KWAME NKRUMAH UNIVERSITY OF SCIENCE ANDTECHNOLOGY, KUMASI

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DEPARTMENT OF CHEMISTRY

FREE AND TOTAL CYANIDE IN ENVIRONMENTAL SAMPLES FROM KENYASE, A MINING COMMUNITY IN THE BRONG AHAFO REGION OF GHANA

Thesis Submitted to the Department of Chemistry, Kwame Nkrumah University of Science and Technology in Partial Fulfilment of the Requirement for the Award of Master of Philosophy in Analytical Chemistry.

BY

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DECLARATION

I hereby declare that this thesis is as a result of my own original research and that it has neither in part nor in whole been presented for another certificate in this university or elsewhere except in situations where due acknowledgement has been made in the work.

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I hereby declare that I supervised this student in undertaking this study. I hereby confirm that the student has my permission to present the thesis for assessment.

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DEDICATION

This work is dedicated to Mad Irene , Mr Aboagye, Jabet and my dear husband Fred. You were my inspiration.

ACKNOWLEDGEMENT

Once again he has done it; God has been faithful in million ways in the course of this write-up. What shall I say? All I have to say is thank you lord.

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ABSTRACT

The occurrence of rampant Cyanide spillage nowadays raises much concern owing to the seriousness of the dangerous effect it has on the environment. Though, it has a short half-life it may accumulate in water, foodstuffs and living organisms after forming stable complexes. Every incident of cyanide spillage introduce large amounts of cyanide into the environment mostly into water bodies and on soil leading to the death of great number of aquatic animals and high concentrations accumulated in foodstuffs. Therefore it is imperative to evaluate the amount accumulated in water bodies and foodstuffs in cyanide spillage prone areas so as to ascertain whether or not they are safe for consumption. Free and total cyanide levels and other physicochemical parameters (pH, conductivity, surrounding temperature, TSS, and TDS) were determined in water (dam and borehole), fish, cassava and cocoyam samples collected from Kenyasi in the Brong Ahafo Region of Ghana. Control samples were collected from Hwidiem also in the Brong Ahafo Region. Free and total cyanide were determined using Skalar San $^{++}$ Automated Wet Chemistry Analyzer with Continuous Flow method. The physicochemical parameters were all determined using probes. The mean free cyanide levels in the dam samples (surface water) range between 0.11 ± 0.01 and 0.22 ± 0.02 mg/L and the mean total cyanide levels ranged between 20.52 ± 0.09 and 22.25 ± 0.02 mg/L, whereas the borehole samples had the mean free cyanide levels between 22.25 ± 0.02 and 0.23 ± 0.02 mg/L and the mean total cyanide levels between 22.82 ± 0.03 and 27.05 ± 1.17 mg/L. The pH ranges were 3.98±0.02 to 6.21 ± 0.01 and 5.97 ± 0.04 to 7.02 ± 0.03 for the dam and borehole samples respectively. The mean free cyanide levels in fish were between 55.81 ± 4.50 and 71.69 ± 2.74 mg/kg and the mean total cyanide levels found between 79.44 ± 4.20 and 110.56 ± 4.00 mg/kg. The pH range was also between 6.69 ± 0.01 and 7.14 ± 0.01. The mean free cyanide levels in cassava were between 05.25 ± 0.02 and 93.88 ± 5.14 mg/kg and the mean total cyanide levels between 06.56

± 0.03 and 165.56 ± 6.89 mg/kg. The pH values also ranged between 5.92 ± 0.02 and 6.89 ± 0.01. Finally the cocoyam samples also recorded mean free cyanide levels between 00.75 ± 0.01 and 34.56 ± 0.73 mg/kg whiles the mean total cyanide levels were between 11.13 ± 0.61 and 59.00 ± 0.57 mg/kg. The pH values were between 6.59 ± 0.03 and 7.21 ± 0.04. From the results, it can be concluded that, dam water, fish, foodstuffs and boreholes close to the dam are all not safe for consumption since they recorded values higher than the set limits of 0.2 mg/L for water and 10 mg/kg for food set by FAO/WHO.

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LIST OF ABBREVIATIONS

A.C.G.I.H American Conference of Governmental Industrial Hygienists

A.P.H.A	American Public Health Association
A.S.T.M	American Society of Testing and Materials
A.T.S.D.R	Agency for Toxic Substances and Disease Registry
C.A.S No.	Chemical Abstracts Service Number
D.W.A.F	Department of Water Affairs and Forestry
E.C.E.T.O.C	European Center for Ecotoxicology and Toxicology of Chemicals
G.N.A	Ghana News Agency
ICMI	International Cyanide Management Institute
I.P.C.S	Internationa programme on chemical society
J.E.C.F.A	Joint FAO/WHO Expert Committee on Food Additives
O.S.H.A	Occupational Safety and Health Administration
UNEP	United Nations Environment Programme
U.S.A.M.R.I.C.D	U.S. Army Medical Research Institute of Chemical Defence
U.S.E.P.A	United States Environmental Protection Agency
W.H.O	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

Cyanide is one of the most toxic substances on earth and it is toxic to most aquatic life and humans even at low concentrations (Hosetti *et al.*, 2011). Unlike toxic metals, cyanide is not an element but a compound however it tends to react readily with many other chemical elements, and is known to form hundreds of different compounds (Flynn & Haslem, 1995). The in-depth chemistry of cyanide, its chemical behaviour in water bodies and its toxicity is complex and influenced by several factors including acidity or alkalinity (Moran, 2004).

Although there are many natural sources of cyanide, including the plants, bacteria and fungi that synthesize and secrete it, the most significant sources of cyanide in the environment are industrial wastes such as mining and oil refineries, as well as municipal wastewaters (Ubalua, 2010). It exists in these effluents in three basic forms: free, simple and complex cyanides. Free cyanide occurs as two species (hydrogen cyanide and the ionized cyanide), depending on the prevailing pH and temperature of the environmental medium (Doudoroff, 1966; Smith & Mudder, 1991). Free cyanide is the most toxic form and the one used in laboratory toxicity tests to derive USEPA water-quality criterion (WQC) (USEPA, 1984). Simple cyanide (e.g., sodium cyanide) normally dissociates into free cyanide and salts in aqueous solutions. Complex cyanides can take a variety of forms, but cyanide-metal complexes (e.g., iron cyanide, nickel cyanide) are the dominant cyanide forms in industrial wastewaters. The latter complexes are normally several orders of magnitude less toxic than free cyanides because their toxicity is largely due to dissociation of free cyanide from these complexes (Doudoroff *et al.*, 1966).

Cyanides are used widely and extensively in the manufacture of synthetic fabrics, electroplating baths, intermediates in agricultural chemical production and the extraction of ores (Silver and

gold), and for some therapeutic applications (Muniswamy *et al.*, 2010). Also in metal processing, photographic processes, chemical synthesis, manufacture of pesticides, dehairing of hides, laboratory processes, the process of chlorination, manufacture of dyes and pigments (USEPA, 1999).

Naturally occurring cyanides (which may be in high levels enough to cause food poisoning) are present in a number of foods and plants (such as spinach, bamboo shoots, apple, mango, peach, almonds, lima beans, spoilt cabbage, cauliflower, fruit pits and tapioca) and are produced by certain bacteria, fungi and algae (Miessler & Tarr, 1991).

Human exposure to cyanide is by breathing air, drinking water, eating food or interacting with soils that contain cyanide. Exposure can also be by absorption through the skin (dermal absorption) or by occupational exposure through electroplating or metallurgy (OSHA, 1998). Chronic exposure to low levels of cyanide is suspected to be responsible for various neuropathic and thyrotoxic conditions in humans (Dube and Hosetti, 2010).

The severity of health effects depends upon the route, duration of exposure, the dose and the form of cyanide coupled with the individual variability in age and sensitivity. However, the cyanide anion CN^- (among forms like HCN, KCN and NaCN) is the primary toxic agent, regardless of origin (Ubalua, 2007) and their toxicity has been unequivocally linked to the tropical ataxic neuropathy and epidemic spastic paraparesis (Okolie & Osagie, 1993). 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 adenosine triphosphate (ATP) (Yen *et al.*, 1995). 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 (Nagasawa *et al.*, 2007). The combined effect of the hypoxia and lactate acidosis is depression of the central nervous system that can result in respiratory arrest and death (Okolie & Osagie, 1999). Sublethal doses cause dizziness and headaches (Nagasawa *et al.*, 2007). Exposure to HCN solutions has caused dermatitis, itching, scarlet rash, papules, nose irritations and bleeding (NIOSH, 2007). Other effects in humans include an enlarged thyroid gland and irritation to the eyes. The acute lethal dose of HCN for humans is reported to be 0.5 to 3.5 mg/kg of body weight (APHA, 1998). However, some of the cyanide is changed to thiocyanate, which is less harmful, and leaves the body in the urine or combine with another chemical to form vitamin B_{12}, which helps maintain healthy nerve and red blood cells (ATSDR, 2004).

Fish and aquatic invertebrates are particularly sensitive to cyanide exposure. The sensitivity of aquatic organisms to cyanide is highly specie specific, and is also affected by water pH, temperature and oxygen content, as well as the life stage and condition of the organism. Immediate responses include an activation of anaerobic glycolysis (Hosetti *et al.*, 2011).

Cyanide in landfills can contaminate underground water and much smaller amounts of cyanide may enter storm water runoff in locations containing cyanide (Environment Australia, 2003). Airborne sources include discharges from organic chemical industries, iron and steel works, and wastewater treatment facilities (USEPA, 1999).The concentration of cyanide in drinking water ranges from 0.001 to 0.011 ppm (Mickelsen *et al.*, 1991).

WHO has set 0.2 mg/L (200 ppb) as maximum limit for cyanide in water and 10 mg/kg in cassava (Baskin, 1992). According to Anhwange *et al.*, (2011), cassava root, a dietary staple in

many tropical regions contains cyanogenic glycosides, such as linamarin, which release cyanide (CN^-) when metabolised endogenously.

This study will therefore focus on the determination of the levels of free and total cyanide in fish, water, and some staple food crops in Kenyasi, a mining community in the Brong-Ahafo region of Ghana.

1.1 STATEMENT OF THE PROBLEM

Mineral resource is the most exploited natural resource due to its immense contribution to the national economy. In spite of its uses, it has a devastating toll on the community. The use of cyanide compounds by the mining industry, coupled with limitations in current analysis and monitoring of these compounds, raises serious concerns regarding public safety and environmental protection at mine sites using cyanide processing (Dube and Hosetti, 2010) since they can contaminate water and food consumed by animals and humans.

There has been several cyanide spillages recorded in Ghana dating from 1989 and 2002. The mining sites which experienced these spills include Obenemase Mine, Bogoso Gold Limited, Teberebie Goldfields limited, Ashanti Goldfields Bibiani Limited, Ashanti Goldfields Corporation, Goldfields Ghana Limited, Satellite Goldfields Limited and Abosso Goldfields limited (Amegbey & Adimado, 2003 cited in Obiri *et al.*, 2006). This includes allegations about cyanide spillages occurring at Newmont Ghana gold Limited – Ahafo Kenyasi over the years to 2012 (GhanaWeb, 8th August, 2012) that needs to be properly investigated.

High levels of cyanide in the soil can only be attained through chemical accidents and once there is cyanide spillage, there is the possibility of contaminating the environment which mostly occurs at the mining areas. Food crops such as cassava, cocoyam and other tuber crops grown in mining communities accumulate this toxic or hazardous chemical from the soil. The cyanide may react to form hydrogen cyanide which has a half-life in the atmosphere of about 1-3 years hence may persist in the environment for long periods and there is an evidence that some forms of cyanide compounds can accumulate in plants and fish (Eisler, 1991; Dube and Hosetti, 2010).

Cassava is a staple food for most Ghanaians including the inhabitants of Kenyasi which is one of the mining areas in Ghana where sodium cyanide is used extensively in the extraction of gold.

The constant reports of cyanide spillage in the area on the news raise much concern in that it renders a very high possibility of cyanide leaching into the soil and contaminating the water bodies which in turn accumulate in foodstuffs and aquatic animals.

Consequently, the determination of cyanide levels in this area is very essential since the main occupation of the people is farming and their sources of drinking water are boreholes and streams.

1.2 AIMS AND OBJECTIVES

The main aim of this study is to determine the extent of cyanide contamination in Kenyasi and its environs.

1.3 SPECIFIC OBJECTIVES

The specific objectives include:

1. To determine cyanide levels (free and total) in fish, water, cocoyam and cassava.

2. To determine cyanide levels (free and total) in water samples from boreholes and the dam.

3. To assess the relationship between free and total cyanide in the various samples.

4. To compare cyanide levels in samples from mining and non-mine areas.

5. To find the relationship between sample properties and cyanide levels.

6. To compare cyanide levels in samples with set limits and make recommendations

1.4 JUSTIFICATION

The health effects from high levels of cyanide exposure can begin in seconds to minutes and small levels may cause cardiac arrest (Sousa *et al.*, 2002). A fatal dose for humans can be as low as 1.5 mg/kg body weight (Crampton *et al.*, 1979). Research has shown that cyanide affects individuals who have regular long-term consumption of cassava (Flynn & Haslem, 1995).

Cyanide is quickly absorbed into the bloodstream and once in the bloodstream prevents the cells of the body from using oxygen by binding to key iron-containing enzymes and halts cellular respiration. When this happens, the cells die and it is more harmful to the heart and brain which use a lot of oxygen (Gosselin *et al*., 1976).

Accidental spills of sodium cyanide or potassium cyanide into rivers and streams have resulted in massive killings of fishes, amphibians, aquatic insects and aquatic vegetation and have occurred in several areas in Ghana (Amegbey & Adimado, 2003). Animal studies have suggested that oral

exposure to cassava (a cyanide-containing vegetable) may be associated with malformations in the foetus and low foetal body weights (Amoore & Hautala, 1983).

Though the effect of cyanide on the individuals in the area is currently not conspicuously seen, yet considering the lethality of cyanide even at smaller doses, the chance of prevention cannot be risked for any other reason. Hence, there is the need to monitor the cyanide levels in this sensitive environment.

CHAPTER TWO

2.0 LITERATURE REVIEW

This section discusses the occurrence of cyanide in environmental and biological samples, the chemistry and its toxicity in these samples. More so cyanide determination using several analytical methods are discussed.

2.1 PHYSICAL AND CHEMICAL PROPERTIES OF CYANIDE

Hydrogen cyanide (HCN) is a colourless or pale blue liquid or gas with a faint bitter almond-like odour. Common synonyms are hydrocyanic acid and prussic acid. Hydrogen cyanide is a very weak acid, with a p*K*a value of 9.22 at 25 °C. It is soluble in water and alcohol. Hydrogen cyanide is commercially available as a gas or as a technical-grade liquid in concentrations of 5, 10, and 96–99.5%. Phosphoric acid is added to liquid hydrogen cyanide as a stabilizer to prevent decomposition and explosion (ATSDR, 2004).

Cyanides are fast-acting poisons that can be lethal. There are several chemical forms of cyanide apart from hydrogen cyanide. Sodium cyanide and potassium cyanide are white powders which may have a bitter almond-like odor. Other chemicals called cyanogens can generate cyanides. Cyanogen chloride is a colorless liquefied gas that is heavier than air and has a pungent odor. While some cyanide compounds have a characteristic odor (ATSDR, 2004). The presence of cyanide can be ascertained by adding iron (II) sulphate to samples and acidified with mineral acid, the formation of prussian blue is a positive test for cyanide (Andreas, 2006).

2.2 THE USE OF CYANIDE

Cyanide was used as chemical weapon for the first time in World War I. Low levels of cyanide are found in nature and in products commonly eaten and used. Cyanide and cyanide-containing compounds are used to make a wide range of chemicals for use in paints, pesticides and fumigants, plastics, electroplating, photo developing, mining and synthetic fibres such as nylon. Dye and drug companies also use cyanides. Some industrial processes, such as iron and steel production, chemical industries and wastewater treatment can create cyanides. During water chlorination, cyanogen chloride may be produced at low levels (USAMRICD, 2000).

Although toxic, cyanide has prominent positive uses which are worthy of note. HCN is mainly used in the manufacture of other chemicals such as methyl methacrylate methionine and its hydroxylated analogues, and potassium cyanide (ATSDR, 1997; ECETOC, 2004)

Sodium ferrocyanide is used in ore floatation, as anti-caking agent in rock salt, and in photography for bleaching, toning, and fixing. Sodium nitroprusside has been used as anti-hypertensive agent and in congestive heart failure and is used for deliberate induction of hypotension during certain nuersurgical procedures. Organic cyanides, called nitriles, are important industrial chemicals used in synthesis and to form polymers such as polyacrylonitrile ("Orlon"). Sodium and potassium cyanide and other cyanide salts may be made from HCN and these are widely used in metal processing including electroplating and hardening the extraction (cyanide-tion) of gold and silver from ores; base metal floatation; coal gasification; and the fumigation of ships, railroad cars, buildings, grain silos, flour mills, seeds in vacuum chambers, and soil. Calcium cyanide is used chiefly as a fumigant, because it readily releases hydrogen cyanide when exposed to air ; as a fertilizer, defoliant, herbicide, and rodenticide; as a stabilizer

for cement; and in stainless steel manufacture (Eisler *et al*., 1999; Patnaik, 1999; ACGIH, 2001; ECETOC, 2004).

2.3 THE CHEMISTRY OF CYANIDE IN THE ENVIRONMENT

Once released in the environment, the reactivity of cyanide provides numerous pathways for its degradation and attenuation. Cyanide undergoes reactions like complexation, precipitation, oxidation, hydolysis and adsorption. These reaction processes are discussed below.

2.3.1 Complexation and Precipitation

Cyanide is very reactive, forming simple salts with alkali earth cations and ionic complexes of varying strengths with numerous metal cations but the stability of these salts is dependent on the cation and on pH. It forms complexes with gold, mercury, cobalt and iron that are very stable even under mildly acidic conditions (ICMI, 2012).

