Uptake of cyanogenic potential by soil in cassava (*Manihot esculenta crantz*) producing sites and health implications

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Cyanogenic glycosides are common plant toxins. The hydrogen cyanide originating from cyanogenic glycosides may affect soil processes and water quality. In this study, hydrogen cyanide concentrations were determined in thirty-six soil samples collected from cassava processing sites in four different towns in Osun State (Osogbo, Oyan, Ada and Ede) by iodimetry. 0.101±0.0115% was obtained as the highest hydrogen cyanide level in Osogbo (mean value of 0.12±0.05%), 0.127±0.014% as the highest level in Oyan (mean value 0.10±0.03%), 0.054±0.005% in Ada (mean value 0.02±0.03%) and 0.030±0.008% in Ede (mean value 0.02±0.01). High level was obtained at 200 m away from the cassava processing area except at Oyan where highest level of hydrogen cyanide was obtained at 0m. The level of hydrogen cyanide in soil samples were in the order Osogbo> Oyan> Ada> Ede. These levels are high compared to the LD50 lethal dose of hydrogen cyanide ingestion and LC50 lethal dose for hydrogen cyanide inhalation except 0m and 100m in Ada. Statistical analysis reveals a high positive correlation between sites studied with no significant difference among the means obtained.

**Key words:** Cassava, hydrogen cyanide, soil, iodimetry, LC50, LD50.

**INTRODUCTION**

Cassava (*Manihot esculenta crantz*) is one of the most important food crops in the tropical countries and is probably the most widely distributed human food crop with high content of cyanogenic glucoside (Akinrele, 1985). Known cyanogenic glycosides in plants include amygdalin, linamarin, prunasin, dhurrin, lotauastralin and taxiphyllin. Transports of cyanide in soils are mostly influenced by volatilization and distribution. FAO (1993) reported a high production of cassava in 1993 with Africa (74.8 million tons, Latin America 28.5 million tons), Asia (50.2 million tons, Oceania (0.2 million tons). High cyanide intake from the consumption of insufficient processed cassava has been advanced as a possible actiologic factor in some diseases such as iodine deficient disorder and tropical ataxic neuropathy (Kamlin, 1995). The leave and root of the plant contain cyanogenic glucoside (linamarin). The linamarin is readily hydrolysed to glucose and acetone cyanohydrin in the presence of the enzyme linemarose, which decomposes rapidly to cyanide ion.

Soil as a habitat for microorganisms, is probably the most complex and diverse on the planet. It is a bio-membrane and can be a source or sink for most gases. A further source of complexity in soil biological activity is the existence of exo-cellular enzymes, presumably derived from past populations of organisms but stabilized by sorption on mineral surfaces and retaining at least part of their activity (Burns, 1978).

Soil is also used for waste disposal, so detoxification and filtering functions are important. A vast range of organic wastes are applied to soil including sewage sludge, composted municipal waste and effluents from biologically-based industries such as the processing of oil palm and cassava (Powolson et al., 2001). Hydrogen cyanide is ubiquitous in nature. Principal natural sources of cyanides are from over 2,000 plant species, including
transports of cyanide in soils are mostly influenced by volatilization and distribution. The sum of the amount (HCN equivalent) of linamarin acetonocyanohydrin, hydrogen cyanide and cyanide ion equals the cyanogenic potential of the cassava sample. HCN equivalents should be processed to reduce the cyanogenic potential before use for human consumption. Some traditional method of processing in South America and West Africa remove nearly all the cyanogens from cassava products, but other methods such as those used for cassava flour production in East Africa and Indonesia reduce, but do not eliminate the cyanogens present. Cyanide enters soil from both natural processes and industrial activities. Cyanide is highly mobile in soil, and can be removed through several processes. Cyanide and cyanide metal complexes are adsorbed on organic and inorganic constituents in soil, including oxygen, aluminium, iron and manganese from certain types of cyanide from inorganic material is unclear, cyanide is strongly bound to organic matter. Cyanide is lost through volatilization from soil surface. Some cyanide in soil can form hydrogen and evaporates where as some cyanide compounds will be transformed into other chemical forms by micro organisms in soil. At high concentration cyanide becomes toxic to soil micro organisms can no longer change cyanide to other chemical forms, cyanide is able to pass through soil into underground water (Callahan et al., 1979). In soil with pH less than 9.2, hydrogen cyanide is expected to be highly mobile and in cases where cyanide levels are toxic to micro organism (i.e. landfills), HCN may leach into groundwater (ATSDR, 1995). Hydrogen cyanide is not strongly partitioned into sediments or suspended adsorbents, primarily due to its high solubility in water (Callahan et al., 1979). Exposure of high levels of cyanide, such as hydrogen cyanide gas for a short time harms the brain and heart and can cause coma and death. The highest proportion of cyanide is found in the peels and the cortex layer immediately beneath the peels (ATSDR, 2006). It is for this reason that the cassava root is always peeled before being processed or consumed. Peeling removes the cortex and the outer periderm layer adhering to it. As this peels are thrown on the ground, HCN accumulate in the soil. Peels can represent 10 to 20% of the fresh root weight, of which the periderm accounts for 0.5 to 2.0%.

