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Leaching of Cyanogens and Mycotoxins from Cultivated Cassava into Agricultural Soil: Effects on Groundwater Quality

Elie F. Itoba-Tombo, Seteno K.O. Ntwampe and
John B.N. Mudumbi

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Abstract

Cyanogens and mycotoxins are vital in protecting flora against predation. Nevertheless, their increased concentrations and by-products in agricultural soil could result in produce contamination and decreased crop yield and soil productivity. When exposed to unsuitable weather conditions, agricultural produce such as cassava is susceptible to bacterial and fungal attack, culminating in spoilage, particularly in arid and semi-arid regions, and contributing to cyanogen and mycotoxins loading of the arable land. The movement of cyanogen including mycotoxins in such soil can result in sub-surface and/or groundwater contamination, thus deteriorating the soil's environmental health and negatively affecting wildlife and humans. Persistent cyanogen and mycotoxins loading into agricultural soil changes its physico-chemical characteristics and biotic parameters. These contaminants and their biodegradation by-products can be dispersed from soil's surface and sub-surface to groundwater systems by permeation and percolation through the upper soil layer into underground water reservoirs, which can result in their exposure to humans and wildlife. Thus, an assessment and monitoring of cyanogen and mycotoxins loading impacts on arable land and groundwater in communities with minimal resources should be done. Overall, these toxicants impacts on agricultural soil's biotic community, affect soil's aggregates, functionality and lead to the soil's low productivity, cross-contamination of fresh agricultural produce.

Keywords: agricultural soil, cassava, cyanogen, groundwater, mycotoxin

1. Introduction

Cyanogens have been widely demonstrated to be an important component within the earth's system. These compounds have been reported to have an influential role in the lives of several organisms on earth [1]. Cyanogens are characterised by the presence of two elements: a carbon: nitrogen functional group held together by a triple bond (─C≡N). The simplest form, which is predominant in the environment, is hydrogen cyanide (HCN), with nitriles and cyanogenic glycosides (CGs) being other forms of these compounds [2–5]. Generally, free cyanide originates from both anthropogenic and natural processes [6]. The anthropogenic sources of cyanide range from effluents discharged from municipal wastewater treatment plants, agricultural run-off, mining activities and electroplating industries [7, 8], including the application of some cyanide containing insecticides in the agricultural industry, which culminates in environmental contamination [9]. Cyanides and CGs have also been generated in plants and agricultural produce such as *Manihot esculenta* (cassava), with the waste generated through processing of such produce contributing to the cyanide load into the environment. During cassava harvesting and processing, plant-borne hydrolases result in CGs' conversion into by-products which are released into the soil, although sometimes this is due to rot produce, a consequence of microbial contamination of the produce and wastewater generated for processing of such produce [10, 11].

As a result of produce-facilitated microbial decay due to the availability of pathogenic organisms in soil where the produce grows, mycotoxins are produced. Mycotoxins are fungal secondary metabolites that also have a negative impact on human and animal wellbeing [12–14]. They co-occur with other bacterial toxins in spoiled agricultural produce such as cassava. Previous studies on mycotoxins revealed that these compounds are hazardous to animals and humans. Generally, it has been reported that CGs as well as mycotoxins occur naturally in flora and organisms (fungi) as a result of biosynthesis, with their prevalence being quantifiable in many agricultural products, such as cassava, apples, spinach, apricots, cherries, peaches, plums, quinces, almonds, sorghum, lima beans, corn, yams, chickpeas, cashews and kirsch [15, 16]. Although some microorganisms and plants synthesise these compounds for their survival when exposed to harsh environmental conditions, their cumulative production can contribute to ecological disturbances. Furthermore, various arthropods and invertebrates were also determined to produce cyanogens as a defence mechanism and for a control of mating behaviour [17, 18], although on a minute scale, with research by Jones [19] indicating that plants including microorganisms are known to be major producers of these compounds owing to their physiology. Thus, the presence and loading of these cyanogens and mycotoxins into terrestrial ecosystems are largely overlooked, although they have some negative effects on the physico-chemical and biological properties of soil, particularly arable land as well as the environment in general [10, 20].