Most cyanide complexes are much less toxic than cyanide, but weak acid dissociable complexes such as those of copper and zinc are relatively unstable and will release cyanide back to the environment. Iron cyanide complexes are of particular importance due to the abundance of iron typically available in soils and the extreme stability of this complex under most environmental conditions. However, iron cyanides are subject to photochemical decomposition and will release cyanide if exposed to ultraviolet light (ICMI, 2012). This decomposition process is reversed in the dark. Similar reaction is withnessed as cobalt complex reacts with hydroxocobalamin to form cyanocobalamin (vitamin B_{12}) (Ringer, 2008).

Iron cyanide complexes form insoluble precipitates with iron, copper, nickel, manganese, lead, zinc, cadmium, magnesium, tin and silver over a pH range of 2-11 (ICMI, 2012) and these complexes are of lower toxicity than primary compounds. The presence of ions of iron (II) or (III) (ferrous (Fe^{2+}) or ferric (Fe^{3+}) cations) in cyanide solutions (depending on pH) the following complexes are formed: hexacyanoferrate (II) ion ($[Fe(CN)_6]^{4-}$) or hexacyanoferrate (III) ion ($[Fe(CN)_6]^{3-}$) in accordance with reactions (I) and (2) (Latkowska and Figa, 2007).

For pH > 8.5 $Fe^{2+} + 6CN^- = [Fe(CN)_6]^{4-}$ (l)

For pH 5-6 $Fe^{3+} + 6CN^- = [Fe(CN)_6]^{3-}$ (2)

Furthermore, depending on pH reaction and oxidation degree of iron ions added or present in excess can lead to the formation of the following insoluble iron salt complexes thus, iron (Ill) hexacyanoferrate (II) ($Fe_4[Fe(CN)_6]_3$ - Prussian blue), Iron (II) hexacyanoferrate (II) ($Fe_2[Fe(CN)_6]$ - Berlin White), iron (III) hexacyanoferrate (III) ($Fe[Fe(CN)_6]$ - Berlin Green), iron (II) hexacyanoferrate (III) – ($Fe[Fe(CN)_6]_2$ - Prussian brown) (Latkowska and Figa, 2007). The process of forming insoluble complex iron salts takes place in accordance with the reactions presented below (3-6).

For pH = 3-4 $3[Fe(CN)_6]^{4-} + 4Fe^{3+} = Fe_4[Fe(CN)_6]_3$ (3)

$[Fe(CN)_4]^{4-} + 2Fe^{2+} = Fe_2[Fe(CN)_6]$ (4)

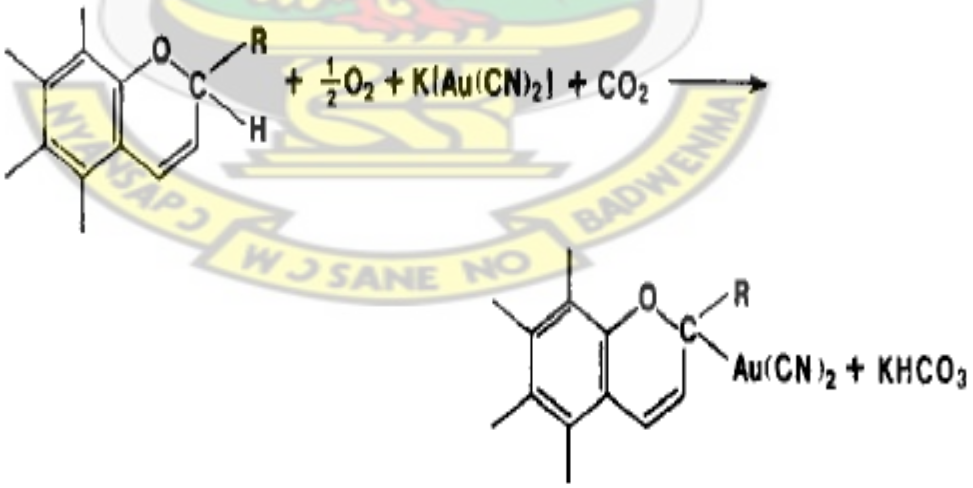
For pH = 3-5 $[Fe(CN)_6]^{3-} + Fe^{3+} = Fe[Fe(CN)_6]$ (5)

$2[Fe(CN)_6] + 3Fe^{2+} = Fe)[Fe(CN)_6]_2$ (6)

Metal cyanide complexes also form salt - type compounds with alkali or heavy metal cations such as potassium ferrocyanide ($K_4Fe(CN)_6$) or copper ferrocyanide ($Cu_2[Fe(CN)_6]$). Heavy metal salts of iron cyanides form insoluble precipitates at certain pH levels (Sousa, 2002). The commonest forms of cyanide include the following complexes: hexacyanides $[M(CN)_6]^{3-}$ (M = Ti, V, Cr, Mn, Fe, Co) which are octahedral in shape, the tetracyanides, $[M(CN)_4]^{2-}$ (M = Ni, Pd, Pt) which are square planar in their geometry and the dicyanides $[M(CN)_2]^{-}$ (M = Cu, Ag, Au) which are linear in geometry (Sousa, 2002).

2.3.2 Adsorption

Cyanide and cyanide-metal complexes are adsorbed on organic and inorganic constituents in soil, including oxides of aluminum, iron and manganese, certain types of clays, feldspars and organic carbon. Although the strength of cyanide retention on inorganic materials is unclear, cyanide is strongly bound to organic matter. According to Mcdougall and co (1980), Cyanide compounds like gold and silver adsorbed on carbon as represented by the equation below.



(2.7)

The reaction involves Firstly, the extraction of Au(CN) and it is strongly enhanced by the presence of electrolytes such as KCI or $CaCl_2$ in the adsorption medium. Secondly, the adsorption is strongly enhanced by an increase in the acidity of the adsorption medium (Mcdougall *et al*., 1980).

2.3.3 Oxidation

Thiocyanates (SCN^-) are a group of compounds formed from a combination of sulfur, carbon, and nitrogen. Thiocyanate dissociates under weak acidic conditions but is typically not considered to be a weak acid dissociable (WAD) species because it has similar complexing properties to cyanide. Oxidation of cyanide to less toxic cyanate normally requires a strong oxidizing agent such as ozone, hydrogen peroxide or hypochlorite. However, adsorption of cyanide on both organic and inorganic materials in the soil appears to promote its oxidation under natural conditions or from the treatment of effluents containing cyanide can produce cyanate, OCN^- (Ringer, 2008).

Various species of bacteria, fungi, algae, yeasts and plants, along with their associated enzymes and amino acids, are known to oxidize cyanide naturally. The predominant mechanism of bio-oxidation is the metabolic conversion of cyanide to cyanate, OCN^- a species less toxic than cyanide:

Pseudomonas paucimobilis bacteria are used to metabolize cyanide in water from tailings and underground mines. This strain of bacteria can treat metal cyanide complexes including total cyanide species and strong acid dissociable cyanide species:

$$M(CN)x^{y-x} + 3xH_2O + x/2O_2(aq) \rightarrow M^{y+} + xNH_4^+ + HCO_3^- + xOH^- \quad \text{.....2.8}$$

Anaerobic bacteria can also be used to oxidize cyanide in oxygen – starved conditions such as ground waters and stagnant tailings ponds (Obiri *et al.*, 2007). Photolysis reaction liberates cyanide ions from ferro- and ferri-cyanide complexes into the environment as shown below:

$$Fe(CN)_6^{3-} + H_2O \xrightarrow{h\nu} [Fe(CN)_5H_2O]^{2-} + CN^- \quad (9)$$

$$Fe(CN)_5H_2O]^{2-} + 2H_2O \xrightarrow{h\nu} Fe(OH)_3(s) + 5CN^- + 3H^+ \quad (10)$$

Reactions 1 and 2 are referred to as photo-equation reactions and have also been observed for cobalthexacyanide, $Co(CN)_6^{3-}$ complexes. Combinations of reactions (1) and (2) liberate cyanide at UV – wavelengths less than 420 nm, thus posing significant health risk to human beings and aquatic organisms who consume the cyanide contaminated water body (White *et al.*, 2000 and Trapp *et al.*, 2003 all cited in Obiri *et al.*, 2007).

2.3.4 Volatilization

At the pH typical of environmental systems, free cyanide will be predominately in the form of hydrogen cyanide, with gaseous hydrogen cyanide evolving slowly over time. The amount of cyanide lost through this pathway increases with decreasing pH, increased aeration of solution and with increasing temperature. Cyanide is also lost through volatilization from soil surfaces (ICMI, 2012).

2.3.5 Biodegradation

Under aerobic conditions, microbial activity can degrade cyanide to ammonia, which then oxidizes to nitrate. This process has been shown effective with cyanide concentrations of up to

200 parts per million. Although biological degradation also occurs under anaerobic conditions, cyanide concentrations greater than 2 parts per million are toxic to these microorganisms (ICMI, 2012).

2.3.6 Hydrolysis

Hydrogen cyanide can be hydrolyzed to formic acid or ammonium formate. Although this reaction is not rapid, it may be of significance in ground water where anaerobic conditions exist (ICMI, 2012).

2.4 SOURCES AND ESTIMATED LEVELS OF CYANIDE IN SOME SAMPLES

Cyanide occurs naturally and anthropogenically. Several levels have been estimated in some samples and have been reported below.

2.4.1 Natural Sources

Cyanide is produced naturally in the environment by various bacteria, algae, fungi and numerous species of plants including beans (coffee, chickpeas and lima), fruits (seeds and pits of apple, cherry, pear, apricot, peach and plum), almond and cashew nuts, vegetables of the cabbage family, grains (alfalfa, and sorghum), roots (cassava, potato, radish and turnip), white clover and young bamboo shoots. The major source of cyanide in food is cyanogenic glycosides (example linamarin and amygdalin), the mostly found in cassava. Some cynogenic glycosides present in major edible foods are Amygdalin occurs in (among others) almonds, dhurrin in sorghum, linamarin in cassava, lotaustralin in cassava and lima beans, prunasin in stone fruits, and

taxiphyllin in bamboo shoots (JECFA, 1993). The structures for these cyanogenic glycosides are shown below.

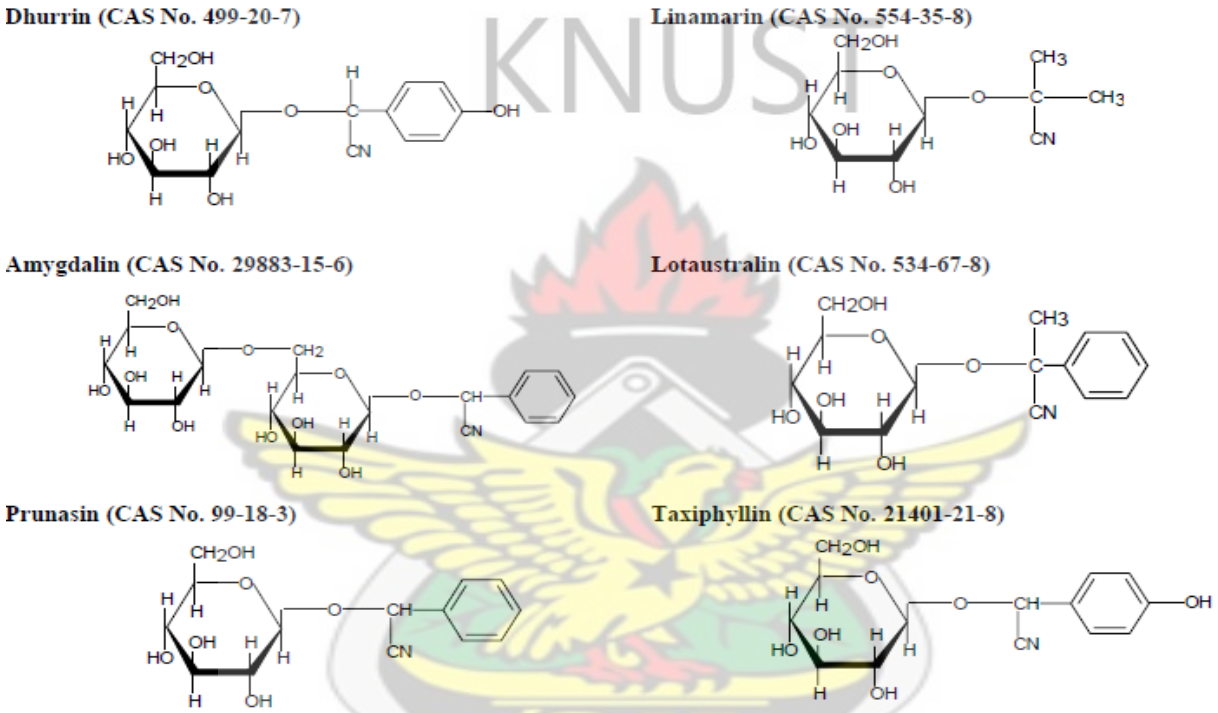


Figure 2.1 Cyanogenic glycosides in major edible plants (JECFA, 1993)

Reported levels of cyanide include: soya protein products (0.07–0.3 µg/g), cereal grains and products (0.001–0.45 µg/g), lima beans (0.1–3 µg/g) and canned fruits (0–4 µg/g) (ATSDR, 1997). It can also be produced by certain bacteria, fungi and algae. HCN is found naturally throughout the environment at low levels as it is released from volcanoes and certain plants and bacteria.

2.4.2 Anthropogenic/Manmade Introduction of Cyanide

Anthropogenic sources are responsible for much of the cyanide in the environment. The major sources of cyanides releases to water are discharges from metal finishing industries, iron and steel mills and organic chemical industries. Effluents from the cyanidation process used in precious metal extraction contain high amounts of cyanide (BioTox, 1998) Much smaller amounts of cyanide may enter water through storm water runoff where road salts containing cyanide are used (Environment Australia, 2003).

Incomplete combustion during forest fires is believed to be a major environmental source of cyanide, and incomplete combustion of articles containing nylon and polyurethane produce cyanide through depolymerization. Other cyanide sources include vehicle exhaust, burning of municipal waste and use of cyanide-containing pesticides (CDCP, 2004).

The levels of cyanide in tobacco smoke vary considerably, with reports of 10 to 550 µg per cigarette for the inhaled smoke, with up to half the concentration found in the non-inhaled or side stream smoke (ATSDR, 1997).

It is also produced industrially by reacting methane and ammonia in air at high temperature over a platinum catalyst (Khoury *et al.,* 2005). Thiocyanates in soil result from direct application of cyanide-containing products like herbicides (weed killers), insecticides, and rodenticides to crops. For instance, cabbage accumulates cyanide from this source and release smaller levels on decay (Kamalu, 1995).

2.5 CYANIDE AND MINING

Cyanide is the most dangerous chemical compound known to mankind, yet it is the chemical of choice for mining companies to extract gold from crushed ore. Sodium cyanide plays a key role in extracting gold and other metals such as silver, copper, and zinc from ores. It is estimated that about 90% of the world's gold production utilizes cyanide in extraction (Environment Australia, 2003).

Many gold-containing ores comprise of finely divided gold particles locked up within other minerals, commonly sulfides. The gold extraction process must separate and concentrate the gold but the low concentrations and particulate nature of the gold mean that purely physical extraction processes are neither economically viable nor quantitatively achievable (Botwick, 2012).

Therefore gold is usually separated from the other constituents of the ore by chemically dissolving it in water and then extracting it with cyanide in conjunction with physical processing (crushing, milling, gravity separation and flocculation) (Evironment Australia, 2003).

Cyanide is still the preferred reagent for extracting gold when leaching step is required. Only 0.3 to 0.4 grams of cyanide per ton of typical ore should be required to dissolve and extract the gold. However, in practice, consumption ranges from 300 grams per ton to more than 2000 grams per ton. The excess cyanide consumption is partly accounted for by oxidation to cyanate and loss through volatilization as HCN gas. Some is also often consumed by complexation with copper, iron, zinc or through reaction with sulfur species to form thiocyanate. Cyanide complexes in particular eventually find their way to tailings dams and then, potentially, into the wider environment (Environment Australia, 2003).

2.6 CYANIDE SPILLS IN GHANA

Between 1989 and 2002, Ghana recorded eight accidental cyanide spills. In 1989 there was a cyanide spillage at the Obenemase Mine (OM) near Konongo after a heavy downpour. The ponds merged and overflowed the embankment draining into the environment. The spillage contaminated a tributary of the Owerri River (Amegbey and Adimado, 2003).

In 1991, there was a cyanide spillage at Billiton Bogoso Gold limited (BGL). In 1994, a truck conveying sodium cyanide to Billiton Bogoso Gold was involved in an accident at Samahu near Tarkwa, resulting in cyanide spill into the environment and causing the death of frogs in a nearby wetland (Amegbey and Adimado,2003).

In 1996 after a heavy downpour, a design flaw in the plastic lining of a solution pond caused a berm failure at Teberebie Goldfiels Ltd (TGL). The entire amount of cyanide solution in the pond was released into the Angonaben stream, a main tributary of the Bonsa River. There was loss of aquatic life (Amegbey and Adimado, 2003).

Two incidents occurred in 1999. The first was a tailings dam pipeline burst at Bibiani, which caused pollution in the Tano River. This accident was detected in the very early stage and was contained. However, death of fish was reported (Amegbey and Adimado, 2003).

The second incident was the spillage from the Dokyiwa tailings dam at Ashanti Golgfields Ltd, Obuasi that polluted river Nyam at Anwona-Sansu and affected vegetation. In 2001, there were two cyanide spillages in two weeks, involving Goldfields Ghana Ltd. (GFGL), Tarkwa in the Wassa West District and Satellite Goldfields Ltd. (SGL), at Akyempim in the Mpohor Wassa East District, both in the Western Region (Amegbey and Adimado, 2003).

In 25th of October 2004, an incident of cyanide spillage occurred at Bogoso Gold Mines Limited at Bogoso/Prestea in the Western Region of Ghana and it was reported that there was loss of aquatic lives but no deaths were recorded for human beings (GCG, 2004).

More recently, on the 3rd January, 2012, an incident of cyanide spillage was reported among several ones over the years which occurred at Newmont Ghana Gold Limited at Ahafo-Kenyasi in the Brong Ahafo Region of Ghana leading to the death of over three thousand fishes (GhanaWeb, 8th August, 2012).

