

Measured parameters		Concentrations of heavy metals ($\mu\text{g}/\text{kg}$)	EDI ($\mu\text{g}/\text{d}$)	EDD ($\mu\text{g}/\text{kg}/\text{d}$)	TRV ($\mu\text{g}/\text{kg}/\text{d}$)*	HQ = R
Lead	Min.	5.37	3.22.10 ⁻³	4.6.10 ⁻⁵	3.5	1.31.10 ⁻⁵
	Avg.	8.03 \pm 0.82	4.8.10 ⁻³ \pm 4.9.10 ⁻⁴	6.8.10 ⁻⁵ \pm 6.9.10 ⁻⁶		1.94.10 ⁻⁵
	Max.	11.21	6.72.10 ⁻³	9.6.10 ⁻⁵		2.74.10 ⁻⁵
Cadmium	Min.	17.49	1.05.10 ⁻²	1.5.10 ⁻⁴	1	1.5.10 ⁻⁴
	Avg.	22.07 \pm 1.54	1.3.10 ⁻² \pm 9.07.10 ⁻⁴	1.9.10 ⁻⁴ \pm 1.33.10 ⁻⁵		1.9.10 ⁻⁴
	Max.	51.01	3.06.10 ⁻²	4.37.10 ⁻⁴		4.37.10 ⁻⁴
Mercury	Min.	19.99	1.20.10 ⁻²	1.17.10 ⁻⁴	0.7	1.67.10 ⁻⁴
	Avg.	27.80 \pm 3.26	1.7.10 ⁻² \pm 1.99.10 ⁻³	2.38.10 ⁻⁴ \pm 2.79.10 ⁻⁵		3.4.10 ⁻⁴
	Max.	40.35	2.42.10 ⁻²	3.45.10 ⁻⁴		4.93.10 ⁻⁴

Min.: Minimum; Avg.: Average; Max.: Maximum; EDI: Estimated Daily Intake; EDD: Exposure Daily Dose; TRV: Toxicity Reference Value; HQ: Hazard Quotient; R: Risk; d: day.*: Codex [39]

The exposure daily doses (EDD) are all lower than the Toxicity Reference Value (TRV) fixed by the Codex Alimentarius regardless of the contaminant. In fact, EDD were $6.8 \cdot 10^{-5} \pm 0.69 \cdot 10^{-5} \mu\text{g/kg/d}$, $1.9 \cdot 10^{-4} \pm 1.33 \cdot 10^{-5} \mu\text{g/kg/d}$ and $2.38 \cdot 10^{-4} \pm 2.79 \cdot 10^{-5} \mu\text{g/kg/d}$ for lead, cadmium and mercury, respectively. Therefore, the average risks of oral exposure to heavy metals from the consumption of the kolanut are all less than 1.

According to the different exposure scenarios, the occurrence of a toxic effect from Pb (HQ = $1.94 \cdot 10^{-5} < 1$), Cd (HQ = $1.9 \cdot 10^{-4} < 1$) and Hg (HQ = $3.4 \cdot 10^{-4} < 1$) due to the kolanut consumption is very unlikely. However, the risk assessment after the consumption of a quantity of kolanuts greater than 1762.59 g/day (70 nuts/day), considering the mass of a nut equal to 25 g [40], would lead to an EDD higher than the TRV for mercury. As for lead and cadmium, it would be necessary to consume more than 30510.85 g/day (1220 nuts/day) and 3171.72 g/day (126 nuts/day) respectively to assess an undesirable effect.

4. DISCUSSION

Heavy metals targeted in this study (Cd, Pb, Hg) are commonly sought out and monitored by public health services responsible for the quality of food because of their toxicity to humans [41]. Quantitative analysis of the different samples revealed the presence of these heavy metals in the kolanut with variable levels according to the collected area and the type of metal. Results showed the average contents of lead, cadmium and mercury lower than the accepted maximum limits fixed at 50 $\mu\text{g/kg}$ for cadmium and 100 $\mu\text{g/kg}$ for lead and mercury [39]. Heavy metals concentrations of the kolanut samples analyzed comply with international specifications. The concentrations observed are lower than those of work of Oti [20] on kolanuts collected from the markets of Abakaliki in the area of Ebonyi Nigeria where the levels of 880 $\mu\text{g/kg}$ and 710 $\mu\text{g/kg}$ were obtained for lead and cadmium, respectively. The difference levels observed could be due to the various conditions of production, post-harvest processing, storage and transportation of kolanuts [4]. However, contamination of kolanuts by heavy metals could result from weathering rocks in the earth's crust [42,43].

