CONCENTRATIONS AND HUMAN HEALTH RISK ASSESSMENT OF CYANIDE IN CASSAVA PEELS, TUBERS AND LEAVES FROM DELTA STATE, NIGERIA.

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ABSTRACT

Cassava (*Manihot esculenta crantz)* is a perennial tropical crop which contains carbohydrates, vitamins, calcium and iron which have of nutritional benefit; in addition to cyanogenic glycosides The latter when hydrolyzed by the enzyme linamarase produces poisonous hydrogen cyanide. The study aimed at assessing the level of hydrogen cyanide in cassava peels, leaves and tubers and the human risk associated. Determination of cyanide levels was done using the Alkaline titration method and data collected were analyzed using SPSS. The concentration of cyanide in cassava fresh peels, fresh leaves, tubers, dried leaves, rotten peels (wet and dried) shows significant differences ($p<0.05$) between the different locations except for tubers ($p>0.05$). The highest level of cyanide was found in fresh peels with 4.75±0.25 mg/kg in Abraka, 4.34±0.32 mg/kg in Obiaruku and 3.89±0.41 mg/kg in Aragba-Orogun. Cyanide concentration in fufu and garri also shows significant differences ($p<0.05$) in the three sampling locations. The daily intake for ingestion of cyanide were evaluated for adults and children, there was no considerable hazard risk arising from the consumption of the cassava leaves and tubers duly processed.

Keywords: Hydrogen cyanide, Cassava peels, Cassava leaf, Cassava tubers, Human risk.

INTRODUCTION

Millions of people in the tropical parts of Africa, Latin America, and Asia rely on cassava, a widely grown tuber crop (Atehnkeng, *et al.*, 2006, Nhassico *et al*., 2008). The edible starchy tuberous root of cassava, a perennial plant that is widely produced as an annual crop in tropical and subtropical areas, provides a significant source of carbohydrates. Cassava which belongs to the Euphorbiaceae family, a major staple food in Africa especially Nigeria. Cassava is one of the most vital food crops consumed in developing countries especially in tropical areas. Its cultivation and processing are usually done into useful products such as garri and fufu (Afuye and Mogaji, 2015).

The chemical element cyanide belongs to the cyanide group (CN^-). The triple bond between the carbon atom and nitrogen atom makes up this group, which is referred to as the cyano group. The cyanide group is present in inorganic cyanides as anion CN^- , and soluble salts like sodium cyanide and potassium cyanide are extremely poisonous. The extremely flammable liquid hydrocyanic acid, often known as hydrogen cyanide or HCN is manufactured on a huge scale in industrial settings.

Depending on the amount of poisonous cyanogenic glucoside present, cassava is either classed as sweet or bitter (Ndubuisi and Chidiebere, 2018). The bitter cultivars may create more than 50 times as much cyanide per kilogramme of fresh roots as compared to the sweet cultivars, which can yield as low as 20 mg (Kamalu and Oghome, 2012). Like other foods, cassava contains antinutritional and harmful elements. The cassava cyanogenic glucosides, linamarin and lotaustralin, are the most significant. When these substances are hydrolyzed, hydrocyanic acid (HCN), which is dangerous for intake by both humans and animals, is released. Between cultivars and environmental situations, the amount of cyanide varies greatly (Kamalu and Oghome, 2012).

Cassava is primarily grown and processed on a small scale in Nigeria by families or peasant farmers. For individuals in Sub-Saharan Africa, especially in Nigeria, where its production, processing, and consumption are most prevalent and significant on a worldwide scale, cassava offers a dependable and affordable supply of carbohydrates. Several tonnes of cassava peels are produced as waste , and they are usually thought to be a major source of environmental contamination (Spencer and Ezedinma, 2017). Cassava peels can be used as livestock feed, but because of the volume produced and the dumping of many around the environment where processing occurs, a lot of waste is left behind and is burned or allowed to decay, which harms the environment (Olukanni and Olatunji, 2018). Tonukari *et al*. (2015) presented a report of a cassava starch production facility with a daily output of 100 tonnes of tubers and roughly 47 tonnes of byproducts. When discarded carelessly or in the vicinity of processing plants, this output could harm the environment.