2.7 CYANIDE IN AIR

The major source of cyanide in air is vehicle exhaust and some lower levels in cigarette smoke (BioTox, 1998). Ambient air monitoring data for cyanides in Bulgaria in areas near petrochemical plants showed concentrations ranging from 0.2 to 0.8 $\mu g/m^3$ (annual average value) (Kaloyanova *et al.*, 1985 cited in WHO, 2004). Cyanide has been detected at levels of 20–46 mg/m^3 in the air near large-scale cassava processing facilities in Nigeria (Okafor & Maduagwu, 2000).

Most cyanide in the atmosphere is expected to exist almost entirely as hydrogen cyanide gas, although small amounts of metal cyanides may be present as particulate matter in air. The reaction of hydrogen cyanide with photochemically generated hydroxyl radicals proceeds fairly slowly. Based on a reaction rate constant of $3x10^{-14}$ m^3/molecules-sec at 25°C and assuming an ambient hydroxyl radical concentration of $8x10^5$ molecules/m^3, the half-life for the reaction of hydrogen cyanide vapor with hydroxyl radicals in the atmosphere has been approximately 334 days (ATSDR, 1995). Hydrogen cyanide is expected to be resistant to direct photolysis. The

relatively slow rate of degradation of hydrogen cyanide suggests that this compound have the potential to be transported over long distances before being removed by physical or chemical processes. Since hydrogen cyanide is miscible in water, it appears that wet deposition may be important fate process. Metal cyanide particle are expected to be removed from air by both wet and dry deposition (ATSDR, 1995).

2.8 THE CHEMISTRY OF CYANIDE IN WATER

The depth at which the water is sampled makes the water surface or ground as shown in fig 2.3.

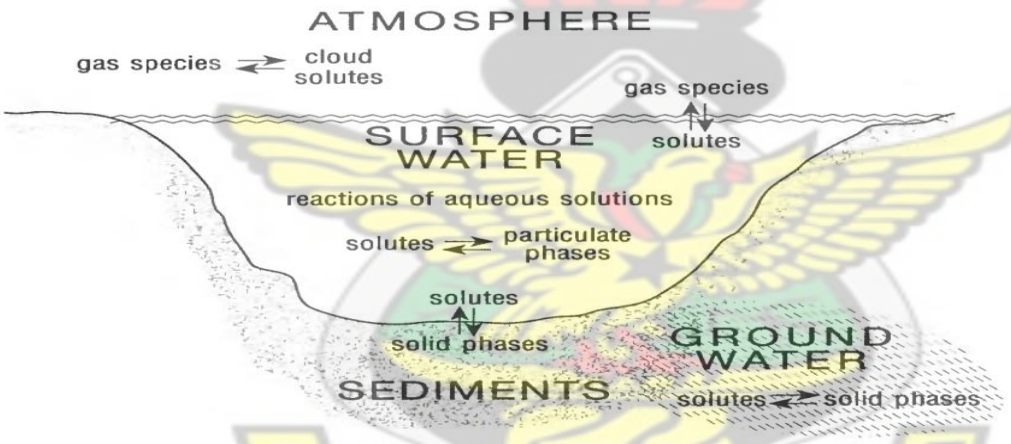


Figure 2.2 The depth of the water makes the water surface/ground water (Johnsons, 1972).

2.8.1 pH of water and Its Effects on Cyanide

When the pH in water decreases, solubility increases and particles become more mobile (APHA, 1998). At pH below 8, most cyanide exists as HCN which is volatile. Hence, under equal parameters, there is an inverse relation between the two parameters.

2.8.2 Conductivity and total dissolved solids of Water and their Effect on Cyanide

Conductivity is a measure of the salts dissolved in a sample. It measures how well water sample conducts an electrical current, a property that is proportional to the concentration of ions in solution and their mobility (Jantz, 1997). It is an indirect measure of the presence of inorganic dissolved solids, such as chlorides, nitrate, sulphates, phosphates, sodium, magnesium, ammonium, potassium, bicarbonate, calcium and iron which make up total dissolved solids. These substances conduct electricity because they are charged in aqueous medium. Sources of these ions include- calcium (gypsum), calcium and magnesium (clay minerals), sulphate (oxidation of sulphide ores, gypsum), chloride (igneous and sedimentary rocks), bicarbonate/carbonate (limestone). These compositions vary with underlying geology and precipitation or evaporation ratios (Weber-Scannell & Duffey 2007).

Conductivity and total dissolved solids are affected by human influences, for example, agricultural runoffs because of the presence of phosphates and nitrates, industry and resource extraction such as mining and gas well development. Generally, conductivity values below 3500 µS/cm are acceptable for drinking water (Fillo *et al.*, 1992).

All electrical conductivity (EC) readings are referenced at 25°C to eliminate temperature differences associated with seasons and depths (Campbell, 1990). All other things being equal, water conductivity rises as cyanide levels rise (Acharyya *et al.*, 2000). Experiment by Mahimairaja *et al.,* (2005) led to the conclusion that water conductivity has poor correlation with cyanide content. They attributed it to the fact that cyanide is generally not a major ion in water so any rise or fall in conductivity value is not likely to be caused by cyanide. The conductivity of natural waters has been found to vary between 50 and 1500 µS/cm (Paul, 1992).

2.8.3 Occurrence of cyanide in water

Although there are many natural resources 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. 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 (Amegbey and Adimado, 2003).

Cyanides, reported as cyanide, hydrogen cyanide, sodium cyanide, potassium cyanide, calcium cyanide, or copper (I) cyanide, have been detected in surface water samples at 70 of the 154 hazardous waste sites studied in the USA; they have also been detected in groundwater samples at 191 of the 419 waste sites studied and in leachate samples of 16 of the 52 sites studied (Stockmann *et al.*, 2011).

Cyanide can exist in water as free cyanide or as complex with other chemicals like metals. All simple cyanides ionize in water to release cyanide ion which, depending on pH, will form hydrocyanic acid (Akcil &Mudder, 2003).

Cyanide ion, CN^-, is the predominant stable form of free cyanide above a pH of about 9.0 to 9.5, depending upon the dissolved solids concentration of the water. As the pH drops, increasing amounts of CN- convert to hydrogen cyanide, HCN. The percentage of HCN continues to increase as the pH drops further, until at a pH of 7.0, about 99.5% of the cyanide exists as HCN. At pH below 7.0, essentially all dissolved cyanide is present as HCN. This is why high cyanide

concentrations are associated with groundwater at sites with alkaline soils (pH 7.5), whereas much lower concentrations have been reported in groundwater with acidic soils (pH 4) (Meeussen *et al*., 1994). Because in this form most of the cyanide volatize contributing to its loss in the water.

Cyanide in water is removed either by ion exchange (where a charged anion resin exchanges cyanide in the water), reverse osmosis where soluble cyanide using a semi-permeable membrane and the application of pressure to a concentrated solution which causes water, but not suspended or most dissolved solids (soluble cyanide), to pass through the membrane (Algar & Kinnear, 1990).

Ground water standards for the United States of America are presented in Table 2.1 below.

Table 2.1 Groundwater Quality Standards (US EPA, 1994).

Constituent	**Units**	**Standard**	**Constituent**	**Units**	**Standard**
Antimony	mg/L	0.006	Manganese	mg/L	0.15
Cyanide	mg/L	0.05	Mercury	mg/L	0.002
Barium	mg/L	2.0	Nickel	mg/L	0.1
Beryllium	mg/L	0.004	Nitrate as N	mg/L	10.0
Boron	mg/L	2.0	Radium-226	pCi/L	20.0
Cadmium	mg/L	0.005	Radium-228	pCi/L	20.0
Chloride	mg/L	200.0	Selenium	mg/L	0.05

Chromium	mg/L	0.1	Silver	mg/L	0.05
Cobalt	mg/L	1.0	Sulfate	mg/L	400.0
Copper	mg/L	0.65	Thallium	mg/L	0.002
Cyanide	**mg/L**	**0.2**	TDS	mg/L	1,200
Fluoride	mg/L	4.0	Zinc	mg/L	5.0
Iron	mg/L	5.0			
Lead	mg/L	0.0075			

2.8.4 Factors Leading to Low Levels of Cyanide in Water

Several factors contribute to the rapid disappearance of cyanide from water. Bacteria and protozoans may degrade cyanide by converting it to carbon dioxide and ammonia under aerobic conditions (Anglogold Ashanti report, 2005).

In water, cyanide is expected to be removed primarily by volatilization mostly under conditions of low pH (acidic), high temperatures, high dissolved oxygen levels, and at increased concentrations of atmospheric carbon dioxide (BioTox, 1998).

Loss of simple cyanides from the water column is primarily through sedimentation, microbial degradation, and volatilization. The rate of volatilization is affected by a number of parameters including temperature, Ph wind speed and cyanide concentration (ATSDR 1995).

2.8.5 Factors Leading to High Levels of Cyanide in Water

In cases where levels of cyanide are toxic to microorganisms (ie., landfills, spills), hydrogen cyanide and nitirles may leach into groundwater (BioTox, 1998).

Cyanide can enter surface water through releases from industries using cyanides (e.g., metal finishing industries, iron and steel mills), runoff from disposal of cyanide wastes in landfills, pesticides and the use of cyanide-containing road salts (Balkau, 2000).

Cyanide binds with numerous other organic and inorganic molecules to form compounds called cyanide complexes. Weak Complexes like $Zn(CN)_4^{2-}$ and $Cd(CN)_3^-$ are readily released in water (ASTM, 1995).

2.9 THE CHEMISTRY OF CYANIDE IN AQUATIC ANIMALS

The concentration of cyanide present in the living fish will vary depending on the concentration of the cyanide solution during exposure, the length of time of the exposure, time for holding and duration of transport, and post-collection treatment. Rates of metabolism may also vary depending on species or size. There is evidence that cyanide is concentrated in several organs and tissues, where it is slowly converted to SCN^- and rapidly excreted through urine (Bruckner and Robert, 2008).

In general, all researchers found a progressive increase in SCN^- in plasma over multiple days, followed by a decline until a period where it was no longer detectable (from 16 days to 16 weeks or more). According to a study reported, cyanide remained detectable in marine fish tissue up to 5-14 days and even 20 days after exposure (Bruckner and Robert, 2008).

Although it has been reported that cyanide induced some histopathological derangements in fish liver, however, the data obtained were not correlated with biochemical alterations (Hosetti *et al.*, 2011).

A report based on the exposure to sub lethal concentrations of copper cyanide to *C. catla* indicated a significant dose dependent alteration in the nitrogenous, as well as carbohydrate metabolism of the fish *C. catla* in all the tissues. There was a significant reduction in protein level while protease activity increased significantly in all tissues of the exposed group when compared to control (Hosetti *et al.*, 2011).

Acute toxicity (96 h LC50) of sodium cyanide for freshwater fish, *L. rohita* was found to be 320µg l-1 in a study. The fish exhibited irregular, erratic and darting swimming movements and loss of equilibrium followed by hanging vertically in water and slowly became lethargic, restless, and secreted excess mucus all over the body following the administration of sub lethal concentration of sodium cyanide. This resulted to respiratory dysfunction (Pube & Hosetti, 2010).

Cyanide is moderately toxic to invertebrates but is highly toxic to freshwater fish regardless the growth stage. However, hydrogen cyanide is not expected to bioaccumulate in living organisms (BioTox, 1998).

2.10 FOOD

Cyanide is present naturally in cassava and cherry laurel and the kernels of apricot, cherry, apple and almond seeds. It is said to have the odour of bitter almonds (Cummings, 2004). The

predominant cyanogenic glycoside in cassava is linamarin. It is present in leaves and tubers, both of which are eaten. In unaffected cassava, cyanogenic glucosides remain intact in the form of linamarin and lotaustralin. When the cellular structure is disrupted, the intracellular glucoside becomes exposed to the extracellular enzyme linamarase. HCN is then produced endogeneously (Kamalu, 1995).

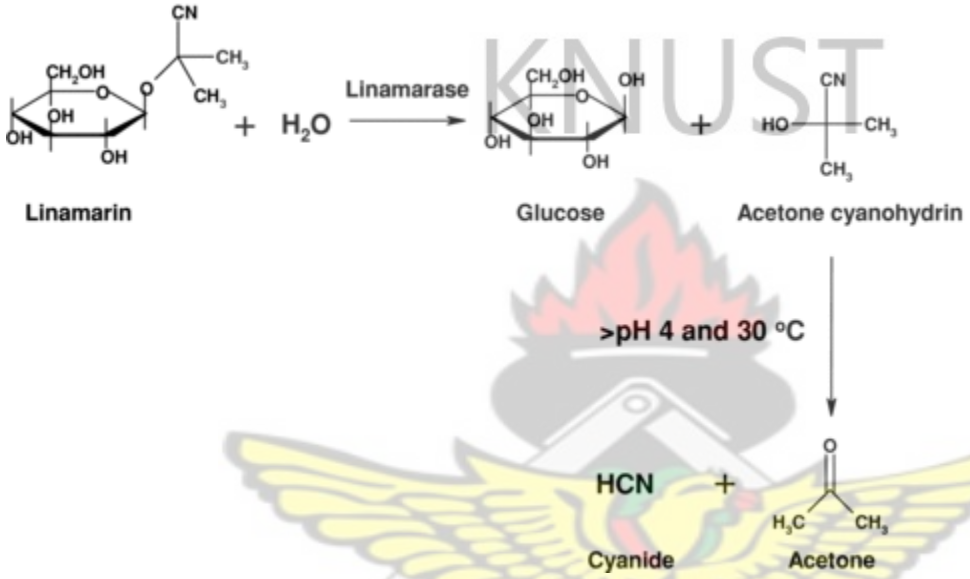


Figure 2.3 Conversion of Linamarin to Cyanide (Montagnac *et al.*, 2009).

Linamarin is the major cyanogenic glucoside found in cassava. According to figure 2.3, during processing, linamarase comes in contact with linamarin and catalyzes the hydrolysis to glucose and acetone cyanohydrin, which subsequently degrades to acetone and cyanide (Montagnac *et al.*, 2009).

It is therefore clear that cyanide in cassava products exist in three forms: the cyanohydrins, the cyanogenic glucosides (95% linamarin and 5% lotaustralin) and free hydrocyanic acid (Montagnac *et al.*, 2009). Higher HCN levels were found in leaves from bitter than in sweeter varieties which are transported to the roots of the plants. However, the HCN concentration and

the bitterness associated with high cyanogenetic glycoside contents in leaves decreases with the maturity of the leaves (Montagnac *et al.,* 2009). The concentrations of these cyanogenic glycosides (CNGs) vary widely in many edible plants due to genetic and environmental factors, location, season, soil types and the nutritional status of the plant (JECFA, 1993). Cassava tubers vary widely in their cyanogenic glycoside content, although most varieties contain 15–400 mg cyanide/kg fresh weight. Occasionally varieties of cassava tubers contain 1300–2000 mg cyanide/kg fresh weight, and cassava leaves contain 1000–2000 mg cyanogenic glucosides/kg on a dry matter basis (Padmaja, 1995). Five varieties of cassava leaves recorded cyanide levels ranging from 40.2 to 60.6 mg/kg with a mean value of 52.9±8.9 (Ayodeji, 2005). Relatively, the amount of cyanide obtained from aerial yam, (49.70 and 32.4 $mg \cdot 100g^{-1}$) cocoyam, (34.10 and 17.3 $mg \cdot 100g^{-1}$) and trifoliate yam, (79.40 and 55.1 $mg \cdot 100g^{-1}$) tubers were lower compared with that of cassava tubers in both fresh and dried (304.60 and 249.50 $mg \cdot 100g^{-1}$) samples (Anhwange, 2011). Uhegbu *et al*., (2012) reported that the cyanide levels found in cocoyam and sweet potato grown on cassava effluent (waste water) contaminated soil were 11.81 ±1.19 and 8.44 ±1.20 mg/kg respectively. Cocoyam incorporated in bread making recorded cyanide level of 3.330±0.200 mg/kg (Nnabuk O.Eddy *et al.*, 2012).

The presence of these CNGs in cassava leaves and roots pose serious health problems in the body if the plants are not processed correctly. WHO limit for cyanide in cassava is 10 mg/kg (WHO, 2001).

2.10.1 Factors Leading to Low Levels of Cyanide in Foods

Cyanide can be reduced in the soil through biological processes. For instance, under aerobic conditions, cyanide salts (especially thiocyanates) in the soil are microbially degraded by fungi: Fusarium solani, Stemphylium loti, and a Pholiota sp., and bacteria species such as

Corynebacterium, Arthrobacter, Bacillus, Thiobacillus, Pseudomonas, Klebsiella, and Escherichia (Akcil & Mudder, 2003) to gaseous nitrites or form complexes with trace metals and not readily released to crops. During metabolism, they use cyanide as nitrogen and carbon source converting it to ammonia and carbohydrate under the appropriate condition (Guilloton *et al*., 2002; Ebbs, 2004). In acidic soils such as pH <7, volatilization of hydrogen cyanide occurs and this process is a significant loss mechanism for cyanides from soil surfaces that leads to a reduction of cyanides in foods. However, the rate of volatilization from soils is complex and depends on many factors (Ubalua, 2010).

Factors like redox conditions, pH as well as soil type are necessary for the leaching and degradation of iron-cyanide complexes in the soil (Kjeldsen, 1999). Cyanide present at low concentrations will be decomposed to ammonia, carbon dioxide, and nitrogen or nitrate under aerobic conditions, and to the ammonium ion, nitrogen, thiocyanate, and carbon dioxide under anaerobic conditions (Rouse & Pyrih 1990).

2.10.2 Factors Leading to High Levels of Cyanide in Foods

Cyanide played a primary role in the evolution of life on earth and remains an important form of nitrogen for microorganisms, fungi and plants (Ubalua, 2010). Soils contaminated with cyanide make this antinutritional substance readily available to plants (Uhegbu *et al*, 2012). Hydrogen cyanide and the alkali metal cyanides are not likely to be strongly absorbed onto sediments and suspended solids because of their high water solubilities. This makes this cyanide readily available for crops (Makkar & Beckar, 1998).