Previous studies have stated that cyanogen and mycotoxin loading in agricultural soil can have a serious impact, disturbing the terrestrial ecosystem functionality [10]. Current evidence suggests that most studies on agricultural produce such as cassava, known for its high cyanogen content, have predominantly focused on the production of the crop for nutritional and

industrial purposes, with its effects on soil (including the surrounding environment) overlooked [2, 10] Accordingly, minimal research has been completed on cyanogen and mycotoxin loading, including their behaviour and movement in soil that can culminate in groundwater contamination. This is because a large amount of agricultural produce, such as cassava tubers, perishes prior to harvesting for a variety of reasons. Although free cyanide and mycotoxin toxicity is widely reported, their level of toxicity is also influenced by cumulative exposure and the continuation of their release from produce into the environment. Cyanogen and mycotoxin loads and their movement in soil, including their potential to contaminate groundwater, which is used in impoverished communities where cassava is cultivated mostly as a source of protein and starch, are largely under-reported.

The highlights of this review are:

- There are similarities in the movement of cyanogens and mycotoxins, including their degradation by-products in soils due to mass transfer processes influenced by the moisture content in the soil, thus;

- Cyanogen and mycotoxins distort the soil's characteristics with seepage into groundwater systems being of paramount concern, negatively impacting terrestrial, aquatic life and water quality, thus;

- Culminate into prolonged cumulative human and animal exposure.

2. Cyanogen and mycotoxin reduction

Several methods of cyanogen reduction have been proposed and include physical, chemical and biological methods [6, 21]. However, it has been reported that some of these methods require high input costs and sophisticated knowledge and/or training to implement successful strategies for their reduction [4]. Meanwhile, scientists have embarked on intense research and simplify reduction methods for these toxicants in the environment by using techniques which are considered environmentally benign, as such novel ways of reducing both cyanogen and mycotoxin levels in the environment, including in agricultural produce destined for consumption, are generally considered cost effective when compared with long-term outcomes of none implementation of control measures [22–24].

2.1. Biological reduction of cyanogens

The biological reduction of CGs as a source of cyanide, as well as mycotoxins, has gained popularity and has been a huge research focus area [17, 22, 23]. As such, genetically modified cassava cultivars, with a suppressed cytochrome P450 gene (producers of enzymes CYP79D1 and CYP79D2) functionality, may inhibit the infiltration of linamarin as it can be converted to free cyanide from valine [25].

Furthermore, other biological treatments for free cyanide involve microorganisms; these organisms are known to be toxin producers and are organisms, such as *Pseudomonas* sp.,

Nocardia sp., *Flavobacterium* sp., *Bdellovibrio* sp., as well as nitrifiers, such as *Nitrosomonas* sp., *Nitrobacter* sp., *Sphingomonas* sp., *Exophiala* sp., *Bacillus* sp., and fungi such as *Aspergillus* sp. and *Penicillium* sp. [4, 8, 22, 26–28]. Among these microorganisms, *Aspergillus* sp. and *Penicillium* sp. are the most prevalent species able to grow successfully in stringent weather conditions, with some, including *Cunninghamella* sp. being common in soil [29], with the ability to grow on a variety of agricultural produce such as maize, peanuts and tubers [30, 31].

In soil consisting of fungal biocatalysts of different origins, scientific evidence seems to indicate that agricultural produce appears to be susceptible to spoilage due to substrate availability, which results in the proliferation of microbial spoilage organisms [32, 33]. It has also been reported that fruit or produce has trace elements, such as Ca, Na, K and Zn, and low relative molecular weight hydrocarbons, including proteins and moisture, providing conditions which facilitate microbial growth and thus spoilage [34, 35]. Owing to this, some microorganisms produce hydrolases, reducing primary compounds in produce to by-products, furthering physico-chemical changes in the environment in which they are leached [30]. These seem to be the ideal conditions in which cyanide reduction biocatalysts proliferate, i.e. conditions that are nutrient rich as a result of nutrient availability from decaying produce.

Some of the cyanogens are reduced to by-products such as bicarbonate and ammonia. The ammonia formed during the process is further utilised by the microorganisms as a source of nitrogen, supporting increased microbial growth [36, 37]. In the agricultural industry, the reduction of both cyanogens and related compounds is complex, as *in-situ* quantification of such processes is minimally reported. The development of processes and strategies that are environmentally benign; i.e. of biological origin, is gaining popularity due to their simplicity and advantages, as they are considered less harmful, and can be beneficial in the economical management urged for, in the improvement of commercial agro-produce manufacturers [28, 38, 39]. Owing to the exposure to primary and by-products of cyanogen conversion/transformation, some species became tolerant, thus biologically evolve.