Farmers in Nigeria typically dump these created wastes carelessly into water bodies, unfinished structures, undeveloped land, and any open places along major roads and streets, despite the harmful effects of these wastes on both the environment and people's general health (Oghenejoboh *et al*., 2021). It is challenging for Nigeria's peasants and smallholder farmers to understand the connection between waste treatment cost-effectiveness and value-added benefit of cassava waste management due to a lack of effective and implementable cassava waste management laws. This ignorance is what FAO (2020) referred to as the knowledge gap. Solid wastes from cassava include the leaves, stems and peels; these wastes constitute more than 18% of the cassava plant (Ezekiel *et al*., 2012).

Cassava that hasn't been properly processed and prepared may include a lot of cyanogenic glycosides, which is dangerous for your health. "Konzo" is a disorder brought on by consuming too much cyanide from improperly prepared cassava, which can lead to a variety of health issues. A disease known as tropical ataxic 12 neuropathy (TAN) is linked to dietary cyanide poisoning from improperly prepared cassava. TAN, as opposed to Konzo, is a chronic condition that primarily impacts senior citizens (Kashala-Abotnes *et al*., 2019). It has been demonstrated that different cassava cultivars contain differing cyanide contents and that the quantification of this level is influenced by climatic circumstances and other variables (Njankouo *et al*., 2019).

The potential contamination of cyanide therefore, has be minimized through proper waste management and using other processing techniques for cassava peels in production of animal feeds. Therefore, the present study was carried out to assess cyanide concentration on cassava, to evaluate the human risk assessment and to assess the concentration in dried leaves, rotten cassava peels dumped in the environment for a period of time.

Hence, the study aimed at investigating the concentration of cyanide in cassava peels both for fresh and rotten, leaves (fresh and dried) and tubers . Also, to determine the risk assessment associated in ingestion of cassava tubers and leaves.

MATERIALS AND METHODS

Study Area

The study was carried out in three farms locations at three Local Government Area (Ethiope East L.G.A, Ukwani L.G.A, and Ughelli North L.G.A) of Delta State, specifically, at; Abraka, Obiaruku, and Aragba-Orogun. (Figure 1).

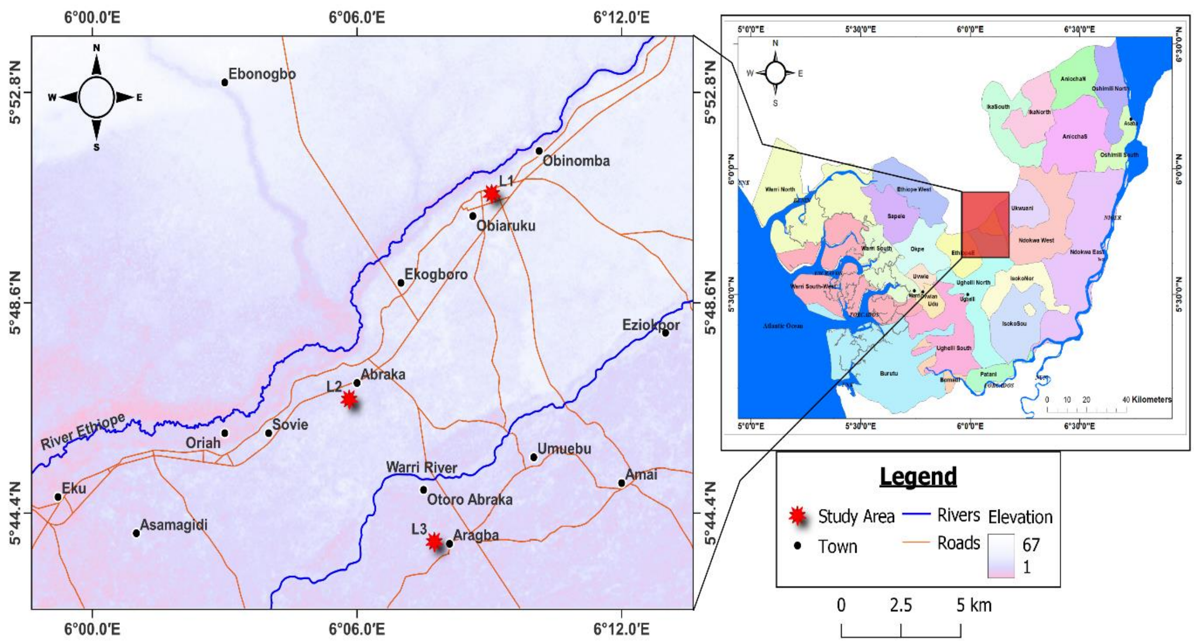

Figure 1: Map of study area

Sample preparation

The cassava tubers were thoroughly rinsed in water, in a basin to get rid of any dirt or sand that could have contaminated them. The outer skin of the cassava tubers was peeled off of the roots. A knife was used to manually complete the process. After being peeled, the cassava was cleaned, split into smaller pieces, and prepared for cyanogenic reduction using a variety of techniques. To determine the degree of cyanide in the cassava leaves and peels, the leaves was cleaned and mashed, and the tubers was weighed, pounded, and soaked for 48 hours. Also rotten peels were cleaned, weighed and soaked.