The presence of cyanide in food of plant origin has been attributed to natural production within the plants and uptake from the surrounding soil. HCN is only weakly absorbed by organic matter

and may be easily absorbed by crops. Cyanide-containing herbicides are potential sources of cyanide in cabbage (Kamalu, 1995).

Table 2.1: Cyanide concentrations in food products [JECFA, 1993; Nartey, 1980]

Type of product	**Cyanide concentration (In mg/kg or mg/L)**
Cereal grains and their products	0.001–0.45
Soy protein products	0.07–0.3
Soybean hulls	1.24
Apricot pits, wet weight	89–2170
Home-made cherry juice from pitted	
Fruits	5.1
Home-made cherry juice containing	
100% crushed pits	23

Commercial fruit juices

Cherry	4.6
Apricot	2.2
Cabbage	7.5
Prune	1.9

Tropical foodstuffs

Cassava (bitter) / dried root cortex	2360
Cassava (bitter) / leaves	300
Cassava (bitter) / whole tubers	380
Cassava (sweet) / leaves	451
Cassava (sweet) / whole tubers	445
Gari flour (Nigeria)	10.6–22.1
Sorghum / whole immature plant	2400
Bamboo / immature shoot tip	7700
Lima beans from Java (coloured)	3000

Lima beans fom Puerto Rico (black)	2900
Lima beans from Burma (white)	2000

2.10.3 Factors Influencing Cyanide Uptake by Plants

Organic matter and soil moisture increase the availability of cyanide to plants while application of iron, zinc minimize the availability of cyanide in soil and its uptake by crops. In general, clay and silt accumulate higher levels of cyanide than sandy soils (Jahiruddin *et al.,* 2000). However, Al Rmalli *et al.,* (2005) discovered lower levels of cyanide in plants grown on clays and silts than in plants grown in lighter soils (sands and sandy-loam). This behaviour reflects the fact that, the amount of elements in a plant depends on the root system and its ability to absorb the elements. Deep-rooted crops like cassava accumulate much cyanide than shallow-rooted ones (Al Rmalli *et al.*, 2005). Roots of plants grown in sandy soils penetrate the soil with ease and can absorb much cyanide making it available to the plant. It is not yet possible to predict cyanide uptake and/or toxicity in plants from soil parameters because cyanide accumulation in plants depends on the plant species, soil composition, planting season, geographical location, growing method and other unknown factors. Generally, crops cultivated in the rainy season have higher levels of cyanide accumulation than those cultivated in the dry season (Reilly *et al.*, 2001). Intensive use of highly cyanide contaminated water to irrigate crops elevates the cyanide content of the crops. However, the loading rate in soil is the paramount factor determining toxicity to microorganisms or hazard for movement into groundwater and food (Ubalua, 2007).

2.11 WAYS TO DETOXIFY CYANIDE IN FOOD FOR SAFE CONSUMPTION

Generally, the decreasing level of cyanide (HCN) in common root tubers is manihort utilissima (Cassava), colocasia esculenta (cocoyam), dioscorea bulbifera (aerial yam) and dioscorea domentorum (trifoliate yam). It is also established that, through boiling, the cyanide levels in foods can greatly be reduced. This shows that the amount of HCN found in the fresh state is higher than when they are in their dried or processed form. This conclusion can be drawn from the work done by Abdulrashid and Agwunobi, (2012) where the cyanide level in cocoyam used as dietary substitute for maize in broiler chicken were reported in both fresh and boiled cocoyam as 1.07 + 0.01 and 1.01 + 0.20 mg/kg respectively. Fermentation of cassava pulp for 96 hours during gari production reduced the hydrogen cyanide content by 50%; soaking of sliced cassava for 24 hours, 40%; and sun-drying, 15% (Kendirim *et al*., 1995). Grafting, Soaking, drying, boiling and fermenting are the best ways to reduce or detoxify cyanide in tuber crops like cassava and cocoyam (Okereke, 2012). Kobawila *et al*., (2005) also attested to this fact by stating that, the cyanide content in cassava roots and leaves is reduced by more than 70% during fermentation through the activities of the bacterial produced linamarase that allows the hydrolysis of cyanogenic glycosides.

Hence, Anhwange *et al*., (2011) based on their findings concluded that, Food crops found to contain high levels of cyanide should be efficiently processed before consumption.

2.12 ROUTE OF EXPOSURE TO CYANIDE AND ITS ABSORPTION

One can be exposed to this deadly chemical through breathing, drinking or eating, touching a substance contaminated with cyanide and occupational exposure. The mode of exposure through the following routes is discussed below.

Breathing: Inhalation is the primary route of exposure for the general population. Nitrogen-containing compounds (melamine, nylon, etc.) contain HCN which is released from a number of combustion processes, such as smoke from cigarettes, fires or from car exhaust fumes (Cummings, 2004).

Cyanide gas can be found in industrial emissions and car exhaust, cigarette smoke and certain papers and plastics as they burn. It is also possible to breathe or eat cyanide dust when working with cyanide powder. If people use a contaminated water supply, they can breathe the cyanide when they cook or shower with the water (WHO, 2004).

Hydrogen cyanide is readily absorbed following inhalation, oral, and dermal exposure. Following exposure to cyanide in the atmosphere, toxic amounts of cyanide are absorbed with great rapidity through the bronchial mucosa and alveoli (ATSDR, 1997).

Drinking/Eating: Cyanide is sometimes found in contaminated drinking water. People can be exposed when they drink contaminated water. People who handle contaminated soil may be exposed when they eat or touch their mouths with dirty hands. Several assidental cases have been reported including transportation accidents, pipe failures, and tailings dam-related releases (Korte *et al.*, 2000; Mudder & Botz, 2000). The use of sodium cyanide in the extraction process of minerals can result to the emission of very high amount of hydrogen cyanide and other forms

of cyanide into the environment. Some of these emissions can result in large cyanide-containing ponds, rivers or leach into groundwaters near the mining operations (Korte *et al.*, 2000; Mudder & Botz, 2000).

Alkali metal cyanides are rapidly absorbed from the gastrointestinal tract. Absorption is affected by the presence of food in the gut, the pH of the gut, and the lipid solubility of the cyanide compound. Gastrointestinal absorption of inorganic cyanide salts is slower than pulmonary absorption, and the onset of symptoms is delayed and the severity of symptoms diminished compared with inhalation. When simple cyanide salts such as potassium and sodium cyanide are ingested, free cyanide ion can rapidly bind hydrogen ion to form hydrogen cyanide in the highly acidic medium of the stomach. Essentially all cyanide ingested as cyanide salts will form hydrogen cyanide and will be quickly absorbed. However, after oral intake, only part of the dose reaches the blood due to first-pass metabolism by the liver (ECETOC, 2004).

Touching: Dermal exposure is a route of cyanide exposure. Cyanide can enter the body through skin when people handle the chemical, contaminated soil or contaminated water. People can be exposed to cyanide if they wash or bathe with contaminated water. Liquid cyanide compounds are easily absorbed through intact skin upon direct contact due to their lipid solubility and rapid epidermal penetration. Skin absorption of vapours of hydrogen cyanide is also possible when the air concentrations are high. Cyanide absorbed through an uninjured and abraded skins may result into breathing abnormalities like peripheral vasoconstriction and plasma extravasation together with deep coma in humans (WHO, 2004). Such situations have been noted after accidents involving immersion in cisterns containing copper or potassium cyanide solutions (ASDTR, 2004). Dermal LD50 following application of cyanides in aqeous solutions to rabbit skin, have been reported in the range of 7-10 mg kg^{-1} (WHO, 2004). Administration of sodium cyanide

(1.7-5.3 mg kg^{-1} day-1) to the inferior conjunctival sac of rubbits resulted in irritation, lacrimation and hyperaemia immediately after treatment (ASDTR, 2004).

Occupational exposure: This may occur with production or application of pesticides containing cyanides. There was a report about a victim who collapsed in some few minutes after operating on hydrogen cyanide in a manufacturing plant. It was presumed that the victim might have ingested the HCN from a 100ml sample containing 82% acetone cyanohydrins which was approximately 25 times the lethal dose (Cummings 2004).

Another victim whose duty was to sample a hydrogen cyanide reactor vessel in an acrylonitrile plant without his breathing apparatus applied was found collapsed in splashes of hydrogen cyanide liquid (Cummings, 2004).

In one study, workers exposed chronically (duration not specified) to 15 ppm hydrogen cyanide reported a range of effects including fatigue, dizziness, headache, disturbed sleep, tinnitus and parathesias (ASDTR, 2004). Similar findings have been reported in another study and also included delay memory and/or visual impairment in around 31.5% of workers. Concentrations of hydrogen cyanide were not, however, specified (ASDTR, 2004). Nuerological features have been reported to persist on cessation of chronic exposure (ASDTR, 2004).

2.13 CYANIDE TOXICITY

Cyanide toxicity is mediated through inhibition of cellular respiration which is potent to cause suicide, homicide and genocide (Bhattacharya, 2000). Exposure to small amounts of cyanide can be deadly regardless of the route of exposure. The severity of the harmful effects depends in part

on the form of cyanide, such as hydrogen cyanide gas or cyanide salts. Cyanide is a rapidly acting toxin (WHO, 1999).

Exposure to a massive concentration of hydrogen cyanide gas may render an individual unconscious within seconds (Seidl *et al*., 2003; Cherian & Richmond, 2000) and may lead to coma and death within minutes. There was a study reporting the time interval between the exposure of different concentrations of cyanide to humans and their subsequence death. On the least, 135 ppm exposure lead to a death in 30 minutes while 270 ppm resulted to immediate death (WHO, 2004).

At short-term exposure levels above the minimum cyanide level (MCL) cyanide causes rapid breathing, tremors, and other neurological effects. Long-term exposure at levels above the MCL, cyanide can cause weight loss, thyroid effects, nerve damage and death. Skin contact with liquids containing cyanide may produce irritation and sores (Wilson, 2011).

Cyanide is an inhibitor of the electron transport chain in the biological metabolic pathway. Cyanide inhibits the enzyme cytochrome oxidase, which catalizes the transfer of electrons from the cytochrome system to molecular oxygen, resulting in cytotoxic anoxia (Wilson, 2011).

Exposure to high levels of cyanide for a short time harms the brain and heart and can even cause coma and death. Cyanide produces toxic effects at levels of 0.05 milligrams of cyanide per deciliter of blood (mg/dL) or higher, and deaths have occurred at levels of 0.3 mg/dL and higher (a deciliter equals 100 milliliters). People who breathed 546 ppm of hydrogen cyanide have died after a 10-minute exposure; 110 ppm of hydrogen cyanide was life-threatening after a 1-hour exposure (WHO, 2004). People who eat small amounts of cyanide compounds in a short time may die unless they quickly receive antidote therapy (WHO, 2004). There are several drugs such

as sodium nitrate, 4-dimethyl aminophenol, sodium thiosulphate (Bhattacharya, 2000) etc, are known to serve as antidotes to cyanide poisoning. However there is a lack of international consensus about the treatment choice and hence there is the need for an agreed local procedure for the emergency treatment of poisoning (Cummings, 2004).

2.13.1 Mechanism of Cyanide Toxicity

Cyanide has a high affinity for certain sulfur compounds (sulfanes, which contain two covalently bonded but unequally charged sulfur atoms) and for certain metallic complexes, particularly those containing cobalt and the trivalent form of iron (Fe^{3+}) (USEPA, 2002). The cyanide ion can rapidly combine with iron in cytochrome a_3 (a component of the cytochrome aa_3 or cytochrome oxidase complex in mitochrondria) to inhibit this enzyme, thus preventing intracellular oxygen utilization. The cell then utilizes anaerobic metabolism, creating excess lactic acid and a metabolic acidosis. Cyanide also has a high affinity for the ferric iron of methemoglobin and one therapeutic strategem induces the formation of methemoglobin to which cyanide preferentially binds (ATSDR, 1995).

2.13.2 Mechanisms of cyanide in plants, insects and higher animals

The figure 2.4 below illustrates the biosynthesis, catabolism and detoxification of cyanogenic glycosides (CNGs) in plants, insects and higher animals.

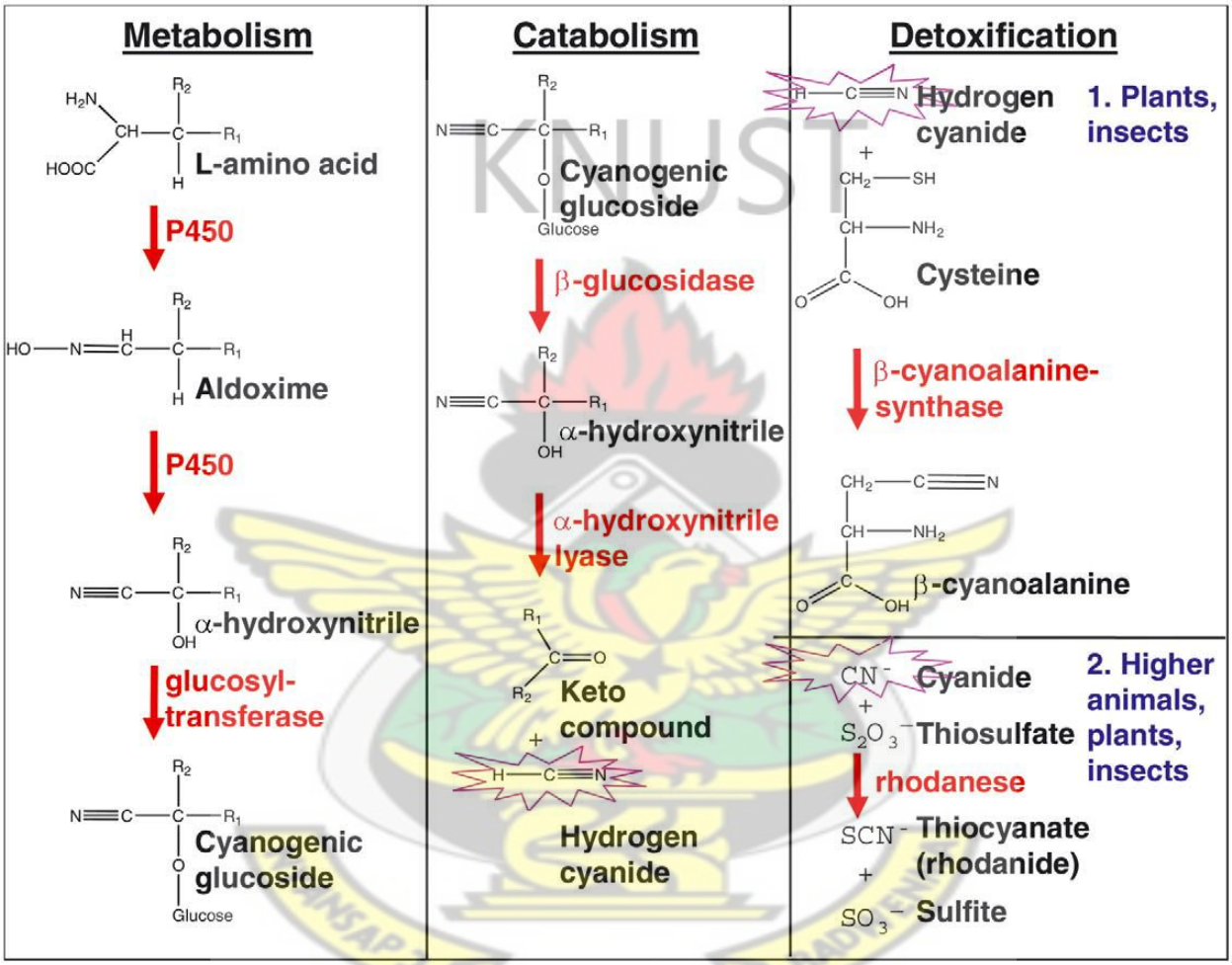


Figure 2.4 Biosynthesis, catabolism and detoxification of cyanogenic glycosides (CNGs) in plants, insects and higher animals. Enzymes involved are shown in red. HCN is highlighted in purple (Zagrobelny *et al*. 2004).

The first two committed steps in CNG biosynthesis are catalyzed by cytochromes P450 (Fig. 2.4). The first P450 catalyzed step proceeds via two successive N-hydroxylations of the amino

group of the parent amino acid, followed by decarboxylation and dehydration (Sibbesen *et al*., 1994). The aldoxime formed is subsequently converted to an ahydroxynitrile through the action of a second cytochrome P450 (Bak *et al*, 1998; Kahn *et al*., 1997). This reaction involves an initial dehydration reaction that forms a nitrile and is followed by hydroxylation of the alpha carbon to generate a cyanohydrin. The final step in CNG synthesis, glycosylation of the cyanohydrin moiety, is catalyzed by a UDPG-glycosyltransferase (Jones *et al*., 1999) (Fig. 2.4). Catabolism of CNGs is initiated by enzymatic hydrolysis by a β-glucosidase to afford the corresponding ȁ-hydroxynitrile, which at pH values above 6 spontaneously dissociates into a sugar, a keto compound, and HCN (Fig. 2.4). At lower pH values, the dissociation reaction is catalyzed by an a-hydroxynitrile lyase. HCN is detoxified by two main reactions (Møller and Poulton, 1993) (Fig. 2.4). The first route involves the formation of β-cyanoalanine from cysteine and is catalyzed by β-cyanoalanine-synthase (Fig. 2.4, route 1). β-Cyanoalanine is subsequently converted into asparagine (Miller and Conn, 1980). The second route proceeds by conversion of HCN into thiocyanate and is catalyzed by rhodanese and is excreted through urine in mammals (Bordo and Bork, 2002) (Fig. 2.4, route 2). The detoxification route involving β-cyanoalanine is common in plants and possibly also in insects.

A similar reaction can also be observed when the seeds of kernels are crushed and moistened. Amygdalin, the CNG present in the seed (which is also present in cassava, bitter almonds, and peach stones) is converted to glucose, benzaldehyde, and hydrogen cyanide (Figure 2.5) (IPCS, 1992) catalysed by the enzyme emulsin (Lasch & El Shawa, 1981).