For example, Sing et al. [30] successfully isolated a fungus, *Cunninghamella* sp. UMAS SD12 from sawdust, with an ability to biodegrade 51.7% pentachlorophenol (PCP) within 15 days in a controlled static environment. However, more research needs to be conducted to assess direct evolvement of the microbial ecosystem, as other microorganisms that constitute a community, for the betterment of soil, can reduce such soils' viability, and/or result in some organisms producing extracellular secondary metabolites such as mycotoxins.

2.2. Biological reduction of mycotoxins

There are numerous mycotoxins known to contaminate agricultural produce such as cassava. Among these mycotoxins, fumonisin B1 and deoxynivalenol (DON) are common. The biodegradation of fumonisin B1 and deoxynivalenol (DON) can be achieved through their direct conversion using detoxification processes with different pathways [22]. For example, fumonisin biodegradation was observed through the elimination of the tricarballylate side chains and amino groups. The enzymatic hydrolysis of such mycotoxins might involve carboxylesterases and aminotransferases from bacteria such as *Sphingomonas* and *Sphingopyxis*

normally found in soil, which have the ability to detoxify recalcitrant persistent organic pollutants (PoPs) such as polycyclic aromatic hydrocarbons (PAHs) [40–43]. Other researchers have reported degradation or detoxification of fumonisin, including by-products, by oxidative deaminase from *Exophiala* sp., a common soil organism [42–44]. *Bacillus* sp., including non-Saccharomyces yeast commonly found in soil, were also suggested to destabilise these mycotoxins' structure, and thus reduce their amino acid functional groups albeit at elevated pH [45].

In most instances, the biodegradation process of most mycotoxins involves a consortium of organisms, which utilises a variety of degradation pathways [42, 44]. Overall, the initial biodegradation stage starts at extracellular level by deamination or facilitation by esterase with the last biodegradation step involving microbial/enzymatic decoupling of the aliphatic chain within the mycotoxin molecule [22]. For example, the first biodegradation steps of DON using *Curtobacterium* sp. and *Eubacterium* sp. were determined to be initiated by the de-epoxidation step which subsequently followed oxidation [22, 46].

3. Toxicity of cyanide as a cyanogen from cassava

3.1. Toxicity of *Manihot esculenta*

Worldwide, cassava is utilised as a primary foodstuff for disadvantaged and needy rural communities of Africa, Asia and South America [23, 47, 48]. Cassava's toxicity is due to cyanogens such as linamarin, lotaustralin and 2-((6-O-(b-ᴅ-apiofuranosyl)-b-ᴅ-glucopyranosyl) oxy)-2-methylbutanenitrile) that are biologically transformed into hydrogen cyanide [25, 49]. As a result of enzymatic hydrolysis, for which the linamarin from the plant tissue is transformed into acetone cyanohydrin through linamarases [3]. At an increased temperature and pH of >30°C and 5, respectively, conditions associated with arid regions which are suitable for microbial proliferation and thus agricultural produce contamination or spoilage, acetone cyanohydrin is released, resulting in its decomposition into acetone and hydrogen cyanide [3, 25] (**Figure 1**). Several studies have been done on the impact of the cultivar on humans as a result of direct ingestion [24, 48, 50], as cyanide concentration in the tuber is estimated to reach 50 mg/kg [51].
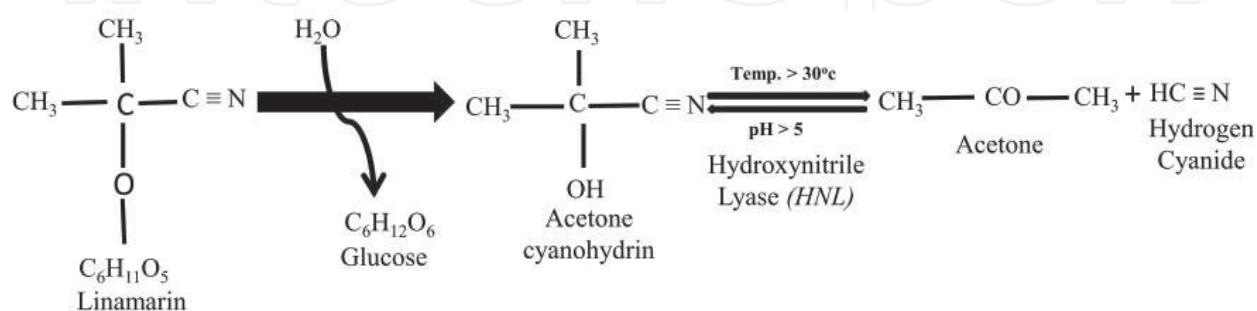

Figure 1. Enzymatic hydrolysis of linamarin to hydrogen cyanide.

