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Original Research Article

Differential Phytochemical Response to Increased Nitrogen in *Manihot esculenta Crantz* (Cassava) Grown in Mining Soil

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ABSTRACT

Ikpeshi and Okpella, communities in Edo State, Nigeria, are areas with active mining operations. These activities can significantly impact the surrounding environment, including the plant life and, consequently, the phytochemical composition of plants growing in these areas. This study investigated the effects of nitrogen amendment on the phytochemical composition of cassava grown in mining soil. The experiment was conducted on loamy sandy soil collected from two different sites each from Ikpeshi and Okpella, and the experiment was arranged in a completely randomized design in triplicate with two treatments of 2 g and 2.5 g nitrogen. Steroids, terpenoids, flavonoids, anthraquinones, phlobatannins, saponins, cardiac glycosides with a steroidal nucleus (CG1), cardiac glycosides with deoxy sugar, cardiac glycosides with cardenolides, and phenols, except for anthraquinone, which was absent, were analyzed in the methanolic extract cassava. The highest concentration of all parameters was recorded for CG1 at Okpella site A (4.91 ± 0.16), and phlobatannin was observed to have the lowest concentration of all parameters, at 0.04 ± 0.02 for Ikpeshi site A at 2 g nitrogen. Increased nitrogen concentration significantly increased the concentrations of alkaloids, flavonoids, and phenols, while other phytochemicals remained relatively stable. At 2.5 g nitrogen, the highest flavonoid concentration was recorded for IKA (1.69 ± 0.02), the highest phenol concentration was recorded for IKB (1.39 ± 0.06), and the highest alkaloid concentration was recorded for OKB (1.69 ± 0.28). This study provides evidence that nitrogen amendment influences the phytochemical composition of cassava grown in post-mining soils from Ikpeshi and Okpella.

Keywords: Mining soil, Nitrogen amendment, Phytochemical composition, Ikpeshi and Okpella

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Introduction

Mining is the extraction of valuable minerals or other geological materials from the Earth, usually from an ore body, lode, vein, seam, reef, or placer deposit.[1] These deposits form a mineralized commodity that is of economic interest to the miner. Ores recovered by mining include metals, coal, oil shale, gemstones, limestone, chalk, dimension stone, rock salt, potash, gravel, and clay. Mining process has undeniably brought affluence and employment prospects in mining areas, but concurrently has led to widespread environmental degradation and the erosion of traditional values in society.[2] Soil pollution has now reached a crisis point. Almost every soil is polluted to an alarming level. Thus, estimation of quality of soil is extremely important for proper assessment of the associated hazards. The extensive mining activities in Edo North also adversely affects the environment. Due to lack of proper planning and negligence of regulations, an appreciable amount of environmental degradation and ecological damage to soil, air and water occurs.

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The problems associated with mining activities are land degradation, disposal of over burden (OB), deforestation, washing rejects, subsidence, water pollution due to wash off, discharge of mine water, acid mine drainage, coal washing operation, air pollution due to release of gases and dust, noise pollution, mine fires, damage to forest flora and fauna, occupational health hazards etc.
All plants utilize nitrogen (N) in the form of NO_3- and NH_4^+. It is most imperative element for proper growth and development of plants which significantly increases and enhances the yield and its quality by playing a vital role in biochemical and physiological functions of plant.[3] Nitrogen is the most important plant nutrient for crop production. It is a constituent of the building blocks of almost all plant structures. It is an essential component of chlorophyll, enzymes, proteins, etc. Nitrogen occupies a unique position as a plant nutrient because rather high amounts are required compared to the other essential nutrients. It stimulates root growth and crop development as well as uptake of the other nutrients. Therefore, plants, except legumes which fix N_2 from the atmosphere, usually respond quickly to nitrogen applications.[4]
Phytochemistry is the study of phytochemicals, these are chemicals derived from plants. In a narrower scene the term is often used to describe the large number of secondary metabolic compounds found in plants.[5] Secondary metabolism produces a large number of specialized compounds (estimated 200,000) that do not aid in the growth and development of plants but are required for the plant to survive in its environment. Secondary metabolism is connected to primary metabolism by using building blocks and biosynthetic enzymes derived from primary metabolism. Primary metabolism governs all basic