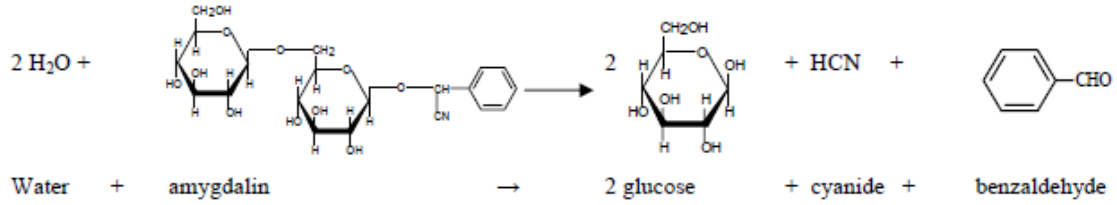


Figure 2.5 Hydrolysis of Amygdalin (WHO, 2004)

2.14 METHODS OF DETERMINING CYANIDE

Cyanide species have been categorized into three main forms based on the analytical methods of determination. These are total cyanide, weak acid dissociable (WAD) cyanide and free cyanide. Total cyanide includes weak and moderately metal-cyanide complexes (WAD) with free cyanide and relatively non-toxic, strong cyanide complexes with iron. Cyanides complex ions with metals are classified depending on bonding strength of the central atom (metal, cation) with ligand (an ion CN^-). Figure 2.6 shows this classification. Several methods are involved in the determination of these classes of cyanide. These includes distillation, extraction, flotation, adsorption and membranous processes (Latkowska & Figa, 2007).

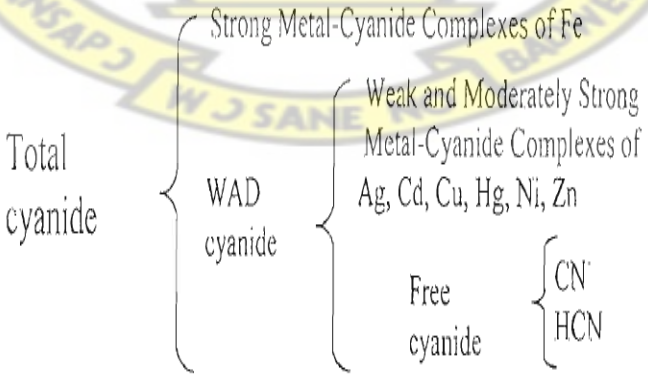


Figure 2.6 Classification of cyanide (Young and Jordan, 1995)

2.14.1 Preservation of water samples

Once samples are removed from their natural environment, chemical or biological reactions can occur to change the composition of the sample, so it is best to analyze the sample as quickly as possible. Preservation of the sample will keep the parameter of interest in the same form as it was prior to the removal from its surroundings. No single preservation technique will preserve all parameters, so each parameter of interest must be considered and preserved specifically. While most soil samples require exclusion of light, air and warmth to preserve the integrity of the sample, aqueous samples require a more concerted effort. Samples of aqueous cyanide species are potentially very reactive and toxic, so safety precautions such as gloves and protective clothing must be rigorously observed. Due to their reactivity, sample solutions must be tested on site prior to cyanide analysis to preserve them against the main interfering substances, oxidizing matter and sulfides (ICMI, 2012).

2.14.2 Interferences

The effects of interferences can lead to false negatives and false positive results. False negative results are those reporting the absence of cyanide when cyanide is actually present in the sample in its natural environment. False positive results are those recording high levels of cyanide in samples in which no cyanide exist in their natural environment (Enviromail 62, 2012). A number of significant interferences can produce these types of inferences during sample analysis with the traditional acid distillation technique, but are minimized or absent with the new analytical method thus the continuous flow method (ASTM 2009). However, increased levels of cyanide were found in some chlorinated wastewaters compared to the levels before chlorination, suggesting a fast reaction mechanism associated with the disinfectant and some precursor in the

wastewater using the same method (Weinberg 2005). Therefore, it is very crucial that all interferences are detected and removed as soon as possible so as to prevent their reactions. Unfortunately the numerous complex mitigation procedures may either themselves introduce more interference into the sample or not mitigate some interference (USEPA 2009). This pose a serious problem in cyanide determination since none of the approved analytical methods guarantee consistent accurate results. The EPA and standards setting organizations, such as ASTM International and Standard Methods, have acknowledged shortcomings in approved methods and are engaged in updating and revising standard approved analytical methods (ASTM 2009). Compounds that can interfere with accurate CN measurement include aldehydes, carbonates, nitrite, nitrate, oxidants, sulfide, sulfur compounds, and thiocyanate (USEPA 2007, WERF 2003, Delaney 2007).

2.14.2.1 Removal of Sulfides

Sulfide can exert either a positive or a negative interference in the determination of Cyanide species. Failure to remove the Sulfide before raising the pH may result in the formation of Thiocyanates that give a positive interference for Cyanide analysis. False negatives can occur when Cyanide reacts with Sulfide in the sample (Enviromail 62, 2012). The presence of thiocyanate can interfere in the reaction of the cyanide with chloramine-T to produce cyanogen chloride for analysis. This is because thiocyanate reacts varyingly with the chloramine-T over a wide range of pH of the solution (Milosavlijevic *et al.*, 1995).

$$CH_3C_6H_4SO_2NClNa + CN^- + 2H_2O \rightarrow 4CH_3C_6H_4SO_2NH_2 + CNCl + Na^+ + SO_4^{2-} + 2OH^-$$

$$4CH_3C_6H_4SO_2NClNa + SCN^- + 4H_2O \rightarrow 4CH_3C_6H_4SO_2NH_2 + CNCl + 4Na^+ + SO_4^{2-} + 3Cl^-$$

For instance, the reaction is rapid in the acidic and weak alkaline region (around pH of 5 and 8) and is slow in the neutral and strong alkaline region (around pH of 7 and 9).The explanation to this is that the thiocyanate competes with the cyanide thereby producing error in the analysis. And this interference lead to a false negative result because there is always a reduction in the concentration of cyanide recovered and such error is recorded mostly in spectrophotometric determination of cyanide such as pyridine-pyrazolone method, pyridine barbituric acid method, pyridine-benzidine method and pyridine-p-phenylenediamine method (Noroozifar *et al*., 2005).

Before the removal of sulfides there must be an indication that this interference is present in the sample. The presence of Sulfides can be confirmed by releasing few drops of the samples on a test strip (moistened with lead acetate). Darkening of the paper indicates the presence of Sulfide in the sample (Enviromail 62, 2012). The first alternative method in the removal of sulfide is to add the sample to sulfide pre-treatment bottle containing lead acetate which reacts with Sulfide in the sample to precipitate insoluble lead Sulfide. The bottle is then capped and mixed by swirling for a few seconds followed by standing for 5-10 minutes to allow any precipitate to settle. After settling, the treated sample is decanted into the second (standard) ALS bottle which already contains Sodium Hydroxide. This will raise the pH to approximately 12 and maintain an alkaline pH to prevent the loss of Cyanide through the formation of hydrogen Cyanide gas (Enviromail 62, 2012).

It must be noted that any used Sulfide pre-treatment bottles must be returned to the zip lock bag in which they were provided and returned to ALS for disposal.

Plate 2.1 shows sample sulphite pre-treatment bottles and the zip lock bag.

Plate 2.1 Sulfide pre-treatment bottle (containing lead acetate) (Enviromail 62, 2012)

2.14.2.2 Oxidizing agents

The presence of oxidizing matter is detected by potassium iodide/starch test papers. This is done by placing a drop of the sample on a moist test paper strip. A blue coloration of the test paper indicates the presence of sufficient oxidizing matter to potentially react with the cyanide present during transport. Oxidizing agents must be reduced prior to sending the sample to the laboratory (O´Dell, 1993).

2.14.2.3 Removal of Oxidizing Matter

The oxidizing agents are removed by first removing and retaining solids by decantation or pressure filtration. About 0.1g/l sodium arsenite is added and retested and if test strip discoloured, more sodium arsenite is added until there is no colouration. The solids are returned to the sample and the pH raise to 12 by adding 1-2 pellets of solid sodium hydroxide.

The presence of sulfides is indicated by lead acetate paper turning black. Place a drop of the sample on the test paper previously moistened with a drop of acetic acid and if the paper darkens, sulfides are indicated. Sulfides are removed by reaction with lead carbonate (ICMI, 2012).

2.14.3 Distillation

This test method covers the determination of cyanide ion in solutions derived from the acid digestion of solid samples like fish, cassava, cocoyam etc. And it has to do with the decomposition of most cyanide compounds in the presence of strong acid, magnesium chloride catalyst, and heat during a one hour reflux distillation (ASTM 1996). Hydrocyanic acid (HCN) vapor is released in the digestion flask and passes through the reflux condenser. Cyanide ion (CN^-) is captured in an absorption tube containing sodium hydroxide (NaOH) solution. The high pH (12-13) of the NaOH ensures that the form of cyanide present in the absorption tube is CN^-.

2.14.4 The Alternate Methods for Cyanide Determination after Distillation.

Various methods such as titrimetric methods, colormetric methods, electrochemical methods (potentiometric, amperometric procedures), gas chromatography, indirect methods involving atomic absorption spectrometry, ion-selective electrode (ISE), ion chromatography – high performance liquid chromatography (IC-HPLC), and infrared spectrometric and automated

methods can be adopted to quantify the cyanide after distillation. Some of them are discussed below.

2.14.4.1Titrimetric methods

This is a primary method, simple and highly recommended for cyanide determination. It is based on the development of turbidity due to silver cyanide that forms as a result of the reaction between the cyanide and a silver nitrate solution as shown below.

$Ag^{+} + 2CN^{-} \rightarrow [Ag(CN)_2]^{-}$

At least the lower quantification level (LQL) of 1mg/l for free cyanide and 0.10mg/l for total must be obtained by all laboratories (Young *et al* 1995 and Trapp *et al* 2003 cited by Obiri *et al.*, 2007). This method has been employed by Obiri *et al* in 2007 where the samples were titrated against $AgNO_3$ using *p*-dimethylaminobenzalrhodanine as indicator. The excess silver ions indicate the end point of the titration. However this method is subjected to some level of inaccuracy since the endpoint determination becomes difficult to detect. It is very imperative to detect the first colour change because that signifies the endpoint. Few seconds after the first colour change, silver ions tend to liberate cyanide ions from other complexes, which lead to a disappearance of the colour. Potentiometric endpoint detection is highly recommended to improve upon the accuracy of the method (ICMI, 2012).

2.14.4.2 Gravimetric analysis of cyanide determination

Gravimetric analysis is the most straightforward kind of quantitative measurements. It consists of isolating in a pure form a species produced stoichiometrically by the analyte, washing it, and calculating the percentage of analyte in the sample. The most common way of obtaining a pure

form of the analyte is formation of a precipitate by a reaction of the analyte in solution. For instance the cyanide content of weighed water can be determined by precipitating the cyanide in the sample with excess silver nitrate solution:

$Ag^{+}_{(aq)} + NO_3^{-}{}_{(aq)} + CN^{-}_{(aq)} \rightarrow AgCN + NO_3^{-}{}_{(aq)}$

The silver cyanide precipitate produces is collected and washed to remove extraneous residual salts. After drying to remove excess water, the precipitate is weighed to get its mass and the percentage of cyanide is calculate by stoichiometric calculation.

2.14.4.3 Electrochemical methods of cyanide determination

Electrochemical analysis of cyanide and thiocyanate concentrations in biological samples, including plasma, tissue, and whole blood have been conducted using ion-selective electrodes potentiometry, amperometry, polarography and coulometry. Two clear benefits of these methods are high and quick analysis time. However, they can be subjected to several interferences from many organic and inorganic ions, including sulfide, Fe^{3+}, ClO_4^-, NO_2^-, N_3^-, and I^- (Mak *et al.*, 2005). Elecrtrochemical methods can also be hampered by narrow working concentration ranges and may require large sample sizes (Ganjali *et al.*, 2002). An application of this method can be seen in Westley and co's work when they used a silver rotating disk electrode, and a dropping mercury electrode for the voltammetric determination of cyanide and thiocyanate in biological samples, including plasma, tissue, and whole blood (Westley and Westley, 1989). Electrochemical detection can also be used for analysis of cyanide and thiocyanate with ion chromatography (Casella, 1998).

2.14.4.4 The use of Ion Selective Electrode (ISE) in Cyanide Determination

Ion Selective Electrodes (ISE) are membrane electrodes that respond selectively to ions in the presence of others. The voltage is theoretically dependent on the logarithm of the ionic activity, according to the Nernst equation. They have the advantage of being inexpensive and not requiring extensive sample preparation. More so the equipment is durable in field settings. However the electrodes lose sensitivity with long use and require frequent recalibration. The results vary with temperature and the electrodes are prone to interference from other ions with iodine levels in seawater causing interference. Numerous methods are available to detect cyanide or cyanide metabolites, but most have been used only for water samples and blood; only limited testing has been done on whole fish and fish organs. The ISE method is the only cyanide test that has been applied on a large scale for Marine Aquarium Fish and LRFF at points of export. The ISE method was successfully used in the Philippines from 1993 to 2001, with at least 48,000 fish tested; the test produced cyanide positive results in fish up to 5 to 14 days after exposure (Rubec *et al*., 2003).

2.14.4.5 Polymeric Membrane Based Ion Selective Electrodes (ISE)

The American Society of Testing and Materials (ASTM) ISE method is the only approach that has been widely used to detect cyanide exposure in marine fishes. The method involves an acid digestion of the fish to liberate hydrogen cyanide gas, and capture of cyanide ions in sodium hydroxide solution after reflux distillation. Chemicals must be added to help remove interfering substances such as chlorine and hydrogen sulfide. An ISE meter manufactured by Thermo-Orion, using a membrane made of Agl or Ag_2S, is then used to analyze cyanide concentrations, based

on its interaction with silver. ISE methods may also work for the determination of thiocyanate (ASTM, 2009).

2.14.4.6 Spectrophotometric and Fluorescence Methods

Fluorescence spectroscopy or fluorometry is a type of spectroscopy used for analyzing compounds that have the ability to fluoresce. Generally, this fluorescence is directly proportional to the concentration of the material in question. It has high sensitivity and permits less interference. However, interference potential still exists without a pre-separation mechanism and this coupled with impurities may diminish flourescence. Sample preparation by microdiffusion is required. Both spectrophotometric and fluorescence methods require extraction techniques to isolate cyanide and eliminate interferences from samples. Spectrophotometric methods have been used for determination of cyanide, thiocyanate and cyanide metabolites. One common approach involves the König dye synthesis to form a cyanide halide that is reacted with an aromatic amine to produce a glutaconic aldehyde product that is measured in the visible region of the spectrum. Spectrophotometric methods have adequate sensitivity, but they may lack specificity due to interferences from other chemical species commonly present during the analysis of cyanide, especially thiocyanate and thiosulfate. They also require lengthy preparation times and the products may be unstable. A number of fluorometric assays are available to determine cyanide, which have several advantages over spectrophotometric methods, including a lack of interference from thiosulfate and greater sensitivity (Wagner, 2003).

2.14.4.7 Flow Injection Analysis (FIA) and Continuous Flow Analysis (CFA)

The FIA method is a simple one with high reproducibility. The approach involves injecting a sample solution containing the target molecule into a flow tube where it reacts with certain chemicals. When the products reach the detector, the target molecules in the sample are measured. The user is able to control the measuring conditions precisely and also has the capability to continuously measure cyanide. In Continuous Flow Analysis (CFA) a continuous stream of material is divided by air bubbles into discrete segments in which chemical reactions occur. The continuous stream of liquid samples and reagents are combined and transported in tubing and mixing coils. The tubing passes the samples from one apparatus to the other with each apparatus performing different functions, such as distillation, dialysis, extraction, ion exchange, heating, incubation, and subsequent recording of a signal. An essential principle of the system is the introduction of air bubbles. The air bubbles segment each sample into discrete packets and act as a barrier between packets to prevent cross contamination as they travel down the length of the tubing. The air bubbles also assist mixing by creating turbulent flow (bolus flow), and provide operators with a quick and easy check of the flow characteristics of the liquid. Samples and standards are treated in an exactly identical manner as they travel the length of the tubing, eliminating the necessity of a steady state signal, however, since the presence of bubbles create an almost square wave profile, bringing the system to steady state does not significantly decrease throughput (third generation CFA analyzers average 90 or more samples per hour) and is desirable in that steady state signals (chemical equilibrium) are more accurate and reproducible (Coakly, 1981). While the CFA uses air segmentation to separate a flowing stream into numerous discrete segments to establish a long train of individual samples moving through a flow channel, FIA systems separate each sample from subsequent sample with a carrier reagent.

And as the air bubbles mix sample homogeneously with reagents in CFA, in all FIA techniques, sample and reagents are merged to form a concentration gradient that yields analysis results. Removal of air segmentation opened the door to instrument miniaturization and inspired further progress towards analytical microfluidics, sometimes termed as "lab-on-chip" (Rulika and Hansen, 1975).

A continuous flow analyzer (CFA) consists of different modules including a sampler, pump, mixing coils, optional sample treatments (dialysis, distillation, heating, etc.), a detector, and data generator. Most continuous flow analyzers depend on color reactions using a flow through photometer, however, also methods have been developed that use ISE, flame photometry, ICAP, fluorometry, and so forth.

2.14.4.8 Biosensors for Determining Cyanide Ion

These methods primarily evaluate chemical reaction products based on the enzyme inhibition of cyanide, cyanide degrading enzymes, and microbial sensors which measure oxygen uptake by bacteria, yeast or other microorganisms. Biosensors have a rapid response, high selectivity, and a pollution free procedure. Most biosensors have the advantages of being portable, low cost, easy to use, and high selectivity. These methods, however, rely on chemical and physical procedures that can be slow, complex, and require the use of expensive equipment and environmental loading reagents. Other limitations of biosensors include degradation of the biological components that make up these sensors, inconsistent electrochemical signals, and difficulty producing sufficient quantities and activities of enzymes or microbes on which these sensors depend. In 2005, one group applied a biosensor approach to marine fishes. Organs of the fish were homogenized with NaOH and a fungal enzyme extract was used to produce formate from

metal-cyanide complexes; the formate was converted using an enzyme (formate dehydrogenase) to nicotinamide adenine dinucleotide (NADH) which was measured spectrophotometrically (Mak *et al.*, 2005).