The research work aims at the determination of the hydrogen cyanide content of soil where cassava is being processed in some towns in Osun State, Nigeria such as Osogbo, Oyan, Ada and Ede.

### RESEARCH METHODOLOGY

Thirty-six soil samples were collected from cassava processing area in four different towns in Osun State namely Osogbo, Oyan, Ada and Ede at 0, 100 and 200 m away from the processing area and labelled A, B, C respectively. Three grab samples were collected at each sampling point and mixed together to form composite. The samples were sun dried for some days before crushing, sieving, labelled and then kept for analysis. The codes used for the samples are as shown in Table 1.

Redox titration (iodimetry) was used in the determination of cyanogenic glycoside (Hydrogen Cyanide) in soil samples collected. 5 g of the sample was weighed into a 250 mL conical flask. Each sample was incubated for 16 h at 38°C. After extraction, filtration was carried out using double layer of hardened filter paper. Distillation was performed with Mark Haw distillation apparatus. Each sample was extracted and transferred into a two-necked 500 mL flask connected with a steam generation. It was steam distilled with saturated bicarbonate solution containing a 50 mL conical flask for 1 h. 1 mL of starch indicator was added to 20 ml of each bicarbonate solution containing a 50 mL conical flask for 1 h. 1 mL of starch indicator was added to 20 ml of each distilled and was titrated with 0.2 M iodine solution. The titration was carried out three times for each sample to be able to calculate the mean and standard deviation. The percentage hydrogen cyanide was calculated using the formula:

\[
\text{HCN} = \frac{\text{Titre value} \times 0.27}{1000 \times \text{weight of sample taken}} \times 100
\]

Soil pH was determined using potentiometric method as described by Brady and Weil (1990). A glass electrode Testronic digital pH meter (Model 511) was used. Particle size and texture class was determined by hydrometer method as described by Bouyoucous (1951) and Agbenin (1995). After, the values for silt and clay were determined, the value of sand was obtained by subtracting the values of silt and clay from 100.

Statistical packages such as SPSS 15.0 and STATISTICA 7 were used to calculate analysis of
## RESULTS AND DISCUSSION

The results obtained for the study showed the level of hydrogen cyanide in soil in the four locations studied in Osun State: Osogbo, Oyan, Ada and Ede. Tables 2 to 5 illustrate the hydrogen cyanide concentration of soil in these locations.

In Osogbo, HCN had its highest concentration at 200 m away from the processing site (0.107±0.003%) and higher than at the processing point, that is 0 m (0.101±0.011%), this is shown in Table 2. This might be due to rainfall that eroded part of the peel that contains HCN to far away distance from the processing site. Table 3 illustrates HCN level in soil collected in Oyan. The HCN content of OyA (0.127±0.014%) was the highest concentration obtained here and higher than OyC (0.117±0.008%). OyB seems to be low compared to others (0.046±0.003%).

In Table 4, HCN had low concentration in all its sampling points except for AdC (0.054±0.005 %). Although there are many natural sources of cyanide, including the plants, bacteria and fungi that synthesize and excrete it, the most significant sources of cyanide in the environment are industrial wastes. Cyanide is one of nature most toxic substances. The level of toxicity of the more stable cyanides depends on the metal present and on the proportion of CN$^{-}$ groups converted to simpler alkali cyanides. The loading rate in soil is the paramount factor determining toxicity to microorganisms or hazard for movement into groundwater and food chains (Ubalua, 2007). High concentrations in the environment usually are associated with accidental spills or improper waste disposal.

In Ada, cassava product might not be so much consumed as compared to Osogbo and Oyan. History had it that Oyan town is well known for its cassava production and processing by rural consumers, who
cassava in subsistence-oriented traditional production system and are largely self-sufficient: their choice of food is often determined by the opportunities for diversification of agricultural production in their area. The mobility of cyanide compounds in soil depends on stability and dissociation characteristics of the compound, soil type, soil permeability, soil chemistry, and the presence of aerobic and anaerobic microorganisms (Fuller, 1984; Higgs, 1992). In aerobic conditions, biodegradation is expected to be an important cyanide process. Experimental studies on the mobility of cyanide in saturated anaerobic soils have shown that aqueous simple cyanides and aqueous ferricyanides tend to be very mobile.