Data obtained indicated that the average content of heavy metals in the kolanut among planters is higher than the levels recorded from other actors

(collectors, stores and storage centers). Indeed, the use of fertilizers and pesticides in kola plantations constitute a contamination source of kola nuts in heavy metals [16,43,6]. As access to the manpower is becoming increasingly unavailable, the majority of producers use phytosanitary products essentially consisting of Callifan super 40 EC, Rund up 360 SL, Thiosulfan 60 EC, Thiametoxam, Durexa, phosphate fertilizers, etc. for the maintenance of the plots. These products contain among other compounds, heavy metals such as arsenic, mercury, cadmium, zinc and lead [44]. According to Wuana *et al.* [45] and Ajani [15], the contamination of kolanut by heavy metals can be attributed to the cultivation techniques used by farmers, marked by excessive use of fertilizers and pesticides. These practices could be justified by a relatively low level of education of the actors and whose ignorance of the main warnings in use of chemicals could derive in serious risks for themselves and for kolanut consumers [17,4]. These chemicals present a good option in agricultural production, but they also represent a source of contamination by heavy metals in soil, with negative impact of the sanitary quality of the raw materials in general [46,47]. Also, the water used in the plantations for post-harvest treatment of kolanuts is another source of contamination in heavy metals. Indeed, the main source of pollution of this water by heavy metals comes from mining sites [43]. Thus, the mining constitutes a contamination factor in heavy metals of kolanut in areas of production. Moreover, this activity releases heavy metals which are concentrated in surface water used for the post-harvest treatment of kola nuts in the plantations [48]. The principal source of cadmium in this medium is phosphate, which is highly concentrated in heavy metals [49].

The high concentration of mercury compared to the lead and cadmium concentrations could be linked to the contact of kolanuts with larger sources of mercury. Indeed, during the storage of kolanuts in peasant kitchens, mercury contamination comes from the smoke produced by the wood fire lit during the preparation of family meals [50]. In addition, on-farm, contamination by mercury comes from the sources of combustion, such as agricultural incinerators [51,52].

Moreover, the pollution caused by the engines with gas emissions from fuels generally containing lead constitutes another source of contamination in the plantations [53,22].

The decrease in heavy metals concentrations during the distribution circuit, from planters to big storage centers, would be due to the post-harvest treatment and the method of packaging kolanuts. Indeed, the conservation of fresh kolanuts for long time and against pests requires several soaking in chemical pesticide often composed of prohibited pesticides such as DDT (dichlorodiphenyltrichloroethane) [17,4]. Thus, the repeated washing of kolanuts during the transit or storage process could justify the drop in concentrations of heavy metals from other actors in the distribution chain by the phenomenon of dilution. However, the use of these solutions represents a source of contamination of other chemical substances such as pesticides toxic to humans [17,18].

Otherwise, estimated daily intakes (EDI) increase with the concentrations in heavy metals present in the kolanut. This result corroborates that of Ake *et al* [54] which stipulates that the exposure level of consumer increases with concentration of the contaminant in the food. The exposure daily doses (EDD) obtained are all lower than the Toxicity Reference Value (TRV) fixed by the Codex Alimentarius to 5 µg/kg body weight/day, 1 µg/kg body weight/day and 0.7 µg/kg body weight/day for lead, cadmium and mercury, respectively [39]. Thus, Hazard Quotient (HQ) calculated from EDD and TRV are less than 1 regardless of the metal. This situation indicates that kolanuts would not represent a health risk for human and would be safe for people consumption.

On the other hand, the regular consumption of a quantity of kolanuts leading to an EDD higher than the TRV would represent a danger for the consumer's health. Taking into account the bioaccumulative and toxic characters of heavy metals, their presence in the body could be a source of varied diseases [55], insofar where these metals can be supplied by other foods. Since kolanut is largely consumed fresh, it is therefore important to intensify efforts to reduce the presence of these toxic metals mainly by raising awareness among actors on good practices for the production and conservation of kola nuts. These good practices pass by the restriction of the use of chemical fertilizers and pesticides in the production of kola nuts, the use of drinking water in the post-harvest treatment and the storage of kolanuts outside of the smoke-producing sources. Failure to follow these good practices could cause a serious health problem for consumer.

5. CONCLUSION

This study revealed the presence of lead, mercury and cadmium at varying levels in kolanuts. Lead, cadmium and mercury concentrations are generally lower than the maximum limits recommended by the Codex Alimentarius. These chemical contaminants in kolanuts come from different lithological sources and anthropogenic activities. Indeed, the safety of kolanuts depends on factors such as agricultural intrants, the water used for the post-harvest treatment of kolanuts, the storage condition near smoke sources, pollution caused by combustion engines and natural sources from weathering rocks. Estimated daily doses in heavy metals from kolanuts, always remains below the different toxicological reference values. Consequently, the occurrence of a toxic effect is very unlikely for the consumer. kolanuts from Côte d'Ivoire would be safe for consumption. However, this satisfaction must not forget the bad practices of the actors in the chain of production and distribution. Thus, the implementation of efficient technical routes throughout the distribution chain of kola nuts, as well during their production, conservation, and marketing will be able to guarantee better sanitary quality for these agricultural food products.

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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