2.14.4.9 Chromatographic Methods

Gas Chromatography (GC), high performance liquid chromatography (HPLC), and ion chromatography (IC) can all be used for determination of cyanide. These methods employ electron capture detectors, electrochemical detectors, UV/VIS detectors, fluorescence detectors, conductivity detectors, or amperometric detectors. Three types of liquid chromatography have been used to analyze cyanide: reverse-phase high-performance liquid chromatography (RP-HPLC), ion chromatography (IC), and capillary electrophoresis (Watanabe *et al.*, 2002) Liquid chromatographic techniques can determine trace amounts of an analyte and can efficiently separate analytes from interfering components in the matrix, offering advantages over spectrophotometric, luminescent, and electrochemical methods. Liquid and gas chromatographic techniques also have the ability to simultaneously analyze for cyanide and thiocyanate. IC methods can determine all species of cyanide by separation. This technique obviates the need for distillation to convert cyanide complexes from metal to HCN. A number of pretreatment steps have been developed to facilitate the analysis of cyanide, thiocyanate, and ATCA using GC. For example, the sampling of cyanide from the sample head space is the most common pre-analysis step when using the GC method (Suzuki and Kumazawa, 1998).

CHAPTER THREE

3.0 MATERIALS AND EXPERIMENTAL METHODS

This chapter describes in detail the study area and the methods adopted in the determination of free and total cyanide levels in the samples alongside other physiochemical parameters like pH, conductivity, total solids etc.

3.1 DESCRIPTION OF THE STUDY AREA

Kenyasi is located in the Brong Ahafo Region. The region lies in the forest zone and is a major cocoa and timber producing area. The northern part of the region lies in the savannah zone and is a major grain and tuber producing region. The total population of the region goes above 1,815,408, representing 9.6 per cent of the country's population. The main occupation of the workforce of the region is in agriculture which employs about 68.6% of the economically active population (MGM, 2010).

Plate 3.1 Shows the dam and fish sampling site.

This dam is located somewhere in between Kenyasi and Ntotoroso and serves as a hydro-power generator for the mining company in the area. The dam was created out of a small stream which once served as a source of drinking water and a medium for fishing for the villages and small cottages located close to it. In replacement to this were boreholes dug for the people in the area as a source of drinking water. There are three different species of fish found in this dam. This is one of the areas cyanide spillages allegedly do occur. Around this dam are various plantations such as cassava, cocoyam, plantain, cocoa etc where the cassava and cocoyam samples were collected.

3.2 EQUIPMENT AND CHEMICAL REAGENTS

The equipments used are listed below:

- Skalar San $^{++}$ Automated Wet Chemistry Analyzer/Continuous Flow Analyzer
- Yogokawa pH meter

Distilled water was used in the preparation of all the solutions

The reagents used:

- Sulphuric Acid (Analar) from BDH, England
- Magnesium Chloride Hexahydrate (Analar) from BDH, England
- Sodium Hydroxide from Fischer Scientific UK.
- Potassium Hydrogen Phthalate from Fischer Science UK.
- Citric Acid from Fischer Scientific UK.
- Sodium Arsenite (Analar) from BDH, England
- Lead Acetate (Analar) from BDH, England
- Lead Carbonate (Analar) from BDH, England
- Zinc Sulphate 7-Hydrate from Surechem
- Potassium Cyanide from Surechem
- Sodium Acetate Anhydrous from Surechem
- Chloramine-T from Acros
- 1, 3-dimethylbarbituric acid from Alderich chemicals, England
- 4-pyridine carboxylic acid/ Isonicotinic Acid from Acros
- Acetic Acid from BDH, England
- Hydrochloric Acid (Analar) BDH, England
- Nitric Acid (Analar) from BDH, England

All the reagents used were of analytical grade (BDH chemical limited, Poole England) unless otherwise stated.

3.3 SAMPLES

The samples used were water, fish, cassava and cocoyam and the procedure employed in the sampling is outlined below.

3.3.1 Water

Black plastic containers of one litter volume were thoroughly washed with detergent, rinsed with distilled water and dried. They were then rinsed with 10% HNO_3 and again thoroughly rinsed with distilled water. In total sixteen water samples were collected from the study area of which seven were from the dam which were labeled from DAM1-7, two from surface water to serve as a control for the dam and seven from boreholes close to the mining area which were labeled as BH1-7. Two water samples from two different boreholes located at a non-mining area (Hwidiem) to serve as control. The pH and conductivity measurements were taken immediately at the sampling site using Yogokawa pH meter and Orion thermoscientific meter.

They were then decanted and potassium iodide test paper used to test the presence of oxidizing matter. The oxidizing matter was removed by the addition of 0.1g/L sodium arsenite to the filtrate. The test strip was used to retest the sample until it discoloured. The presence of sulfides was also tested using lead acetate test strip and subsequently removed by the addition of 0.1g/L lead carbonate to the filtrate until there was no colouration after retesting using the test strip. The solids were returned to the sample and the samples preserved by adding 1-2 pellets of sodium

hydroxide which increased the pH to about 12. All the samples were kept in an ice cooled container and transported to the laboratory for cyanide analysis.

3.3.2 Fish Samples

A total of thirty three samples of fish were sampled from the dam with hook and line into a black polyethene bag. They were then kept in an ice cooled container and transported to the laboratory at Kwame Nkrumah University of Science and Technology and refrigerated after identification at the department of fisheries, faculty of Renewable Natural Resources. The samples were found to belong to the specie, *Oreochromis niloticus.* Three *Oreochromis niloticus* were obtained from a pond in Kumasi to serve as control. All the samples were preserved in the freezer till chemical analysis.

3.3.3 Cassava and Cocoyam Samples

A total of 24 randomly selected cassava and cocoyam tubers were harvested from local farms close to the mining area. This was done by picking four samples from four divided portions of a farm. In all, six different farms were considered each for cassava and cocoyam. Adhering soils were thoroughly washed with distilled water. The cassava samples were then labeled CvF1(i) indicating cassava from the first (of six) farm and the first sample among the four samples picked from the four different portions of the farm up to CvF6(iv) also denoting the fourth cassava sample picked from the sixth farm and among the four samples picked from that particular farm. The cocoyam samples were also labeled in a similar manner starting with CyF1(i) up to CyF6(iv) where Cy represent cocoyam. Three samples each of cassava and cocoyam were collected from a non-mining area (Hwidiem) treated the same way and labeled Cv1 to Cv3 for cassava and Cy1 to

Cy3 for cocoyam to serve as control. They were wrapped in a black polyethene bag and kept in an ice cooled container and transported to the project laboratory of KNUST for further analysis.

3.4 pH AND CONDUCTIVITY MEASUREMENT OF THE SOLID SAMPLES

Approximately 20 g of each solid sample was ground and put into 100 mL of distilled water in a 500 mL pyrex beaker and stirred for 5 to 10 minutes using a magnetic stirrer to ensure complete homogeneity. The mixture was then filtered into a small sterilized plastic container and the pH of the filtrate determined by immersing the pH electrode in the filtrates of the solid samples. A steady reading was recorded in each case. The electrodes were washed thoroughly with distilled water after every determination to avoid cross contamination. For each sample, the pH determinations were made in triplicate and the mean taken to represent the pH of the sample. The conductivity readings of both the solid and water samples were also taken in the same manner using the Orion thermoscientific meter.

3.5 DETERMINATION OF MOISTURE CONTENT OF CASSAVA AND COCOYAM

About 5g of each cassava and cocoyam samples were grated into crucible and dried in an oven at a temperature of 105^{o}C for 72 hours. The samples were then cooled in a desiccator and then reweighed. The drying process was repeated at the same temperature and at time intervals of 1 hour until a constant weight was recorded for each of them.

3.6 DETERMINATION OF CYANIDE CONCENTRATION IN WATER, FISH, COCOYAM AND CASSAVA SAMPLES

Total cyanide concentrations were determined in the water, fish, cocoyam and cassava samples from Kenyasi and Hwidiem. The solid samples were first distilled before the cyanide concentrations were determined using simple distillation setup. A continuous flow method was adopted for the cyanide determination using the Skalar san ++ automated wet chemistry analyzer.

3.6.1 Treatment of the Solid Samples

The Skalar instrument has in-built facilities that support distillation coupled with an autosampler that picks liquid samples into the chemistry section for the analysis to begin. Therefore the water samples required no further treatment once the oxidizing matter and sulfides were removed. The fish, cassava and the cocoyam needed further treatment since they were all solid samples. An appropriate procedure was required to bring them into the liquid form taken into consideration the exact concentration of cyanide present in the samples. Hence a distillation process was adopted to trap all the cyanide present in each sample into sodium hydroxide solution. The procedure is elaborated below.

3.6.2 Distillation of Cassava, Cocoyam and Fish

Approximately 40.0 g each of fish, cassava and cocoyam samples were blended with 250 mL distilled water and th mixture transferred into a 500 mL round bottom flask. To each sample in the round bottom flask was added 12.5 mL concentrated (98%) sulfuric acid and 5 mL of 2.5M magnesium chloride hexahydrate (127 g of $MgCl_2.6H_2O$ dissolved in 250 mL distilled water). 12.5 mL aliquot of 1.25M sodium hydroxide (12.5 g of solid NaOH dissolved in 250 mL

distilled water) was put into the glass collector and the delivery tube was positioned such that the tip was in contact with the sodium hydroxide solution to enable it trap the hydrogen cyanide released without any escape. The sample mixture in the round bottom flask was heated at a temperature of 100^0C in a heating mantle for an hour. The heating mantle was then turned off; the distillate cooled for 15-20 minutes, poured into a 250 ml volumetric flask and diluted to the mark with distilled water.

Blanks were prepared with the same procedure without the sample. The samples were kept in the freezer to await the final determination using the Skalar San ++ Automated Wet Chemistry Analyzer.

3.6.3 Determination of Cyanide using the Skalar Methods

The ISO 14403 method was adopted in the determination of both free and total cyanide using the Skalar San ++ automated wet chemistry analyzer which has the detection limit of 0.03 mg/L. The specific steps involved in the method are detailed under the principle of operation.

3.6.4 The Principle of the Operation of the Analyzer for the Determination of Total Cyanide

The automated procedure for the determination of total cyanide is based on the following reaction; complex bound cyanide is decomposed in a continuously flowing stream at pH of 3.8 by the effect UV light. A UV-B light lamp (312 nm) and decomposition spiral of borosilicate glass is used to filter out UV light with a wavelength less than 290 nm thus preventing the conversion of thiocyanate in cyanide. The hydrogen cyanide present at pH of 3.8 is separated by in-line distillation at 125^oC under vacuum. The hydrogen cyanide produced react with

chloramine-T leading to the formation of cyanogens chloride. This reacts with 4-pyridine carboxylic acid and 1, 3-dimethyl barbituric acid to give a red colour which has a maximum absorbance at wavelength of 600 nm.

3.7 PREPARATION OF REAGENTS

The reagents involved are outlined below alongside their preparation

3.7.1 Distillation Reagent

The distillation reagent used was prepared from citric acid ($C_6H_8O_7.H_2O$), 2.5M NaOH and distilled water. 5 g of Citric acid was dissolved in 700 mL distilled water and120 mL of 2.5M NaOH solution added. The solution was brought to a pH of 3.8 using 1M HCl or NaOH and4made to 1liter mark with distilled water.

3.7.2 Buffer Solution PH 5.2

The buffer solution used was prepared from NaOH, potassium phthalate ($C_8H_5KO_4$), Brij 35 (30%) and distilled water. 2.3 g of NaOH and 20.5 g of potassium phthalate ($C_8H_5KO_4$) were dissolved in 975 mL distilled water and the pH adjusted to 5.2 using 1M HCl. 1 mL of Brij 35 (30%) was then added to the solution and made to one litter mark with distilled water.

3.7.3 Chloramines-T Solution

The chemical used was Chloramine-T ($C_7H_7ClNNaO_2S.3H_2O$) and distilled water. It was prepared by dissolving 2.0 g of Chloramine-T in 1litter of distilled water.

3.7.4 Colour Reagent

The chemicals used were NaOH, 1,3- dimethylbarbituric acid ($C_6H_8O_3N_2$), 4-pyridine carboxylic acid ($C_6H_5NO_2$) and distilled water. The preparation was done by dissolving 7.0 g of NaOH, 16.8 g of 1,3- dimethylbarbituric acid and 13.6 g of 4-pyridine carboxylic acid in 975mL of distilled water. The solution was then stirred for an hour and the pH of the solution was then adjusted to 5.2 using 1M HCl or 1M NaOH. It was then filled to 1litter mark with distilled water and filtered over a pleated filter.

3.7.5 Sodium Hydroxide Solutions

Concentrations of 0.01, 0.1 and 1M Sodium Hydroxide solutions were prepared by dissolving 400 mg, 4 g and 40 g of NaOH in 1Liter of distilled water respectively.

3.7.6 Calibration Standard (Working Standard)

A stock of 1000 ppm CN solution was prepared from Potassium Cyanide (KCN). This solution was scaled down to six different concentrations by the Auto-sampler and used to calibrate the equipment. This was prepared by dissolving 2.5028 g KCN and 400 mg NaOH in 1Liter of distilled water.

3.8 DETERMINATION OF FREE CYANIDE

Free cyanide consists of the ionized cyanide (CN^-) and hydrogen cyanide. The principle underlining the determination is detailed below.

3.9 THE PRINCIPLE OF THE METHOD

The automated procedure for the determination of free cyanide is based on the following reactions; a zinc sulfate solution is added to the sample flow so any iron cyanide present are precipitate as the zinc cyanoferrate complex. The hydrogen cyanide present at pH of 3.8 is separated by in-line distillation at 125^oC under vacuum. The hydrogen cyanide is then determined photometrically. The photometric determination is based on the reaction of cyanide with chloramine-T leading to the formation of cyanogen chloride. This reacts with 4-pyridine carboxylic acid and 1, 3-dimethylbarbituric acid to give a red colour which has maximum absorbance at wavelength of 600 nm.

3.10 PREPARATION OF REAGENTS FOR FREE CYANIDE DETERMINATION

This method uses the same reagents as the Total cyanide except Zinc sulfate solution.

3.10.1 Zinc Sulfate Solution

The zinc sulfate solution used was prepared from zinc sulfate (ZnSO4.7H20), HCl (32%) and distilled water. The preparation was done by dissolving 10 g of zinc sulfate in 700mL of distilled

water. 10 mL of HCl was added, mixed and the solution made up to 1litter mark with distilled water.

3.11 OPERATION OF THE INSTRUMENT

Plate 3.2 Skalar San ++ Automated Wet Chemistry Analyzer

The Skalar instrument, plate 3.2, consists of three main parts which are the autosampler (where the samples are picked into the chemistry section), the chemistry section (where the analysis take place) and the monitor (which presents the result). The Skalar instrument is able to run the determination from start to finish without the users attendance. Hence the samples (water and distillates) were fixed in the sample positions in the racks demarcated on the sampler. Standard positions were as well indicated on the sampler where the auto-sampler prepared standards for the determination by sucking the 25 ppm working standard solution (prepared by dissolving 25 ml of 1000 ppm in 1L distilled water) and diluting them into 0.0, 0.5, 1.0, 1.5, 2.0 and 2.5 ppm.

With this done, the calibration curve was plotted by the instrument itself and displayed on the computer. The auto-sampler began the analysis by picking samples one after the other according to the arrangements of the samples with each sample separated by air from the other. The continuous flow technique offered a continuous stream of samples divided by air bubbles into discrete segments in which chemical reactions occured. The chemical reactions occurred between the samples and the reagents in the chemistry unit of the instrument. In the chemistry unit, each sample is distilled at 125^{o}C under vacuum to release the cyanide at pH of 3.8 in the form of hydrogen cyanide. The hydrogen cyanide produced reacts with chloramine-T leading to the formation of cyanogens chloride:

$$CH_3C_6H_4SO_2NClNa + CN^- + 2H_2O \rightarrow CH_3C_6H_4SO_2NH_2 + CNCl + Na^+ + 2OH^-$$

This reacts with 4-pyridine carboxylic acid and 1, 3-dimethyl barbituric acid to give a red colour. This product moves into the photometric detector for the determination of cyanide concentration present in each sample at 600 nm. The results were displayed on the computer. The determination was repeated for each sample twice and their means taken.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

This chapter presents the results obtained for the physicochemical parameters determined in water, fish cassava and cocoyam and their cyanide concentrations. The interpretations of the findings are also discussed.

4.1 RECOVERY

The reliability of the instrument was obtained by performing the recovery of 0.5, 1.0, 1.5, 2.0 and 2.5 ppm standard cyanide solutions. 250 mL of each standard was taken through the Skalar methods as the liquid samples described above. The recovery rate was then determined.

Table 4.1 Recovery of Free Cyanide Concentration in the Recovery Studies. Average Recovery=99.9

Concentration	Amount recovered in mg/L				Percentage
In mg/L	**1**	**2**	**3**	Mean	recovered
0.50	0.50	0.51	0.50	0.50±0.01	100.0
1.00	1.00	1.00	1.00	1.00±0.00	100.0
1.50	1.51	1.50	1.49	1.50±0.01	100.0
2.00	2.00	1.99	1.98	1.99±0.01	99.5
2.50	2.49	2.50	2.51	2.50±0.01	100.0

Table 4.2 Recovery of Free Cyanide Concentration in the Recovery Studies Average Recovery=100

Concentration	Amount recovered in mg/L				Percentage
In mg/L	**1**	**2**	**3**	Mean	recovered
0.50	0.50	0.49	0.50	0.50±0.01	100.0
1.00	1.01	1.01	1.00	1.00±0.01	100.0
1.50	1.53	1.49	1.50	1.50±0.02	100.0
2.00	1.97	2.01	2.02	2.00±0.02	100.0
2.50	2.49	2.50	2.49	2.50±0.01	100.0

4.2 STATISTICAL ANALYSIS

The results recorded were analyzed using ANOVA with the aid of MINI TAB. Least significant different (LSD) was determined using the turkey method to determine the differences between the means of the measured parameters.