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physiological processes that allow a plant to grow and set seeds, by translating the genetic code into proteins, carbohydrates, and amino acids. Specialized compounds from secondary metabolism are essential for communicating with other organisms in mutualistic (e.g., attraction of beneficial organisms such as pollinators) or antagonistic interactions (e.g., deterrent against herbivores and pathogens). In any case, a good balance between products of primary and secondary metabolism is best for a plant's optimal growth and development as well as for its effective coping with often changing environmental conditions. Well, known specialized compounds include alkaloids, polyphenols including flavonoids, and terpenoids. Humans use quite a lot of these compounds, or the plants from which they originate, for culinary, medicinal and nutraceutical purposes.[6]

Cassava is among the major staple food crops in Nigeria and in most tropical countries. Cassava (*Manihot esculenta Crantz)* is a woody shrub native to South America [80]. The plant is known by its edible starchy tuberous root. It is a drought tolerant, staple food crop grown in tropical and subtropical areas where many people are afflicted to under nutrition, making it a potentially valuable food for developing countries.[7] Cassava plays a significant role in making sure food security in a developing country; namely, Nigeria, is sustained. Approximately 750 million people, of which 45% of sub-Saharan Africans, depend entirely on cassava as a primary food source.[7] The research specifically examines the combined effects of *mining soil, cassava as a target crop, and nitrogen amendment on the detailed phytochemical composition* of the cassava, within the specific geographical context of Ikpeshi and Okpella and also explores a potential strategy (nitrogen amendment) for improving cassava production (a staple food) in areas affected by mining, while also considering the impact on the nutritional and potentially medicinal properties (phytochemical composition) of the cassava produced.

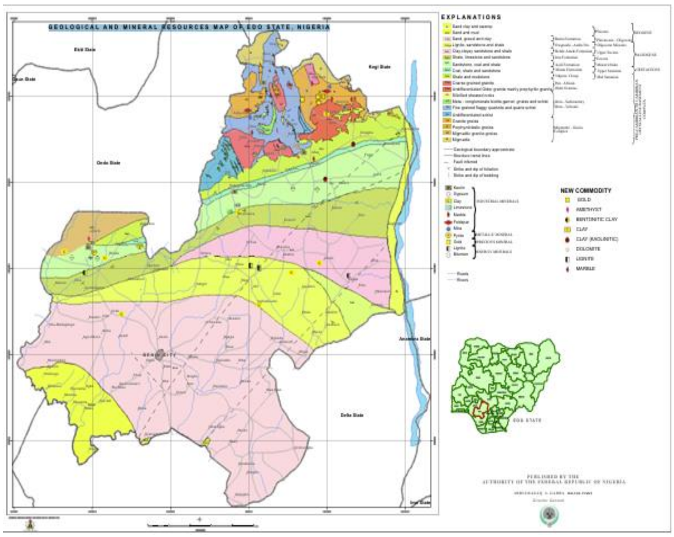

Figure 1: Mineral resources map of Edo State, Nigeria[2]

Materials and Methods

Chemical and Reagent

Chemicals such as 1,1-diphenyl-2-picrylhydrazyl (DPPH), 1,10 phenanthroline, trichloroacetic acid (TCA), ferric chloride reagent, Dragendorff's reagent, pyridine, sodium nitroprusside reagent, glacial acetic anhydride, and other chemicals/reagents are all products of Evans Medical PLC, Lagos, Nigeria

Sample Collection and Identification

Healthy cassava stems (with voucher number; 3035614) were obtained from the Department of Crop Science, Leventis Farm, Agenebode, Edo State, Nigeria, in July, 2024 and was identified by the head of the unit Mr. Kenneth. The soil collected from each location was loamy sandy soil. It was homogenized, crushed and dried in the dark at room temperature under a fume hood for 7days. 1kg of each soil was measured into a planting bag where the cassava was grown for twelve weeks.

Sample preparation

At twelve weeks post-planting, all aerial parts of the cassava were harvested. Following harvest, the plant material was meticulously washed with tap water to remove any adhering soil or debris. Subsequently, the washed plant material was subjected to a shade-drying process for a duration of approximately six weeks at ambient temperature. Upon thorough drying, the plant material was finely pulverized using a blender.

Sample Extraction

Hundred grams (100 g) of the powdered sample was extracted successively with100 mL of a methanol-water solvent mixture (4:1 v/v) using Soxhlet extractor for 24 hours and the extracts were concentrated by the use of vacuum rotary evaporator at 50^0C.