Boiling of Cassava Tubers

Fifty grams (50 g) from each portion were weighed into different beakers containing 100 mL of distilled water. These were boiled at a constant temperature of 95 ˚C for 10, 15, 20 and 25 minutes respectively. After cooling, the boiled tubers were sliced into small fragments and mashed. The mashed sample was soaked and fermented for 48 hours. The filtrate was then titrated to determine the cyanide content.

Pounded Leaves

Fifty grams (50 g) of homogenised leaves was pounded . The pounded leaves were then placed in various beakers containing 100 mL of distilled water, soaked for 48 hours, and then prepared for cyanide determination using an alkaline titration.

Fresh Cassava peels

Fresh cassava peels were cut into small pieces and 50 g of each portion weighed into a mortar and pestle. After being transferred into beakers and filled with 100 mL of distilled water and soaking for 48 hours, which was then ready for the Alkaline titration test to determine the amount of cyanide present

Dry and Wet Rotten Cassava Peels

The dry and wet rotten cassava peels were weighed separately and 50 g of each was put in a beaker. To this 100 mL of distilled water was added and then the filtrate was used for the determination of cyanide.

Selected Products of Cassava

Fifty grams (50 g) of garri and fufu were weighed separately and put in a beaker. 100 mL of distilled was added and allowed to remain in the beaker for 48 hours before the cyanide determination.

Determination of Cyanide

Fifty grams (50 g) each of samples of cassava, comprising peels, tubers, and leaves were crushed with a mortar and pestle. Each was put into a beaker and allowed to sit for 40 hours to liberate any leftover cyanoglucosides in the samples. After that, it underwent filtering to produce 70 mL of filtrate. In a volumetric flask filled with distilled water, each filtrate was added 20 mL of 0.02 M sodium hydroxide. Two aliquots of 30 mL each were obtained. 3 mL of 6 M of ammonium solution and 1 mL of 5% of potassium iodide were added to the 30 mL of aliquots. This was titrated using 0.02 M of silver nitrate in duplicates. The readings were taken. An equation was used to get HCN concentration in mg: 1 mL of 0.02 M of silver nitrate = 1.80 mg HCN. (AOAC, 1990). Cyanide was detected by a little colour change from yellow to faint reddish brown. Due to changes in concentration, there were colour variations from yellow to faint yellow and then faint red colour . Each concentration was read from the burette after the colour. Concentrations were measured in milligrams per kilogramme (mg/kg) and the average for each area was calculated using the formula below.

$$\text{Total HCN Content} = \frac{13.5 \times TV}{Mass} \quad (1)$$

Where TV is titre value

Health Risk Assessment

The average daily dose (ADD) of cyanide via ingestion (ADD_{ing}) for both adult and children were evaluated by the following equation:

$$ADD_{ing} = \frac{C \times IngR \times EF \times ED}{BW \times AT} \quad (2)$$

To estimate the health risk from consuming cyanide in different parts of cassava, the target hazard quotient (THQ), the Noncarcinogenic risk was calculated as follows (USEPA IRIS 2011).

$$THQ = \frac{C \times IngR \times EF \times ED \times Cf}{BW \times AT \times RfD} \times 10^{-3} \qquad (3)$$

Where Cf is the conversion factor (0.208).

RESULTS AND DISCUSSION

The concentration of cyanide in the fresh peels, fresh leaves, tubers, dried rotten leaves, wet rotten leaves and dried rotten peels in Abraka, Obiaruku and Aragba-Orogun are presented in Table 1. There were statistically significant differences ($p<0.05$) in the cyanide concentrations between all parts of the plants evaluated except the tubers ($p>0.05$) in all the sampled locations.

Table 1: Concentration of Cyanide in Cassava (*Manihot esculenta Cantz*) fresh peels, fresh leaves, tubers, dried leaves, wet rotten peels and dried rotten peels.