Thus, its prolonged consumption may be toxic. However, there is minimal information on hydrogen cyanide loading into irrigable land in which cassava is cultivated. Free metal ions in such soil exposed to hydrogen cyanide can form metallic cyanide complexes under suitable conditions, further prolonging cyanide-based compounds' prevalence in the soil, which might leach into groundwater.

3.2. Production of mycotoxins

Terrestrial ecosystems are populated by a diversity of microorganisms that contribute to and maintain the ecological and biological balance. These organisms contribute to the characteristics of the soil that directly influence soil productivity and crop yield in the agricultural sector [52–54], although some have been shown to exhibit pathogenicity toward mature produce. For example, during the growth and up to the harvest stage of cassava tubers, several pathogenic organisms with mycotoxin-production potential can dominate several other types of bacteria and fungi on the tuber and in cassava-cultivated soils [30]. Some of these organisms are resistant even to the free cyanide in cassava, and with their inherent characteristics, such as their predisposition for survival, they produce mycotoxins such as ochratoxin A, aflatoxins, fumonisin B, pyranonigrin A, tensidol B, funalenone, naphtho-y-pyrones, deoxynivalenol (DON) and malformins [55–57]. Research revealed that exposure to mycotoxins pre/post-harvest and their presence in soil can render the cassava tubers inedible [58, 59], leading to their cumulative and increased levels due to sustained use of pre-recovery land for cultivation to produce an essential food source—a method that will affect the soil's ecology.

3.3. Mycotoxins' effects on soil ecology

Soil ecology is influenced by the biochemical including biotic relationships and physical conditions paramount for its good health [52, 54]. The biochemical aspect of soil used for cultivation is related to its microbial diversity as well as its chemical/pollutant content [53], with the soil's microbial community playing a transformative role with regard to the soil nutrient availability, health and fertility, which enhance the soil's quality, including its productivity [52]. The microbial ecology of any soil facilitates nutrient flow through immobilisation processes, which may result in its bioaugmentation [54, 60, 61], contributing to suitable soil structure that assists in the formation of nutrient-rich aggregates. According to Knudsen [53], soil aggregates are created by microbial activity, albeit at a microscopic level, linking soil particles, while the external polysaccharide tissues of bacterial cells play a role in holding soil aggregates together [52], with subsequent structuring and compaction, parameters influencing the quality of the soil's texture, porosity, aeration, moisture permeability, water circulation and organic matter content [52]. Soil grain cohesion, porosity, permeability and organic matter content are vital for soil quality and fertility, particularly for soil demarcated for sustaining the production of agricultural produce.

All these parameters are indispensable for sustainable use of arable soil for food production and productivity for crop yield [52, 53]. Additionally, soil health can also be affected by surface, subsurface and groundwater supply, including quality.

Soil moisture content is vital for soil functionality as it serves as a water reservoir for the terrestrial ecosystem, playing a major role in the water cycle between surface and subsurface water, thus affecting the quality of groundwater [62, 63]. High mycotoxin loading into the soil may impact its functionality. Thus, the interaction of microorganisms, invertebrates, vertebrates, and planted crops, which eventually leads to the depletion of groundwater quality, leads to sustained leaching or periodic contamination of the water, which can easily lead to human exposure. The disturbance in a terrestrial hydrological movement may have long-term disastrous consequences for surface, subsurface and groundwater supplies [62, 64].

Previous studies on mycotoxin mobility in soil revealed that the movement of these contaminants is influenced by processes such as deposition, decomposition, distribution and accumulation [2, 65], while the compounds' concentration increases with depth. A soil with a high moisture content creates conditions that lead to the furtherance of the contaminants' ability to be transferred, based on processes such as infiltration, percolation and leaching into groundwater [62, 63]. The detoxification bioprocesses and strategies may involve extended periods during which the land is unusable. Furthermore, several studies on the effects of cassava effluents on soil, including microbiota, stated that a high mycotoxin concentration in soil is harmful to these soil microorganisms. Some of these mycotoxins are produced because of inhibitive competition, i.e. organisms will produce these mycotoxins to limit the proliferation of others, particularly under nutrient-depleted conditions.