4.3 WATER SAMPLES FROM THE DAM (SURFACE WATER) AND BOREHOLE (GROUND WATER)

The water samples consist of dam (surface water) and borehole (ground water) samples. Tables 4.3 and 4.4 show the results of the samples below.

Table 4.3 The result from the analysis of water samples from the dam (surface water) and SW1 and SW2 serving as controls.

Sample	pH	Cond. (μS/cm)	Temp (^{0}C)	TDS (mg/L)	TS (%)	CN^- Conc. in mg /L	Total CN^- Conc. in mg/L
Dam 1	5.88±0.03^d	222.7±5.42	30.0±0.2	65±4.0	525±10.9	0.17±0.02	22.25±0.02
Dam2	6.21±0.01^b	214.2±7.12	30.8±0.1	63±2.1	611±15.4	0.11±0.01	22.13±0.03
Dam3	6.20±0.02^b	210.0±5.42	30.2±0.1	61±4.0	571±12.4	0.12±0.02	22.30±0.27
Dam4	4.11±0.01^e	274.7±6.00	30.5±0.2	69±3.0	634±15.7	0.21±0.02	21.71±0.03
Dam5	6.02±0.01^c	117.1±6.12	30.1±0.1	54±5.0	514±10.6	0.15±0.01	21.47±0.05
Dam6	5.95±0.04^{c,d}	111.5±7.10	30.3±0.3	52±3.0	504±12.4	0.14±0.01	21.54±0.04
Dam7	3.98±0.02^f	301.5±10.41	30.5±0.1	70±5.0	617±8.51	0.22±0.02	20.52±0.09
SW 1	6.96±0.02^a	119.5±5.70	22.7±0.2	55±4.0	245±14.5	0.09±0.01	12.38±0.01
SW2	6.99±0.01^a	118.9±8.42	22.1±0.2	52±2.0	224±8.7	0.10±0.01	13.57±0.02

4.4 The result from the analysis of water samples from the borehole (ground water) and Cw1 and Cw2 serve as controls.

Sample	pH	Cond. (µS/cm)	Temp (^{0}C)	TDS (mg/L)	TS (%)	CN^- Conc. in mg /L	Total CN^- Conc. in mg/L
BH1	5.97±0.04	298.0±10.6	26.9±0.1	65±0.1	401±8.4	0.23±0.02	26.07±0.02
BH2	6.31±0.06	261.5±9.01	26.4±0.0	63±0.5	355±6.2	0.17±0.02	24.43±0.98
BH3	6.38±0.02	221.1±10.2	26.7±0.1	58±0.4	445±8.4	0.16±0.01	27.05±1.17
BH4	6.80±0.02	170.2±6.40	26.0±0.0	51±0.2	330±4.6	0.16±0.02	25.57±0.02
BH5	6.72±0.07	172.0±5.00	26.3±0.1	55±0.4	505±7.1	0.17±0.07	25.55±0.40
BH6	6.93±0.01	202.3±5.12	26.4±0.1	60±0.5	321±9.1	0.19±0.07	24.15±0.08
BH7	7.02±0.03	116.8±8.41	26.8±0.1	49±0.4	312±4.3	0.15±0.07	22.82±0.03
Cw1	7.05±0.01	109.0±5.10	26.2±0.2	24±0.3	241±4.1	0.09±0.01	3.13±0.010
Cw2	7.21±0.01	88.2±4.21	26.1±0.1	26±0.1	235±5.0	0.09±0.00	2.96±0.020

4.3.1 The pH of the Water Samples (Surface and Ground Water)

The pH value of the samples ranged from 3.98 to 6.21 which fall within the acidic region. Four out of the seven samples from the dam had low pH values (below pH 6) with the other three (dam2, dam3 and dam5) having pH values of 6.21, 6.20 and 6.02 respectively. The control samples from Hwidiem, SW1 and SW2 gave pH values of 6.96 and 6.99 respectively, which were almost neutral. It can be observed that the pH differ from one part of the dam to the other. This could be explained based on the fact that the water is contained and for that matter, do not mix properly. Nonetheless the pH values indicated that the dam samples were acidic. An increase

in acidity increases the dissociation of cyanide compounds and this increases free cyanide concentrations in the water (Meeussen *et al*., 1994). This explains why samples with the lowest pH values recorded the highest free cyanide concentrations. The pH values of the borehole samples ranged from 5.97 to 7.02. The borehole samples are less acidic in comparison with the dam samples and this could be why generally the free cyanide concentrations were high because high pH values decrease the extent of dissociation of the cyanide complexes which lower the chances for the free cyanide to be released into the water. The more easily it is for the free cyanide to be released into the water the more volatile it becomes and hence decrease its concentration in the water (Biotox, 1998). Therefore, the free cyanides are held in complexes until the distillation process where it is released and trapped. The pH of the control samples (Cw1 and Cw2) were also within the neutral point, far above the acidic medium and yet recorded the lowest cyanide concentration values.

There was significant difference ($p < 0.05$) among all the pH values for the dam samples. The borehole samples and their corresponding pH values also showed significant difference. Comparing the pH values of both borehole and dam samples showed results that differed significantly (appendix).

4.3.2 Conductivity of the Water Samples

Conductivity depends on the presence of ions, their total concentration, mobility, valence and relative concentrations and on the temperature of the measurement (APHA, 1998). High conductivity corresponds to high concentration of ions in solution. The conductivity values for the dam samples are within wider range than those of the borehole samples thus: from 111.5 to 301.5µS/cm for the dam samples whereas those of the boreholes are within 116.8 and 298.0µS/cm. This shows that the ions in the dam samples move more freely than those in the

borehole samples because of their complex nature. From the result (Tables 4.3 and 4.4), there is no clear relationship between the conductivity values which could mean that the conductivity values cannot be attributed to the presence of cyanide ions alone since cyanide is generally not a major ion in water (Mahimairaja *et al.*, 2005) but all other ions like chlorides, nitrate, sulphates, phosphates, sodium, magnesium, ammonium, potassium, bicarbonate, calcium and iron present in the water could contribute it. However, it can be concluded in general that, the higher conductivity values for these samples are due to the higher concentrations of the cyanide ions present comparing the conductivity values and the cyanide concentrations recorded for the control samples. The conductivity values were significantly different ($p<0.05$) (appendix).

4.3.3 Total Dissolved Solids (TDS) of Water Samples

Total dissolved solids contribute to the conductivity levels of samples. The total dissolved solids values increase from 52 to 70% for the dam samples and those of the borehole samples increase from 51 to 65%. Even though there is this common trend linking an increase in total dissolved solids to increasing values of cyanide concentrations yet it cannot be ascertained that these levels of total dissolved solids are due to cyanide ions present alone. The total dissolved solids of the control samples of the dam, SW1 and SW2 are 55 and 52% respectively which are higher than those of the control samples for the borehole samples, thus 24 and 26% respectively. This suggest that surface water contain higher levels of total dissolved solids than ground water probably because it is more exposed to the environment and also dissociation probability due to high pH and increase in temperature.

4.3.4 Free Cyanide Levels in the Water Samples

According to data available, the acceptable free cyanide level for drinking water is 0.2 mg/L (USEPA, 1994). The free cyanide levels of the dam samples range from 0.11 to 0.22 mg/L (Table 4.3). With the exception of dam 4 (0.21 mg/L) and dam 7 (0.22 mg/L), all the cyanide levels of the other dam samples are below the acceptable level. On the part of the borehole samples, the cyanide levels ranged from 0.15 to 0.23 mg/L (Table 4.4). These results also appear to fall below the acceptable level excluding BH1 which is even higher than all cyanide levels of the dam samples. Averagely the cyanide levels of the borehole samples appear to be higher than those of the dam samples. This could be explained by the fact that cyanide may leach into the subsurface ground water and hence causing an increase in the cyanide concentration. More so the borehole sample (BH1) that recorded 0.23mg/l is located closer to the dam far more than the rest and this can also account for the higher levels. The presence of alkali metals and other metals in the soil facilitate the formation of metallocyanide compounds which do not easily volatilize due to their stability (APHA, 1998). The free cyanide levels of the control samples, SW1 and SW2 are averagely greater than those of Cw1 and Cw2 but are all lower than the results of all the samples and fall far below the acceptable level. There was significant difference ($p<0.05$) in free cyanide levels among all the water samples (appendix).

4.3.5 Total Cyanide Levels in the Water Samples

Candidly, there is no any written evidence stating the acceptable level of total cyanide level in water. It seems that the necessity to determine this acceptable level has not been a serious issue over the years till now probably because total cyanide in nature is not that poisonous compared to free cyanide. However, total cyanide only comprises of 8-12% of the free cyanide (FAO, 1998). Hence the results obtained cannot be compared with any standard. Total cyanide

comprises of all forms of cyanide including complexes hence their results are higher than those of the free cyanide. The total cyanide level of the dam samples ranged from 20. 52 to 22.30 whereas those of the borehole samples are between 22.82 mg/L and 27.05 mg/L (Tables 4.3 & 4.4). Significantly, the result of the borehole samples are higher than those of the dam samples probably because of the complex nature of the cyanide species in the water. The results of the control samples (Tables 4.3 & 4.4) were very low compared to those of both (dam and borehole) samples. There was also a significant difference between the control samples of the dam samples (SW1 and SW2) and those of the borehole samples (Cw1 and Cw2). There was significant difference ($p<0.05$) in total cyanide level among all the water samples (appendix).

4.4 FISH SAMPLES

The table below shows the result of fish samples in terms of pH, conductivity, free cyanide concentration and total cyanide concentration.

Table 4.5 The result from the analysis of fish samples and fish C serving as control.

sample	pH	Cond. (µS/cm)	CN^- Conc. in mg /kg	Total CN^- Conc. in mg/kg
Fish 1	6.88±0.01	201.2±5.00	59.81±4.10	79.44±4.20
Fish 2	6.87±0.02	215.8±9.20	61.50±2.04	103.44±5.12
Fish 3	6.69±0.01	117.4±4.64	71.69±2.74	110.56±4.00
Fish 4	7.14±0.01	119.0±9.00	55.81±4.50	84.31±2.12
Fish 5	6.90±0.02	114.8±6.57	66.63±3.24	90.25±3.11
Fish C	7.22±0.01	111.0±5.41	<0.03±0.01	<0.03±0.01

4.4.1 pH values of Fish Samples

The pH values of the fish samples ranged from 6.69 to 7.14 which showed a inverse relationship between the pH values and the cyanide levels. Conversely, the control sample (fish C) was pH of 7.22 but had both free and total cyanide levels below detection limit. The pH of a fish is influenced by its environment. When the pH is too low, it becomes lethal to the fish (Ikuta *et al.*, 1992). In that case, the fish has to regulate its acid-base balance in order to maintain normal pH. Consequently chloride cells in the gill tissue take up HCO_3^- from the outside to neutralize the hydrogen (H+) ion flowing in the body (Ikuta *et al.*, 1999). Now there is a high possibility for the fish to rather take up CN- ions if the immediate surroundings of the fish is contaminated and

predominated with cyanide ions rather than HCO_3^- since they are all anionic compound with the same charge that is capable of neutralizing the excess hydrogen ions. As a result, cyanide is introduced and accumulated in the fish. Under typical conditions in natural waters (pH 6 to 8.5 and 4 to 10°C), over 90% of cyanide is in the form of molecular HCN (APHA, 1998). The pH values for all the fish samples were significantly different ($p<0.05$) (appendix).

4.4.2 Conductivity of Fish Samples

The conductivity values increased from 111.8 to 215.8µS/cm. Specific conductance (µS/cm) increases with increasing concentrations of total dissolved solids (Lind, 1979). These parameters in the fish are highly affected by the conductivity and total dissolved solids of the water in which the fish habit. The two major factors influencing the composition of ions are geology and precipitation/evaporation ratios. Common ions in freshwater include bicarbonate, sulfate, calcium, sodium, and silica (Weber-Scannell and Duffey, 2007). Changes can occur from a variety of anthropogenic sources including industry and resource extraction such as mining and gas well development (Fillo *et al*., 1992) and this may lead to an increase in conductivity. There were also significant difference ($p<0.05$) among the conductivity values.

4.4.3 Cyanide Levels in Fish Samples

The free cyanide levels ranged from 55.81 to71.69 mg/kg. Free cyanide is acutely and almost instantaneously poisonous to living organisms, including humans. Once in the body, it blocks the ingestion of oxygen by cells. Fish are about one thousand times more sensitive to cyanide than humans (Ikuta *et al*., 1999).

Exposure to sub lethal concentrations of copper cyanide (0.253 and 0.152 mg/L), caused significant dose dependent alteration in the nitrogenous, as well as carbohydrate metabolism of

the fish *C. catla* in all the tissues (Hosetti, 2011). Exposure of 12.5 to 50mg/l levels of cyanide led to the mortality of some fish in 60 s (Vaz *et al*, 2012). It must be noted that, the effect of cyanide on fish depends on the cyanide solution during exposure, the length of time of the exposure and the size of the specie (Bruckner and Robert, 2008). Fish like humans have a way of eliminating cyanide from the body. They do so by after accumulating this undesirable substance, they slowly convert it to SCN and rapidly excrete. (Bruckner and Robert, 2008). On the other hand, total cyanide remains inactive in the body because of its complex nature until it is excreted and so therefore do not harm the organism. Hence higher levels can be found in the fish and yet may not kill it as seen in the result. There were significant difference ($p<0.05$) among the cyanide levels (appendix).

4.5 CASSAVA

The table below shows the result of cassava samples in terms of pH, conductivity, moisture content, tree cyanide concentration and total cyanide concentration**.**

Table 4.6 The result from the analysis of cassava samples CwC serving as control

Sample	pH	Cond. (μS/cm)	Moisture content (%)	CN^- Conc. in mg /kg	Total CN^- Conc. in mg/kg
CvF1(I)	6.65±0.02	231.0±8.43	53.90±0.80	16.63±0.68	17.13±0.07
CvF1(II)	6.11±0.04	213.8±6.42	56.30±0.52	50.44±3.14	51.38±4.17
CvF1(III)	6.88±0.02	113.5±5.24	54.60±0.60	05.25±0.02	08.06±0.01
CvF1(IV)	6.55±0.01	118.7±5.00	53.30±0.45	15.75±0.17	16.25±0.07
CvF2(I)	6.76±0.02	118.2±6.24	55.90±0.60	09.19±0.07	09.94±0.06
CvF2(II)	6.20±0.01	191.6±6.27	54.10±0.95	44.56±0.85	49.00±2.15
CvF2(III)	6.67±0.01	199.4±7.20	59.20±1.52	10.31±0.12	10.63±0.07
CvF2(IV)	6.77±0.01	94.3±4.02	54.90±2.00	06.25±0.04	06.75±0.03
CvF3(I)	6.33±0.01	120.9±7.07	52.80±0.95	34.80±1.24	35.81±1.20
CvF3(II)	6.05±0.03	114.5±5.80	53.00±0.83	86.50±4.85	123.63±5.14
CvF3(III)	5.92±0.02	187.2±6.17	53.40±0.74	93.88±5.14	165.56±6.89
CvF3(IV)	6.01±0.02	182.0±6.24	55.80±1.02	88.38±5.23	128.63±8.12
±CvF4(I)	5.96±0.03	134.3±5.67	55.50±1.10	93.56±4.20	132.63±10.1
CvF4(II)	6.14±0.02	209.5±8.72	58.00±1.20	37.31±1.52	39.19±4.24
CvF4(III)	6.84±0.01	239.5±6.27	54.80±0.67	09.63±0.03	10.69±0.09
CvF4(IV)	6.89±0.01	130.4±5.00	51.20±0.88	06.19±0.04	06.56±0.03

CvF5(I)	6.72±0.01	114.7±5.42	55.40±1.24	11.06±0.05	11.19±0.10
CvF5(II)	6.86±0.02	164.9±7.87	54.30±0.65	09.75±0.07	10.25±0.04
CvF5(III)	6.74±0.04	132.8±10.41	52.40±1.70	11.69±1.12	11.88±0.08
CvF5(IV)	6.24±0.04	125.3±8.07	58.42±1.34	24.06±1.54	24.31±0.18
CvC	7.01±0.02	111.2±4.20	55.80±0.97	4.75±0.02	4.94±0.03

4.5.1 pH of Cassava Samples

The cassava samples appeared to be slightly acidic with their Ph values ranging from 5.92 to 6.89 (Table 4.6). This pH region favours the hydrolysis of the cyanogenic glycosides (dorminated by linamarin and lotaustralin) present to hydrocyanic acid (HCN) catalysed by endogeneous linamarase (Ayodeji, 2005). This increases the free cyanide concentration in the cassava samples. However, it may subsequently decrease the free cyanide concentration in a very short time since free cyanide easily volatilizes at higher temperatures. Distinctively, the pH of the control sample (CvC) was 7.01 indicating its neutrality. There were significant difference ($p<0.05$) among all the pH values (appendix).

4.5.2 Conductivity and Moisture Content of the Cassava Samples

The conductivity values of the cassava samples fall within 113 .5 and 239.5 µS/cm (Table 4.6). These values do not correlate with the concentration of cyanide present in the samples probably because cyanide might not be the only ions responsible for the conductivity value as explained above for the water samples. A high moisture content ensures easy distribution and movement of substances (free cyanide) within the cassava and the surrounding soil. The moisture content also ranged from 51.20 to 59.20% (Table 4.6). The conductivity values were not significantly different ($p<0.05$) from each other (appendix).