Concentration of Cyanide (mg/kg)	**Abraka**	**Obiaruku**	**Aragba-Orogun**	**p-values**
Fresh Peels	4.75 ± 0.25^{a}	4.34 ± 0.32^{ab}	3.89 ± 0.41^{b}	0.01
Fresh Leaves	3.42 ± 0.36^{a}	2.99 ± 0.07^{b}	2.82 ± 0.19^{b}	0.01
Tubers	1.15 ± 0.08^{a}	1.24 ± 0.14^{b}	1.33 ± 0.35^{c}	0.47
Dried Leaves	1.82 ± 0.07^{a}	0.97 ± 0.09^{b}	0.94 ± 0.05^{b}	0.00
Wet Rotten Peels	2.17 ± 0.12^{a}	1.52 ± 0.14^{b}	1.08 ± 0.02^{c}	0.00
Dried Rotten Peels	1.82 ± 0.07^{a}	0.95 ± 0.06^{b}	0.94 ± 0.05^{b}	0.00

The mean sharing similar superscripts on the same row are not statistically different at p=0.05

The concentration of hydrogen cyanide (HCN) in cassava sample from Abraka has a mean and standard deviation value of 4.75±0.25 mg/kg for cassava peels, 3.42±0.36 mg/kg for cassava leaves and 1.15±0.08 mg/kg for cassava tubers. For cassava samples taken from Obiaruku, the concentration of hydrogen cyanide is 4.34±0.32 mg/kg in cassava peels, 2.99±0.07 mg/kg in leaves and 1.24±0.14 mg/kg in cassava tubers. While in cassava samples from Aragba-Orogun the cyanide contents is 3.89±0.41 mg/kg for cassava peels, 2.82±0.19 mg/kg for cassava leaves and 1.33±0.35 mg/kg for cassava tubers. The concentration of hydrogen cyanide from the three studied areas for cassava peels, leaves and tubers were all within the recommended levels by World Health Organization of 10 mg/kg for hydrogen cyanide (HCN) body weight (WHO, 2012).

The results from this study was consistent with Williams *et al.,* (2021) findings, which also noted a greater level of cyanide in fresh cassava peels at 5.25 $\pm$ 1.78. Cassava peels are known to have a higher amount of cyanogenic glucoside, which makes it not suitable in feeding of animals directly but must be processed to reduce cyanide content (Twenyongyere and Katongle, 2002 ; Okpako *et al.,* 2008). It is also known that cyanide concentration tends to increase during drought or extended dry periods due to water stress of plants (Njankouo *et al.,* 2019).

The results from Table 1, shows the concentration of rotten peels of cassava (wet and dried). The result revealed that concentration of wet rotten peels were higher compared to the dried ones. This was as a result of the wet rotten peels have been exposed to waste water or effluent from different garri mill site. This was carried out to evaluate if the concentration of cyanide were within the WHO standard and can still be used for the production of animal feeds in order to reduce environmental pollution.

Previous studies revealed that boiling has been one of the most effective method used to reduce cyanide concentration in leaves for consumption or cooking it for a longer period of time (Ojiambo *et al.,* 2017). From Table 1, the study shows that leaves that are dried directly from the sunlight and pounded reduced the cyanide level in the different locations. The pounding is known to rapture the cells compartment of the leaves and allows the linamarin and enzyme linamarase, which catalyzes the hydrolytic breakdown of cyanogenic glycosides to come into direct contact (Cardoso *et al.,* 2005).