A study by Knudsen [53] revealed that mycotoxins from cassava are mobile in soil and destroy the resident soil's organisms. Additionally, Okechi et al. [10] showed that the effects of cassava effluents on soil microbial populations revealed a discrepancy in bacterial and fungal populations at different pH levels and soil depths. This indicates that the bacterial populations from the upper layers of soil counts revealed an increase in comparison with those recorded in the lower soil layers, with high concentrations of the mycotoxins observed on the lower soil layers, a process furthered by leaching. Similar total fungal population counts revealed a similar phenomenon with surface, subsurface and deeper soil layers.

3.4. Impacts of hydrovgen cyanide on biochemical and physical properties of agricultural soil

Although the conditions and diversity of habitats contribute to and thus influence the biochemical and physical properties of arable soil [62, 64], a high cyanogen load in soil can have a negative impact on soil microbial populations, with sustained exposure and an increased concentration of cyanogens hindering the microbial activity, and thus the functionality of soil microorganisms, leading to the deformation of the biochemical and physical properties of the soil. A high hydrogen cyanide concentration load in such soil was determined to contribute to an increase in the total oxygen carbon (TOC) and chemical oxygen demand (COD), reducing the ability of *Nitrobacter* sp. to sustain nitration processes [29, 64]. Therefore, an increase in the hydrogen cyanide loading could lead to an imbalance between *Nitrospira* and *Nitrobacter* sp., resulting in a higher count of species with a hydrogen cyanide-resistant ability. The change in the microbial population balance could lead to stunted growth and/or variations in the growth of a cultivar. This can easily culminate in the dominance of the

species, which can be a spoilage organism with free cyanide-resistant characteristics contributing to spoilage patterns/microbial contamination of the produce of interest.

For mitigation strategies in the post-harvesting period, preparation of soil for re-cultivation could lead to inadequate organic matter (OM), variation in total nitrogen (TN) content and availability, which could interfere with soil biochemical and physical properties [64]. Research on the physico-chemical characteristics of cassava-cultivated soil has shown a correlation between continuous cassava cultivation and a decline in the soil's physico-chemical properties (Haplic Acrisols) [64]. Therefore, continuous cultivation of cassava, which normally happens in impoverished communities, could result in a decrease in soil quality, bulk density, organic carbon (OC), OM, trace elements, moisture, infiltration rate, including holding capacity, and aggregate stability. Howeler [66] further reported that the average nutrient removal rate per ton of cassava tuber harvested is equivalent to: N=2.53 (38%), P=0.37 (49%), K=2.75 (56%), Ca=0.44 (16%) and Mg=0.26 (30%). Thus, cyanogen loading indirectly has an impact on C:N ratio, which will result in a pH increase with depth, while OC, nitrogen (N) and OM distortions will be entrenched.

Similarly, Boadi et al. [67] examined the relationship between cyanogen concentration, pH and soil moisture, determining that with an increase in cyanogen values, soil pH increases with moisture content, further supporting the retention of cyanogens at a lower pH. The concentration of cyanogenic compounds was shown to be varied from soil to groundwater and from one site to another [61–63, 68], which suggested that the discrepancies in distribution could be due to the mobility of the contaminant [67].

3.5. Behaviour of cyanogen and mycotoxin in soil

Cyanogen and mycotoxin behaviour in soil, groundwater and the environment is largely controlled by a multitude of chemical reactions and processes. There are similarities and differences between the processes involved for the behaviour of each contaminant, which is largely controlled by conditions the contaminant undergoes when in soil and groundwater. These processes are primarily influenced by numerous biochemical processes and by the compounds' structures, properties and behaviour in the environment. According to Kjeldsen [65], the behaviour of cyanogen and mycotoxins from soil into groundwater is largely influenced by processes such as deposition, dissolution, infiltration, leaching, degradation, transformation and complexation (see **Figure 2**).