4.5.3 Cyanide Levels in Cassava Samples

The free cyanide levels in the cassava samples ranged from 5.25 to 93.88 mg/kg (Table 4.6). Considering the range, 70% of cassava samples contain cyanide levels far above the set limit which is 10 mg/kg (Baskin, 1992). The implication here is that, the cassava samples with the cyanide levels above the set limit could pose serious health effect to any human or animal that may feed on it. These high levels of cyanide in the cassava may result from cyanide contamination of soil, Soil nature and climate conditions. The total cyanide levels also ranged from 6.75 to 165.56 mg/kg (Table 4.6). In comparison, the free cyanide levels were slightly lower than the total cyanide levels. This is an expected result because total cyanide should comprise all forms of cyanide including free cyanide and so therefore even a wider disparity is expected. Thus, the slight difference recored here can be attributed to the process through which the analysis was done. Strong acid together with heat was used in the distillation process hence almost all the cyanide complexes were converted to free cyanide. The only few left not converted added up to the free cyanide to give the total cyanide. The control sample (CvC) recorded lower values for both free and total cyanides. Both free and total cyanide levels differed significantly ($p<0.05$) (appendix).

4.6 COCOYAM

The table below (Table 4.7) shows the result of the cocoyam samples in terms of pH, conductivity, moisture content, free and total cyanide concentrations.

Table 4.7 the result from the analysis of cocoyam samples and CyC serve as control.

Sample	pH	Cond. (µS/cm)	Moisture content (%)	CN^- Conc. in mg /kg	Total CN^- Conc. in mg/kg
CyF1(I)	6.64±0.01	112.7±3.24	49.70±0.94	34.56±0.73	47.19±0.85
CyF1(II)	6.52±0.01	64.3±1.14	46.50±0.56	13.44±0.65	33.69±0.72
CyF1(III)	6.59±0.04	81.4±4.20	47.501±0.87	25.38±0.87	48.38±0.40
CyF1(IV)	6.71±0.02	101.3±6.32	44.30±1.50.	18.13±0.53	40.00±0.23
CyF2(I)	6.99±0.02	98.2±6.12	47.10±0.87	12.75±0.24	28.44±0.35
CyF2(II)	6.97±0.01	78.2±3.12	48.40±1.68	11.81±0.41	29.88±0.61
CyF2(III)	7.01±0.04	121.0±4.12	50.20±0.40	04.50±0.03	30.19±0.45
CyF2(IV)	6.88±0.05	34.3±0.07	46.60±0.60	16.25±0.06	25.75±0.74
CyF3(I)	6.59±0.03	64.6±3.00	46.20±1.35	22.63±0.85	36.31±0.12
CyF3(II)	6.66±0.04	77.6±3.40	48.40±1.35	31.38±1.12	59.00±0.57
CyF3(III)	6.50±0.06	59.0±1.40	52.30±0.90	27.75±0.98	43.63±0.37
CyF3(IV)	6.53±0.05	84.2±4.21	47.10±1.42	25.50±0.65	39.44±0.42
CyF4(I)	7.21±0.04	74.1±2.40	45.30±0.65	00.75±0.01	11.13±0.61
CyF4(II)	7.03±0.01	44.9±0.71	56.37±0.74	10.63±0.07	14.63±0.10
CyF4(III)	6.95±0.01	66.5±3.17	43.10±1.70	14.63±0.24	24.63±0.42
CyF4(IV)	6.85±0.03	79.2±3.14	50.10±0.68	13.13±0.45	17.94±0.35
CyF5(I)	6.91±0.01	125.9±6.14	62.00±1.68	06.38±0.03	18.63±0.71

CyF5(II)	6.95±0.04	47.9±2.14	54.90±0.90	06.13±0.03	13.25±0.35
CyF5(III)	6.93±0.06	55.8±2.01	56.70±1.35	06.19±0.03	14.06±0.76
CyF5(IV)	6.89±0.02	49.4±0.45	53.70±0.64	06.88±0.04	16.31±0.45
CyC	6.99±0.04	50.9±1.54	59.10±0.50	0.500±0.01	00.69±0.01

4.6.1 pH values of Cocoyam Samples

The pH values of the cocoyam samples ranged from 6.50 to 7.21 (Table 4.7). The pH values here showed a direct variation of cyanide concentrations in the samples. Cocoyam like any cyanide contained plant (cassava for instance) accumulate cyanide in the form of cyanogenic glycosides. The reactions of these glycosides are affected by pH. And some pH values recorded here fall within the optimum range within which cyanide can easily be released or remain in the complex form. There were significant difference ($p<0.05$) among all the pH values (appendix).

4.6.2 Conductivity and Moisture Content of Cocoyam Samples

The conductivity values begin from 34.3 to 125.9µS/cm whereas the moisture content increase from 43.10 to 62.00% (Table 4.7). these two parameters do not have a direct link to the concentration of cyanide in the sample. There were significant difference ($p<0.05$) among both the conductivity and the moisture content values (appendix).

4.6.3 Cyanide Levels in Cocoyam Samples

The free and total cyanide concentrations ranged from 0.75 to 34.56 and 11.13 to 59.00 mg/kg respectively. The ledal dose of hydrogen cyanide in cocoyam was was reported to be 35.0 mg/kg (Goesaert *et al.*, 2005). This gives cocoyam a safe margin compared with cassava. According to Anhwange (2011), cocoyam recorded hydrogen cyanide level of 34.10mg/kg which is the least

result among those of cassava and yam. Echendu *et al*., (2009) also recorded 0.87 mg/kg as the highest cyanide level in cocoyam among plantain, yam and sweet potato. Abdulrashid and Agwunobi (2009), Olajide *et al*., (2011) reported wide variation (2.10 – 17.13mg/100g) in the level of cyanide in cocoyam. A study reported in October, 2012 by Uhegbu *et al* showed higher levels of cyanide (15.19±1.61 mg/kg) of cocoyam grown on a cassava effluent waste water contaminated soil. Aside all these levels, literature is somehow silent on stating a well established limit value for cyanide content in cocoyam. This notwithstanding, one thing is clear which is that plants take up cyanide from soils polluted by organic salts containing cyanide ion or cyanides from industrial wastes as reported by El-Sharkang (2004). The cyanide levels of these root tubers may not pose any considerable danger to consumers since their average cyanide content (Table 4.7) is still below the estimated maximum sub-lethal dose of 20 mgHCN/kg (WHO, 2001). Both free and total cyanide levels showed significant difference ($p<0.05$) (appendix).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

This study was carried out to determine the free and total cyanide concentrations in water, fish, cassava and cocoyam samples which were collected from Kenyase in the Brong Ahafo region, a mining community. pH, conductivity, total dissolved solids and total suspended solids were determined.

Based on the results obtained from the study and the subsequent discussions made, the following conclusions can be established:

- The free cyanide levels found in the dam samples were below the set limit (0.2 mg/L for drinking water) except two results; 0.21 and 0.22 mgCN/L that were slightly above the set limit. These samples are not safe for drinking.
- With the exception of the result of one borehole sample, BH1, the free cyanide levels in the rest of the samples were below the set limit and the therefore can be recommended for drinking.
- The results from the control samples were all generally lower than those of all the samples with the control fish recording result below detection limit.
- The total cyanide levels recorded from each determination were all higher than levels recorded for the free cyanide.
- There was an inverse relationship between the pH values and the cyanide levels in all the samples including the control samples. The other parameters like conductivity, total dissolved solids and total suspended solids were relating very well with the cyanide contents in the samples.

- Among all the solid samples, cassava recorded the highest cyanide level followed by the fish and the cocoyam containing the least. Apart from the results from the fish samples, only few of samples of cassava and cocoyam recorded levels higher than the set limit in food. These few samples were collected from farms closer to the dam than the rest hence such samples are not safe for consumption.

5.2 RECOMMENDATION

Based on the research findings it can be recommended that:

- Boreholes that are located very close to the dam should be banned from being used.
- Further determinations should be extended to other crops like plantain, cocoa, pepper, tomatoes, cabbage which are very common in the area and also to farms located a little farther away from the dam to ascertain the level of contamination of the cyanide.
- People are advise to properly cook their foodstuffs especially cassava and cocoyam no matter where they are coming from in order to reduce the cyanide content that may be present in them. High temperature is an optimum condition for the breakdown of cyanide complexes to release hydrogen cyanide.
- Similar study should be carried out in all mining towns including galamsey operating areas so as to make known the extent to which the environment is being polluted with such poisonous substances.
- Further investigations into the level of exposure of consumers and the systemic bio-availability of the cyanide upon consumption of these foods and water and the short and long term toxicological implications and impact on consumers' health are recommended among the population.

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APPENDIX

1. Dam Samples

One-way ANOVA: pH versus sample

Source	DF	SS	MS	F	P
sample	8	27.77976	3.47247	2473.79	0.000
Error	18	0.02527	0.00140		
Total	26	27.80503			

S = 0.03747 R-Sq = 99.91% R-Sq(adj) = 99.87%

Grouping Information Using Tukey Method

sample	N	Mean	Grouping
SW2	3	6.9867	A
SW1	3	6.9633	A
D2.	3	6.2100	B
D3	3	6.1433	B
D5	3	6.0267	C
D6	3	5.9500	C D
D1	3	5.8767	D

Means that do not share a letter are significantly different.

One-way ANOVA: cond versus sample

Source	DF	SS	MS	F	P
sample	8	128864.2	16108.0	19405.10	0.000
Error	18	14.9	0.8		
Total	26	128879.2			

S = 0.9111 R-Sq = 99.99% R-Sq(adj) = 99.98%

Grouping Information Using Tukey Method

sample	N	Mean	Grouping
D7	3	302.307	A
D4	3	274.210	B
D1	3	222.293	C
D2	3	214.997	D
D3	3	211.987	E
SW1	3	119.127	F
SW2	3	118.767	F

One-way ANOVA: Conductivity versus Sample

Source	DF	SS	MS	F	P
sample	8	128864.2	16108.0	19405.10	0.000
Error	18	14.9	0.8		
Total	26	128879.2			

sample	N	Mean	Grouping
D7	3	302.307	A
D4	3	274.210	B
D1	3	222.293	C
D2	3	214.997	D

D3	3	211.987	E
SW1	3	119.127	F
SW2	3	118.767	F
D5	3	118.723	F
D6	3	112.890	G

One-way ANOVA: FCN versus sample

Source	DF	SS	MS	F	P
sample	8	0.056719	0.007090	40.73	0.000
Error	18	0.003133	0.000174		
Total	26	0.059852			

S = 0.01319 R-Sq = 94.76% R-Sq(adj) = 92.44%

Grouping Information Using Tukey Method

sample	N	Mean	Grouping
D7	3	0.22333	A
D4	3	0.20667	A B
D1	3	0.17333	B C
D5	3	0.15000	C D
D6	3	0.13667	C D E
D3	3	0.11667	D E F
D2	3	0.11000	E F

One-way ANOVA: TCN versus sample

Source	DF	SS	MS	F	P
sample	8	364.1560	45.5195	4622.14	0.000
Error	18	0.1773	0.0098		
Total	26	364.3333			

S = 0.09924 R-Sq = 99.95% R-Sq(adj) = 99.93%

Grouping Information Using Tukey Method

sample	N	Mean	Grouping
D3	3	22.2967	A
D1	3	22.2500	A
D2	3	22.1333	A
D4	3	21.7133	B
D6	3	21.5400	B
D5	3	21.4667	B
D7	3	20.5167	C

2. Borehole Samples

One-way ANOVA: pH versus Sample

Source	DF	SS	MS	F	P
sample	8	4.004674	0.500584	2252.63	0.000
Error	18	0.004000	0.000222		
Total	26	4.008674			

S = 0.01491 R-Sq = 99.90% R-Sq(adj) = 99.86%

Grouping Information Using Tukey Method

sample	N	Mean	Grouping
CW2	3	7.2100	A
CW1	3	7.0567	B
BH7	3	7.0167	B
BH6	3	6.9300	C
BH4	3	6.8333	D
BH5	3	6.7200	E
BH3	3	6.3833	F
BH2	3	6.3067	G
BH1	3	5.9767	H

Means that do not share a letter are significantly different.

One-way ANOVA: Conductivity versus Sample

Source	DF	SS	MS	F	P
sample	8	120224.1	15028.0	12052.12	0.000
Error	18	22.4	1.2		
Total	26	120246.6			

S = 1.117 R-Sq = 99.98% R-Sq(adj) = 99.97%

sample	N	Mean	Grouping
BH1	3	297.01	A
BH2	3	262.39	B
BH3	3	221.66	C
BH6	3	203.78	D
BH4	3	172.51	E
BH5	3	172.17	E
BH7	3	117.29	F
CW1	3	109.17	G
CW2	3	88.92	H

One-way ANOVA: Temperature versus Sample

Source	DF	SS	MS	F	P
sample	8	2.12000	0.26500	79.50	0.000
Error	18	0.06000	0.00333		
Total	26	2.18000			

S = 0.05774 R-Sq = 97.25% R-Sq(adj) = 96.02%

Grouping Information Using Tukey Method

sample	N	Mean	Grouping
BH1	3	26.8667	A
BH7	3	26.7667	A
BH3	3	26.7333	A
BH2	3	26.4333	B
BH6	3	26.3667	B C
BH5	3	26.3667	B C
CW1	3	26.2333	C

One-way ANOVA: FCN versus sample

Source	DF	SS	MS	F	P
sample	8	0.046763	0.005845	56.37	0.000
Error	18	0.001867	0.000104		
Total	26	0.048630			

S = 0.01018 R-Sq = 96.16% R-Sq(adj) = 94.46%

Grouping Information Using Tukey Method

sample	N	Mean	Grouping
BH1	3	0.23333	A
BH6	3	0.18667	B
BH5	3	0.16667	B C
BH2	3	0.16667	B C
BH4	3	0.16333	B C
BH3	3	0.16000	B C
BH7	3	0.14667	C
CW1	3	0.09333	D
CW2	3	0.09000	D

One-way ANOVA: TCN versus Sample

Source	DF	SS	MS	F	P
sample	8	2319.456	289.932	1173.57	0.000
Error	18	4.447	0.247		
Total	26	2323.903			

S = 0.4970 R-Sq = 99.81% R-Sq(adj) = 99.72%

Grouping Information Using Tukey Method

sample	N	Mean	Grouping
BH3	3	27.050	A
BH1	3	26.070	A B
BH4	3	25.573	B
BH2	3	25.433	B C
BH5	3	25.220	B C
BH6	3	24.147	C D
BH7	3	22.820	D
CW1	3	3.133	E
CW2	3	2.977	E

pH values of Water Samples

One-way ANOVA: pH of Dam versus pH of Borehole

Source	DF	SS	MS	F	P
pH_2	19	27.78436	1.46233	495.31	0.000
Error	7	0.02067	0.00295		
Total	26	27.80503			

S = 0.05434 R-Sq = 99.93% R-Sq(adj) = 99.72%

Conductivity of Water Samples

One-way ANOVA: Borehole versus Dam

Source	DF	SS	MS	F	P
cond_1	25	120241.6	4809.7	957.25	0.026
Error	1	5.0	5.0		
Total	26	120246.6			

Free Cyanide of Water Samples

One-way ANOVA: Borehole versus Dam

Source	DF	SS	MS	F	P
dam	13	0.035771	0.002752	2.78	0.038
Error	13	0.012858	0.000989		
Total	26	0.048630			

S = 0.03145 R-Sq = 73.56% R-Sq(adj) = 47.12%

Total Cyanide of Water Samples

One-way ANOVA: Borehole versus Dam

Source	DF	SS	MS	F	P
dam	13	0.035771	0.002752	2.78	0.038
Error	13	0.012858	0.000989		
Total	26	0.048630			

S = 0.03145 R-Sq = 73.56% R-Sq(adj) = 47.12%

Fish Samples

One-way ANOVA: pH versus SAMPLE

Source	DF	SS	MS	F	P
SAMPLE_1	5	0.5992444	0.1198489	1268.99	0.000
Error	12	0.0011333	0.0000944		
Total	17	0.6003778			

S = 0.009718 R-Sq = 99.81% R-Sq(adj) = 99.73%

One-way ANOVA: Conductivity versus Sample

Source	DF	SS	MS	F	P
SAMPLE	5	35123.43	7024.69	7635.34	0.000
Error	12	11.04	0.92		
Total	17	35134.47			

S = 0.9592 R-Sq = 99.97% R-Sq(adj) = 99.96%

One-way ANOVA: FCN versus TCN

Source	DF	SS	MS	F	P
TCN	14	10368.59	740.61	7691.58	0.000
Error	3	0.29	0.10		
Total	17	10368.88			

S = 0.3103 R-Sq = 100.00% R-Sq(adj) = 99.98%

3. Cassava Samples

One-way ANOVA: pH versus Sample

Source	DF	SS	MS	F	P
SAMPLE	20	7.72268	0.38613	334.61	0.000
Error	42	0.04847	0.00115		
Total	62	7.77114			

S = 0.03397 R-Sq = 99.38% R-Sq(adj) = 99.08%

One-way ANOVA: Conductivity versus Moisture Content

Source	DF	SS	MS	F	P
MOIST	58	116601	2010	1.11	0.530
Error	4	7232	1808		
Total	62	123832			

S = 42.52 R-Sq = 94.16% R-Sq(adj) = 9.48%

One-way ANOVA: FCN versus TCN

Source	DF	SS	MS	F	P
TCN_1	61	60148.20	986.04	1232545.15	0.001
Error	1	0.00	0.00		
Total	62	60148.20			

S = 0.02828 R-Sq = 100.00% R-Sq(adj) = 100.00%

4. COCOYAM SAMPLES

One-way ANOVA: pH versus Sample

Source	DF	SS	MS	F	P
SAMPLE	20	2.433298	0.121665	298.24	0.000
Error	42	0.017133	0.000408		
Total	62	2.450432			

S = 0.02020 R-Sq = 99.30% R-Sq(adj) = 98.97%

One-way ANOVA: Conductivity versus Moisture Content

Source	DF	SS	MS	F	P
MC	59	37571	637	3.39	0.171
Error	3	563	188		
Total	62	38134			

S = 13.70 R-Sq = 98.52% R-Sq(adj) = 69.47

One-way ANOVA: FCN versus TCN

Source	DF	SS	MS	F	P
Factor	1	5780	5780	37.91	0.000
Error	124	18904	152		
Total	125	24684			

S = 12.35 R-Sq = 23.42% R-Sq(adj) = 22.80%