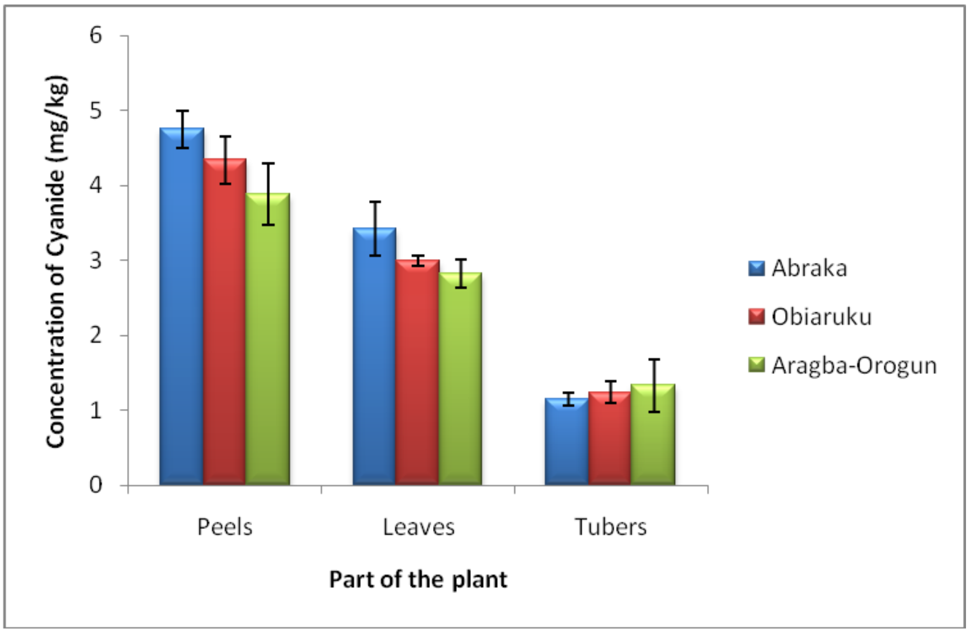

Figure 1: Concentration of cyanide in different parts of *Manihot esculenta* Cantz

The result of the concentration of cyanide in the peels, leaves and tubers in Abraka, Obiaruku and Aragba-Orogun is presented in Figure 1. In peels, Abraka has a substantially greater cyanide content than Obiaruku and Aragba-Orogun. In terms of the leaves, Abraka has a substantially higher cyanide content than Obiaruku and Aragba-Orogun. Cyanide levels in tubers are lowest in Abraka and greatest in Aragba Orogun.

Table 2: Cyanide concentration in Fufu and Garri in Abraka, Obiaruku and Aragba-Orogun

Concentration of Cyanide	Abraka	Obiaruku	Aragba-Orogun	p-value
Fufu	1.11±0.02[a]	0.49±0.04[b]	0.51±0.02[b]	0.00
Garri	0.82±0.06[a]	0.67±0.02[b]	0.56±0.01[c]	0.00

From Table 2, the concentration of cyanide in Fufu and Garri in different locations shows that the Cyanide concentration found in both Fufu and Garri is higher in Abraka than in Obiaruku and Aragba. The Cyanide concentration is however lowest in Fufu in Obiaruku but lowest in Garri in Aragba-Orogun. The result revealed that fufu contains more cyanide content 1.11±0.02 mg/kg and 0.51±0.02 mg/kg than garri 0.82±0.06 mg/kg and 0.56±0.01 mg/kg in samples collected in Abraka and Aragba-Orogun. While samples obtained in Obiaruku show a slightly lower concentration of hydrogen cyanide in Fufu (0.49±0.04 mg/kg) than in Garri (0.67±0.02 mg/kg). The concentration of hydrogen cyanide in Garri and Fufu follows the order: Abraka > Aragba-Orogun > Obiaruku. This may be due to the processing techniques involved in processing these cassava products which could be environmental and fermentation conditions. The statistical analysis also revealed that the raw cassava peels, leaves and tubers obtained from Abraka contain more cyanide content than the processed cassava product (fufu and garri). According to Ojiambo *et al*. (2017), heat increases the evaporation of hydrogen cyanide and cyanohydrins therefore, the cassava products would not contain free cyanides and cyanohydrins and this explains why the cyanide content in the raw cassava (peels, leaves and tuber) is higher than the processed product (fufu and garri).