Furthermore, human, wildlife exposure and environmental contamination are directly associated with other pathways, such as volatilisation, dermal contact and ingestion of other degradation by-products from the transformation of the primary source due to the transportation pathways facilitated by leaching mechanisms into groundwater [62, 65]. Thus, when not monitored, the environmental prevalence and exposure of these contaminants can be harmful to human health/wildlife. For example, the concentration of leached iron-cyanide complexes in groundwater ranged between 2 and 12 mg/L [59, 69]. The prevalence of such complexes is influenced by the reactivity of free metal ions and free hydrogen cyanide from cassava. These compounds may be transformed (through decomposition) to free cyanide at a later stage, although most are stable with longer half-life, thus they enter an aquifer through processes such as infiltration and leaching.
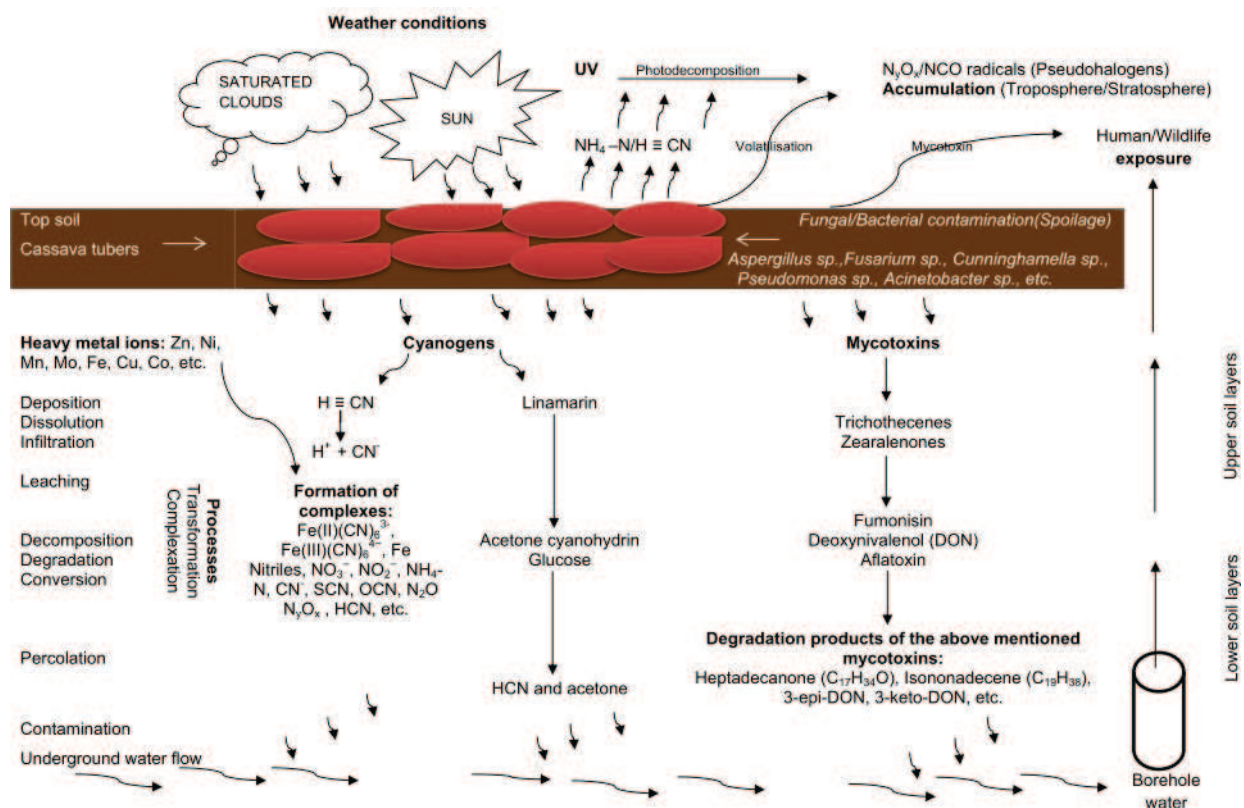
Figure 2. Cassava cyanogen and mycotoxin movement in agricultural soil. Key: NO_3^- (nitrate), NO_2^- (nitrite), Fe(II)(CN)63− (ferrocyanide), Fe(III)(CN)64− (ferricyanide), SCN (thiocyanide), NH_4-N (ammonium nitrogen), CN- (cyanide ion), -OCN (cyanate), N_2O (nitrous oxide) and N_yO_x (nitrogen oxides).