Ede town had low HCN level as compared to Osogbo and Oyan with least concentration to be 0.014±0.002% (EdA) and 0.014±0.005% (EdB) as illustrated in Table 5. The kind of trend here shows an increase from 0 to 200 m away from the site. Ede can be termed as an urban area compared with towns such as Ada because of the institution being sited there. The urban consumers buy most of their requirements and often consider cassava difficult to store, sometimes wasteful because of spoilage and inconvenient to prepare and use when compared with other staples and the increasing prevalence of convenience foods which can lead to lower rate of cassava production compared to Osogbo and Oyan. Some of the reactions attributed to the low levels of cyanides in soils are: Biological dissemination and assimilation (metabolism), Microbial transformation to CO₂, H₂O, NH₃, and metals, (hydration and oxidation), dispersion to the atmosphere as gases and/or to water sources (translocation, volatilization, and dispersion), complex formations with metals (chelation), chemical combination and precipitation (precipitation), adsorption to surfaces (surface physical chemistry) and photodegradation (Fuller, 1984).

The study revealed high percentage content of sand and low clay and silt contents, at the various distances from the mill. This agreed with the report that sand particles take large proportion of the first three-layers in cassava effluent polluted soils (Uzoije et al., 2011). Weather conditions might equally be responsible for the soil texture observed as reported by (Jungerius and Levett, 1964).

Samples collected 200 m away from the mill recorded less acidic pH except at Ada and Ede compared to those obtained 0 m as shown in Tables 2 to 5. This could be attributed to the cassava mill effluent as there was a corresponding increase in the concentration of cyanogenic glycosides in the impacted samples. Similar results have been reported by other authors (Utu-Baku et al., 1995; Okafor, 2008; and Uzoije et al., 2011). The hydrogen cyanide content of the impacted soil collected 200m away from the cassava processing site was higher than the ones collected at 0 and 100 m. Such higher concentrations have been observed near cassava mills (Nwaugo et al., 2008; Uzoije et al., 2011).

The HCN level in Oyan is not high as compared to what was obtained in Osogbo an urban area and densely populated compared to Oyan. The demand for food in large urban areas has increased and is continuing to increase due to the large population migration from the rural areas, which has continued for thirty years and shows no signs of abating. The migrating rural population takes with it its traditional eating habits, particularly until it becomes urbanised despite the opportunities for diversification of eating habits with the choice of food available in the towns. Cassava, remain in demand, but this demand is often unsatisfied because of the inherent limitations of traditional production systems which improve serious constraints in marketing and processing. The general pattern of supply of cassava from the surpluses of subsistence farming leads to high marketing costs and high consumer prices. As a consequence in urban communities the consumption of cassava tends to be replaced by imported cereals, rice and wheat flour, this will invariably result in low HCN in soil in cassava processing area than might expected. EPA regulates that HCN in a work place should not be more than 10 ppm (ATSDR, 2006).

Cyanide is produced in the human body and exhaled in extremely low concentrations with each breath. It is also produced by over 1,000 plant species including sorghum, bamboo and cassava. Relatively low concentrations of cyanide can be highly toxic to people and wildlife. The mean levels of cyanide in these towns are high. The toxicity of hydrogen cyanide to humans is dependent on the nature of the exposure. Due to the variability of dose-response effects between individuals, the toxicity of a substance is typically expressed as the concentration or dose that is lethal to 50% of the exposed population (LC50 or LD50). The LC50 for gaseous hydrogen cyanide is 100 to 300 parts per million (that is 0.01 to 0.03%), this implies that inhalation of hydrogen cyanide at this concentration results in death within 10 to 60 min, with death coming more quickly as the concentration increases, though there has not been a reported case such as this. The LD50 for ingestion is 50 to 200 mg (that is 0.005 to 0.02%) or 1 to 3 mgkg⁻¹ of body weight, calculated as hydrogen cyanide. For contact with unabraded skin, the LD50 is 100 mg (as hydrogen cyanide) per kilogram of body weight (that is 0.01%). There should be urgent need of bringing awareness to those involved in this processing industry. Most of them are not educated, they eat at the site where they produce this cassava products and the food can come in contact with the waste from these cassava peels or the effluent. Cyanide is acutely toxic to humans. Although the time, dose and manner of exposure may differ, the biochemical action of cyanide is
Figure 1. Spatial distribution of HCN in soil of sampling sites.

The same upon entering the body. Once in the bloodstream, cyanide forms a stable complex with a form of cytochrome oxidase, an enzyme that promotes the transfer of electrons in the mitochondria of cells during the synthesis of ATP. Without proper cytochrome oxidase function, cells cannot utilize the oxygen present in the bloodstream, resulting in cytotoxic hypoxia or cellular asphyxiation. The lack of available oxygen causes a shift from aerobic to anaerobic metabolism, leading to the accumulation of lactate in the blood. The combined effect of the hypoxia and lactate acidosis is depression of the central nervous system that can result in respiratory arrest and death. At higher lethal concentrations, cyanide poisoning also affects other organs and systems in the body, including the heart.