Phytochemical Screening

The methanolic extract of cassava were screened for the quantity of steroids, terpenoids, flavonoids, anthraquinones, phlobatannins, saponins, cardiac glycosides with a steroidal nucleus, cardiac glycosides with deoxy sugar, cardiac glycosides with cardenolides, and phenols according to the procedure described by.[8]

Results and Discussion

Quantitative Phytochemical Screening

Quantitative phytochemical screening of the cassava extract is shown in Table 1 and Table 2 at different concentration of nitrogen. All the phytochemicals analyzed were virtually present in all cassava extract at different sites with exception of anthraquinones which was found to be absent in all the samples.

Table 1: Phytochemical screening of Cassava amended with 2.g nitrogen

Ster = Steroids, Terp= Terpenoids, Fla= Flavonoid Anthr =

Parameter	OK SITE A	OK SITE B	IK SITE A	IK SITE B
Alka	0.99 ± 0.01^{a}	1.12 ±0.09*	0.85 ± 0.06^{a}	0.97 ± 0.02*^{a}
Sapo	1.22 ± 0.02	1.21 ± 0.02	1.19 ± 0.01	1.21 ± 0.01
Phlo	0.58 ± 0.03	0.58 ± 0.03	0.04 ± 0.02^{a}	0.07 ± 0.02^{a}
Tan	0.07 ± 0.01	0.07 ± 0.01	0.08 ± 0.00	0.08 ± 0.00
Anthr	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Ster	1.02 ± 0.02	1.02 ± 0.02	1.10 ± 0.02^{a}	1.03 ± 0.03*
Ter	0.97 ± 0.06	0.97 ±0.06	0.89 ± 0.01^{a}	1.10 ± 0.01*^{a}
Flav	1.16 ± 0.06	1.16 ± 0.06	0.89 ± 0.10^{a}	1.08 ± 0.09
CG1	4.91 ± 0.16	4.91 ± 0.16	3.22 ± 0.08^{a}	3.99 ± 0.11*^{a}
CG2	2.65 ± 0.01	2.65 ± 0.01	3.11 ± 0.01^{a}	3.06 ± 0.03^{a}
CG3	1.77 ± 0.07	1.77 ± 0.07	2.13 ± 0.03^{a}	1.70 ± 0.13*
Phenol	0.60 ± 0.14	0.60 ± 0.14	0.20 ± 0.02^{a}	0.41 ± 0.08*^{a}

Anthraquinones, Phlo = phlobatannins, Sapo= Saponin, CG1= Cardiac glycosides with steroidal nucleus, CG2 = Cardiac glycosides with deoxy sugar, CG3 = Cardiac glycosides with cardenolides

When soil was amended with 2g and 2.5g nitrogen cassava extracts shows the presence of steroids, terpenoids, flavonoids, anthraquinones, phlobatannins, saponins, cardiac glycosides with a steroidal nucleus (CG1), cardiac glycosides with deoxy sugar, cardiac glycosides with cardenolides, and phenols. The existence of phytochemicals in cassava has varying levels of protective antioxidants and antimicrobial molecules.[9]

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Table 2: Phytochemical screening of Cassava amended with 2.5g nitrogen

Parameter	OK SITE A	OK SITE B	IK SITE A	IK SITE B
Alka	2.03 ± 0.12	1.69 ± 0.28*	1.36 ± 0.07[a]	1.11 ± 0.01*[b]
Sapo	1.22 ± 0.0	1.33 ± 0.05*	1.19 ± 0.01	1.21 ± 0.01[a]
Phlo	0.58 ± 0.03	0.71 ± 0.05*	0.04 ± 0.02[a]	0.07 ± 0.02
Tan	0.07 ± 0.01	0.03 ± 0.02*	0.08 ± 0.01	0.08 ± 0.00[a]
Anthr	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Ster	1.01 ± 0.00	1.15 ± 0.01*	1.10 ± 0.01[a]	1.03 ± 0.03*[a]
Ter	0.97 ± 0.06	1.06 ± 0.02*	0.89 ± 0.01[a]	1.10 ± 0.01*[a]
Flav	1.30 ± 0.03	1.64 ± 0.18*	1.69 ± 0.02[a]	1.39 ± 0.06*[a]
CG1	4.91 ± 0.16	4.86 ± 0.08	3.22 ± 0.08[a]	3.99 ± 0.11*[a]
CG2	2.65 ± 0.01	2.35 ± 0.12*	3.08 ± 0.05[a]	3.06 ± 0.03[a]
CG3	1.77 ± 0.00	2.04 ± 0.01*	2.13 ± 0.03[a]	1.70 ± 0.13*[a]
Phenol	1.34 ± 0.05	1.15 ± 0.22*	1.11 ± 0.10[b]	1.39 ± 0.06*[a]