It is also important to point out that the behaviour of contaminant movement in terrestrial or/ and aquatic ecosystems and the environment in general is also influenced by parameters such as wash-off, periodic moisture saturation and time. Based on the stability of each individual contaminant, including its by-products, the mobility can also be spontaneously influenced by the rate of conversion, thus degradation, and can even become volatilised under suitable conditions [65, 70], depending on vapour pressure. Time or length of exposure is a very important aspect, particularly where human exposure is assessed, which is generally neglected or unclear in many recent studies. Similarly, contamination gradients must be established because of groundwater variations in the water flow, as well as the influence of the insolation surrounding the water body that might contribute to acute exposure levels. Furthermore, from produce itself, volatilised compounds can undergo photodecomposition due to UV effects contributing to pseudohalogen accumulation in the troposphere/stratosphere.

3.6. Cyanogen and mycotoxin effects on humans and animals

The focus of this review is that cassava can be toxic when consumed in large quantities owing to its cyanogen content [49]. The prolonged consumption of cassava in different forms can be harmful for humans in particular, owing to inadequacies in post-harvest treatment techniques [8, 21]. For instance, studies on cassava-cyanide effects in humans revealed that a permanent consumption of low-level concentrations of cyanide from poorly processed cassava could result in goitres and Tropical Ataxic Neuropathy (TAN) [24, 59], whereas a high consumption

of the produce could result in neurological disorders, such as konzo [23, 50]. Most post-harvest cyanogen removal techniques focus on free-cyanide removal techniques, without accounting for transformed varieties of the cyanogens (see **Figure 2**), such as thiocyanate, etc.

A team of researchers conducting studies on the thiocyanate concentration in urine samples of pupils, who consumed cassava in Mozambique, revealed that a mean concentration of urinary thiocyanate in school children ranged from 225 to 384 mol/L, whereas mean total cyanogen concentrations in processed cassava flour varied with seasons and years from 26 to 186 mg/L [71]. Similarly, a study by Shifrin et al. [59] revealed that mycotoxin can easily be absorbed through dermal contact, ingestion and inhalation. Some mycotoxins are hazardous and are proposed to be carcinogens facilitating mutation in human cells—an effect that can be postulated to suggest their facilitation of cell mutation in humans.

In animals, on the other hand, an increased consumption of tuber debris and waste by-products of produce processing could lead to neuronal disturbances, weight loss and dysfunctional thyroid [23, 50, 72]. Observations reported by Wade et al. [73] on cassava waste in fish, i.e., in the Nile tilapia (*Oreochromis niloticus*), revealed that some cyanogens caused oedema, gill lamellae telangiectasia, gill enlargement, formation of vacuoles and liver cell deterioration. Similar health outcomes for humans and animals observed in acute mycotoxin exposure, including ingestion, were inter alia, weight loss, internal organ bleeding, respiratory diseases (asthma, pneumonia), diarrhoea, liver and kidney cancer and skin irritation [74–76]. Therefore, a large-scale propagation of agricultural produce with cyanogens, which is susceptible to a high concentration of spoilage organisms, particularly mycotoxin producers, requires continuous monitoring to ascertain its quality. Such produce should be free of both cyanogen and mycotoxins, primarily if it is destined for human and animal consumption and/or exposure. In this case, required strategies for the reduction of exposure must be implemented.

4. Conclusion

Cassava, in general, and cassava tubers, in particular, are indispensable for daily self-nourishment of several poor communities worldwide owing to their nutritional value. However, when exposed to environmental processes and bacterial and fungal attacks that can occur prior to harvesting, the produce is susceptible to release cyanogen and mycotoxin compounds that are hazardous to humans, animals and the environment. These contaminants and their by-product mobility into the terrestrial ecosystem are similar and are facilitated by environmental processes such as transformation, complexation, percolation and volatilisation as they can travel from surface and subsurface to groundwater level, which can result in exposure to both animals and humans. The presence of these compounds in arable land can lead to their accumulation, which can negatively affect soil properties, groundwater quality and the environment, thus contributing to a decline in the production of useful produce, such as cassava. Monitoring, particularly in communities that use such arable soil on a continuous basis, can mitigate intoxication of humans and animals, by effectively implementing suitable reduction strategies thus prevent environmental pollution. Therefore, continuous monitoring, quality assurance and a novel *in-situ* biological method (for treatment of the contaminants) are paramount to ensure a healthier agricultural soil, clean surface and groundwater quality.

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Declaration of conflicting interests

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Author details

Elie F. Itoba-Tombo*, Seteno K.O. Ntwampe and John B.N. Mudumbi

*Address all correspondence to: itobatomboe@cput.ac.za

Bioresource Engineering Research Group (BioERG), Department of Biotechnology, Cape Peninsula University of Technology, Cape Town, , South Africa

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