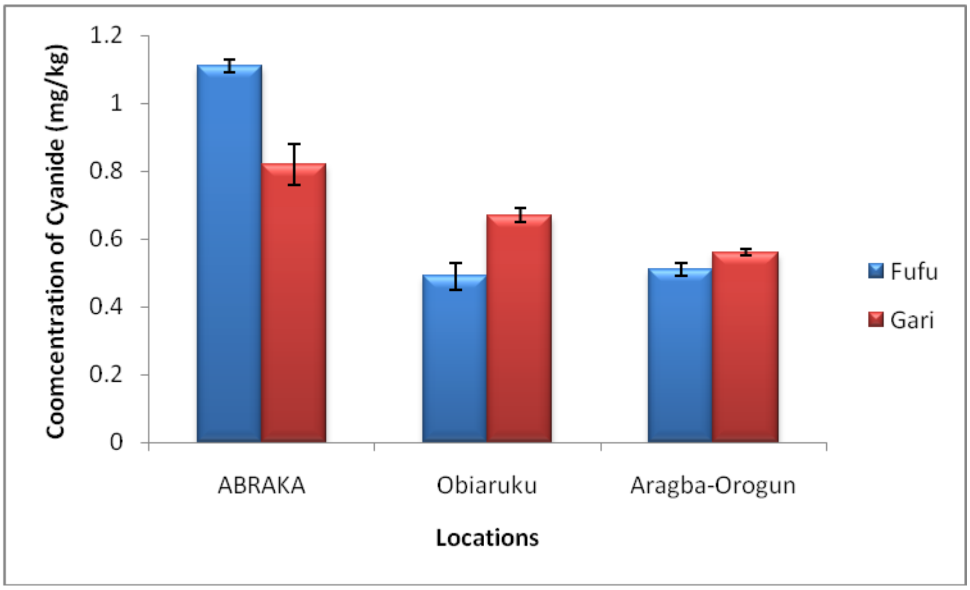

Figure 2: Cyanide concentration in Gari and Fufu within different locations

The following parameters were used to calculate the average daily dose ingestion rate and the Target Hazard Quotient.

Table 3: Exposure Factors and Reference Value of Parameters Used for the Human health Evaluation of Cyanide

Factor	Definition	Unit	Value	
			Children	Adult
C	Cyanide concentration	mg /kg mg/L		
IngR	Ingestion rate	g /day	0.2	0.1
EF	Exposure frequency	days /year	350	350
ED	Exposure duration	Kg	6	24
BW	Body weight	Days	15	55.9
AT	Average time	cm^2	365 × ED	365 × ED

Source: (Kwaansa-Ansah *et al.*, 2017)

Table 4 Daily intake of cyanide (g/day) from cassava

Parts used	Location	ADD_{ing}		THQ	
		Adult	Children	Adult	Children
Fresh leaves	Abraka	0.0059	0.0437	0.0019	0. 0144
	Obiaruku	0. 0051	0. 0382	0.0017	0.0126
	Aragba-Orogun	0.0048	0. 0360	0.0016	0. 0119
Dried leaves	Abraka	0.0031	0.0233	0.0010	0.0768
	Obiaruku	0.0016	0. 0124	0.0053	0. 0409
	Aragba-Orogun	0.0017	0. 0120	0.0055	0. 0397
Tubers	Abraka	0.0019	0.0147	0.0019	0.0485
	Obiaruku	0. 0021	0.0158	0.0021	0. 0523
	Aragba-Orogun	0.0023	0.0170	0. 0023	0.0560
Boiled tuber	Abraka	0.0057	0.0422	0.0019	0.0139
	Obiaruku	0.0055	0.0409	0.0018	0.0135
	Aragba-Orogun	0.0051	0.0384	0.0017	0.0127
Garri	Abraka	0.0014	0.0105	0.0046	0. 0346
	Obiaruku	0.0015	0. 0857	0.0038	0. 0283
	Aragba-Orogun	0.0096	0.0716	0.0032	0. 0236
Fufu	Abraka	0.0019	0.0142	0.0063	0. 0469
	Obiaruku	0.0084	0.0626	0.0028	0. 0207
	Aragba-Orogun	0.0087	0.0652	0.0029	0. 0215

Children's exposure to cyanide from the consumption of cassava leaves and tubers was assessed using the exposure factor handbook (USEPA IRIS, 2011). The findings showed that in adults, fresh cassava leaf values which ranged from 0.0059 mg/kg to 0.0048 mg/kg, were within the WHO limit (10 mg/kg). Using the exposure factor handbook, the average daily cyanide dose for children ingesting cassava leaves and tubers were calculated (USEPA IRIS, 2011). According to the findings in adults, fresh cassava leaves values ranged from 0.0059 mg/kg to 0.0048 mg/kg, which was within the WHO limit of 10 mg/kg. Therefore, indicating that the target harzard quotient for both children and adults were less than one (< 1), they can all be consumed after proper preparation.

CONCLUSION

From this research study, the concentration of cyanide in cassava peels, leaves and tubers were evaluated, specifically in rotten peels that are dumped in the environment over a long period of time. To reduce the threat of cassava peels in the environment, the concentration of cyanide in wet and dried rotten peels were investigated. The results showed that these peels can further be used as animal feed since they had a lower amount of concentration. The study further investigated on leaves that are fresh and dried. This was done to compare which one had a lower concentration. The study revealed, leaves that are sun dried had a lower concentration compared to other research work that was carried out on cassava leaves that were boiled at different intervals. It was also noted that the target harzard quotient for children and adults for products of cassava, boiled tubers and dried leaves were all less than one (USEPA IRIS, 2011) , showing that they can be consumed when properly prepared.

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