Initial symptoms of cyanide poisoning can occur from exposure to 20 to 40 ppm of gaseous hydrogen cyanide, and may include headache, drowsiness, vertigo, weak and rapid pulse, deep and rapid breathing, a bright-red colour in the face, nausea and vomiting. Convulsions, dilated pupils, clammy skin, a weaker and more rapid pulse and slower, shallower breathing can follow these symptoms. Finally, the heartbeat becomes slow and irregular, body temperature falls, the lips, face and extremities take on a blue colour, the individual falls into a coma, and death occurs. These symptoms can occur from sublethal exposure to cyanide, but will diminish as the body detoxifies the poison and excretes it primarily as thiocyanate and 2 amino thiazoline 4 carboxilic acids, with other minor metabolites (ICMC, 2013).

The body has several mechanisms to effectively detoxify cyanide. The majority of cyanide reacts with thiosulfate to produce thiocyanate in reactions catalyzed by sulfur transferase enzymes such as rhodanese. The thiocyanate is then excreted in the urine over a period of days. Although thiocyanate is approximately seven times less toxic than cyanide, increased thiocyanate concentrations in the body resulting from chronic cyanide exposure can adversely affect the thyroid. Cyanide has a greater affinity for methemoglobin than for cytochrome oxidase, and will preferentially form cyanomethemoglobin. If these and other detoxification mechanisms are not overwhelmed by the concentration and duration of cyanide exposure, they can prevent an acute cyanide-poisoning incident from being fatal.

Some of the available antidotes to cyanide poisoning take advantage of these natural detoxifying mechanisms. Sodium thiosulfate, administered intravenously, provides sulfur to enhance the sulfur transferase-mediated transformation of cyanide to thiocyanate. Amyl nitrite, sodium nitrite and dimethyl aminophenol (DMAP) are used to increase the amount of methemoglobin in the blood, which then binds with cyanide to form non-toxic cyanomethemoglobin. Cobalt compounds are also used to form stable, non-toxic cyanide complexes, but as with nitrite and DMAP, cobalt itself is toxic.

Cyanide does not accumulate or biomagnify, so chronic exposure to sublethal concentrations of cyanide does not appear to result in acute toxicity. However, chronic cyanide poisoning has been observed in individuals whose diet includes significant amounts of cyanogenic plants such as cassava. Chronic cyanide exposure is linked to demyelination, lesions of the optic nerve, ataxia, hypertonia, Leber's optic atrophy, goiters and depressed thyroid function.

There is no evidence that chronic cyanide exposure has teratogenic, mutagenic or carcinogenic effects.

Statistical analysis using Pearson correlation revealed a strong positive and significant correlation between the following pairs at p<0.01, r = +1.000: AdA/EdA, AdB/AdA, EdA/AdB and a strong, significant but negative correlation at p<0.05, r = -0.999 for the following pairs: AdA/OsA, OsA/AdB. Figure 1 shows the spatial distribution of HCN in each sampling site at different
distances from the processing area. The distribution is such that HCN was very high in Oyan at 0 m that is at the sampling site (0.185%). HCN was however noticed from the graph in Figure 1 to have its lowest concentration in Ada at 0 m that is at the processing site (0.003%).

The mean values of HCN in each town are as illustrated in Figure 2 with Os having the highest mean value and Ad the least mean hydrogen cyanide content in soil.

The analysis of variance (ANOVA) shows that there were no significant differences between the means obtained with F value 5.95 at p<0.05 and this is supported by the box plot shown in Figure 3. The pattern of the quantitative data is as displayed in the box plots in Figure 3. Figure 3 show distributions are skewed right for Ad, Ed and Os while distribution showed skewness to the left for Oy.

**Conclusion**

Hydrogen cyanide has the potential to be transported over long distances before being removed by physical or chemical processes due to its relatively slow rate of degradation. Adsorption of hydrogen cyanide by montmorillonitic clay is fairly weak and is decreased by the presence of water, and this explains why HCN level was low in some towns investigated such as Ada and Ede. Among the four towns studied for their HCN content, Osogbo was found to have the highest level because food made of cassava are highly being produced in this town in large quantity because of the population of inhabitants and the indiscriminate disposal of cassava waste peels and subsequently more HCN gets into the soil of the processing area. Ada had the least HCN content and might probably be due to the fact that not so much cassava products are being eaten because of students leaving there might prefer other foods to cassava made food and so low HCN would be expected.

**REFERENCES**


Agency for Toxic Substances and Diseases Registry