Ster = Steroids, Terp= Terpenoids, Fla= flavonoids Anthr = Anthraquinones, Phlo = phlobatannins, Sapo= Saponin, CG1= Cardiac glycosides with steroidal nucleus, CG2 = Cardiac glycosides with deoxy sugar, CG3 = Cardiac glycosides with cardenolides

In addition, these phytochemicals serve as the best antioxidants and protect cells from free radical damage or to minimize the risk of cancer by inhibiting tumor development or hormonal stimulation and antibacterial activity.[10] The quantitative phytochemical screening of the cassava extract after treatment of soil with 2g nitrogen shown in Table 1 revealed the highest phytochemical content was recorded for CG1 at Okpella site A (4.91 ± 0.16) and the lowest concentration was recorded for phlobatannin at Ikpeshi site A (0.04 ± 0.02). However, the difference between the groups was significant. Increase in the concentration of nitrogen from 2g to 2.5g in Table 2 showed significant increase in alkaloids, flavonoid and phenol while other phytochemicals relatively remained stable. Extract of plant from IK site A present the highest flavonoid content (1.69 ± 0.02) and OK site A had the lowest value (1.30 ± 0.03). Extract from IK site B has the highest in phenol (1.39 ± 0.06) and IK site A had the lowest value (1.11 ± 0.10) while extract from OK site B has the highest for alkaloid (1.69 ± 0.28) and IK site B had the lowest value (1.11 ± 0.01).

Mining activities significantly impact soil quality by causing contamination with heavy metals, altering soil structure, increasing erosion, and disrupting the natural soil ecosystem, often leading to decreased soil fertility and land degradation; essentially, mining can severely damage soil quality through the introduction of toxic chemicals and disruption of the natural soil profile. Soil serves a vital function in nature, providing nutrients for plants to grow, as well as millions of micro and macro-organisms. Soil is fundamental to ecosystem, agricultural sustainability and production. Nitrogen is one of the most important nutrients required by plants. Nitrogen fertilizers influence the productivity and yield of various plants worldwide, Nitrogen level can also influence plant secondary metabolites (phytochemicals).[3] The result confirmed the presence of alkaloid, flavonoid, saponin, tannin and phenol in the cassava extracts, this is in accordance with the findings of[11] who identified the presence of alkaloid, flavonoid, saponin, tannin and phenol in cassava. The significant increase in phenols and flavonoids is in agreement with[12] who reported that nitrogen fertilizers have a significant effect on the level of total phenols and flavonoids while working on effects of nitrogen application on phytochemical component levels and anticancer and antioxidant activities of *Allium fistulosum.* The Increase of these compounds contribute to defense against pests and diseases.[11]. Phenolics and flavonoids can deter herbivores and pathogens. Alkaloids can have toxic effects on insects and other pests, protecting the plant. They also contribute to the sensory properties (e.g., taste, color) of cassava.[11] The observed increase in alkaloids, flavonoids, and phenols with the 2.5g nitrogen treatment can be explained by the established roles of nitrogen in the biosynthesis of these specific compound classes. Nitrogen is a fundamental component of amino acids, which serve as precursors for various secondary metabolites, including alkaloids, flavonoids, and phenolic compounds.[12] The study revealed that increased phytochemical levels are likely due to nitrogen's crucial role in the biosynthesis of these compounds and while nitrogen was the main driver, some variation in phytochemical levels was observed between different cassava growing sites.

Conclusion

In conclusion, this study demonstrates that nitrogen fertilization significantly influences the phytochemical composition of cassava extracts. While cassava is inherently a source of various beneficial phytochemicals, increasing nitrogen application from 2g to 2.5g notably enhanced the levels of alkaloids, flavonoids, and phenols, important compounds associated with antioxidant, antimicrobial, and pest-deterrent properties. This increase is likely attributable to nitrogen's role in the biosynthesis of these compounds. Although some site-specific variations in phytochemical content were observed, the overall trend indicates that nitrogen fertilization can be a viable strategy for enhancing the phytochemical profile of cassava, potentially improving its nutritional and medicinal value. These findings contribute to a better understanding of how agricultural practices can be optimized to maximize the beneficial properties of cassava.

Conflict of Interest

The authors declare no conflict of interest.

Author's Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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