Journal of Medicinal Plants for Economic Development
ISSN: (Online) 2616-4809, (Print) 2519-559X

Secondary metabolites profiling of *Vernonia amygdalina* Del. in response to copper-induced abiotic stress

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Dates:
Received: 17 May 2020
Accepted: 14 Dec. 2020
Published: 17 Feb. 2021

How to cite this article:
Adu, A., Ojekale, A., John, B. & Avoseh, O.N., 2021, 'Secondary metabolites profiling of *Vernonia amygdalina* Del. in response to copper-induced abiotic stress', *Journal of Medicinal Plants for Economic Development* 5(1), a91. https://doi.org/10.4102/jomped.v5i1.91

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Background: *Vernonia amygdalina* is a green leafy vegetable that grows in tropical Africa and popularly cultivated in the southern part of Nigeria for its economic, nutritional and ethnomedicine value.

Aims: This study analysed the influence of copper-induced abiotic stress on the deoxyribonucleic acid and secondary metabolites of *V. amygdalina*.

Setting: Analyses of plant material were carried out in the Department of Botany, Lagos State University.

Methods: Deoxyribonucleic acid (DNA) extraction and Inter Simple Sequence Repeat (ISSR) amplification were performed. Copper (Cu) concentration in leaves and stem of *V. amygdalina* was determined using inductively coupled plasma-optical emission spectroscopy (ICP-OES), secondary metabolites were determined using gas chromatography–mass spectrometry (GC-MS) and the effect on DNA amplification and profiling was analysed using gel electrophoresis.

Results: After 41 days of cultivation, the stem of *V. amygdalina* significantly bioaccumulated 37 mg/L, 50 mg/L and 215 mg/L of Cu after spiking with 400 mg/L, 1000 mg/L and 2000 mg/L, respectively, whilst the leaves accumulated 52 mg/L, 100 mg/L and 350 mg/L of Cu, respectively. Copper-impregnated soil influenced phytochemicals of the plants by causing inconsistent increase and decrease in specific compounds such as levomenthol, methyl stearate and glycerine. Deoxyribonucleic acid bands of the stem and leaf of *V. amygdalina* from control site showed no band shift, whilst band shift occurred in the stem and leaf of the Cu-spiked *V. amygdalina.*

Conclusion: This study revealed the leaves of *V. amygdalina* having higher Cu accumulation capacity than the stems. Also, Cu alters the quality and quantity of phytochemicals in plant parts.

keywords: *V. amygdalina*; copper; phytochemicals; abiotic stress; bioaccumulation.

Introduction

Vernonia amygdalina, commonly called 'bitter leaf', is a member of the daisy family. It is a shrub that may grow to a height of 2.5 m when matured and bears small white flowers that form small fruits with little nuts inside. The plants usually domicile in the forest zone, woodlands and open grassland in the wild or cultivated (Akachukwu 2001). It is indigenous to tropical Africa (Kokwaro 2009) and propagated by seeds and stems cuttings, which grow faster than seedlings. It is cultivated as food and medicines amongst the local people of Nigeria (Ewuro in Yoruba, Chusar-doki in Hausa & Onugbu) and Cameroon (Lusoga) (Constance et al. 2020). It grows very well under full sunlight and humid environmental conditions. Goats feed on the leaves, dry stem provides fuel, young twigs serve as toothpicks, or chewing sticks and the branches are used as stakes to line fields (Burkill 1995). Phytochemicals reported in the plants includes alkaloids, saponins, terpenes, coumarins, flavonoids, phenolic acids, lignans, Xanthones and anthraquinones and steroids (Ijeh & Ejike 2011). The production of phytochemical is affected by factors such as nature and type of soil, climatic conditions, type, plant parts, the plant's geographical location, age of the plant and its biosynthetic pathways.

In recent years, there has been an increasing ecological and global public health concern associated with environmental contamination of heavy metals (Tchounwou et al. 2012). Heavy metals such as mercury (Hg), lead (Pb) and calcium (Ca) have no severe or beneficial effect on the plants. Still, their accumulation over time in mammals' bodies that consume them may cause serious illness

(Kabata-Pendias & Pendias 2001). Copper (Cu) is one of the micronutrients needed in minimal quantities by plants. The acceptable range, according to the World Health Organization (WHO 2005) in the growing medium is 0.05 ppm – 0.5 ppm, while in most tissues the range is between 3 ppm and 10 ppm. Cu metal rarely caused high-toxicity effect and its deficiency is barely reported; hence, it is best to avoid either extreme as both can affect crop growth and quality (Mccauley 2011).

Reports have shown that abiotic environmental stress such as salinity, low and elevated temperatures, drought, alkalinity and increased metal concentrations are potentially harmful to plants and its phytochemicals (Christie, Alfenito & Walbot 1994). For instance, nutrient stress has a marked effect on phenolic levels in plant tissues, as reported by Bryant, Chapin and Klein (1983). The concentration of heavy metals in soil seldom reaches a level sufficient to cause an osmotic disturbance in plants, through the downward sipping into the root. Decreased elongation of the primary roots, impaired accordance growth, increased root dieback or reduced root hair caused by toxic ions all exert a deleterious effect on the root absorbing area and water uptake (Dhawale 2013).

Sequel to the above background, this study aimed to evaluate the influence of Cu-induced abiotic stress on the DNA and secondary metabolites of *V. amygdalina*.

Materials and methods

Experimental site

The research project was carried out in the botanical greenhouse garden of the Department of Botany, Lagos State University (GPS location of 6°27′57.8″N, 3°12′00.4″E, Ojo) between April and May 2018. The stem of *V. amygdalina* used was collected from a cultivated farm on 17 April 2018 at Ijanikin, Otto/Awori LCDA, Lagos state. Four specimens of *V. amygdalina* were taken to the Lagos State University (LSH) Herbarium for identification and authentication. The plant materials were identified, and the following voucher numbers, LSH20/0016, LSH20/0017, LSH20/0018 and LSH20/0019, were issued as sample markers for ease of reference. The loamy soil used for the experiment was collected at the vegetable farm, Lagos State University main gate, Ojo, on 18 April 2018.

Planting procedure and spiking

Ten previously perforated, pre-washed plastic buckets were used. Copper sulphate ($CuSO_4$) of 400 mg/L, 1000 mg/L and 2000 mg/L were prepared using standard procedure. In brief, 400 mg, 1000 mg and 2000 mg of $CuSO_4$ anhydrous were dissolved in 1 L of distilled water to make the respective concentrations and standardised using the titrimetric method (Danny 2020). Each plastic bucket was preloaded with 500 g of soil previously spiked with varying copper sulphate concentrations except the control sample that was unspiked with Cu solution. The stem of *V. amygdalina* was cut into 10 pieces, each 6 cm long, and was planted in the already spiked soil samples to a depth of about 16 cm. Distilled water was used to wet the plant for 3 days, according to Burkill methodology. The plant was allowed to germinate for about 14 days.

Analysis of soil, leaves and stems of *Vernonia amygdalina*

Laboratory analysis of the unspiked soil sample and fresh *V. amygdalina* stem was first carried out before planting to determine the initial composition of Cu before subjecting them to spiking. After 41 days of planting, following spiking, Cu analysis of the soil and *V. amygdalina* stem and leaves was carried out to ascertain the influence of absorption of materials by the plant on the translocation of the copper in *V. amygdalina* (Towolawi et al. 2017).

Preparation of samples

All the apparatus used in the analysis were immersed in 10% of nitric acid (HNO_3) and left for a day. The apparatus was taken out and washed with distilled water (Talbot & Weiss 1994). The plants were divided into two parts, that is, the stem and the leaves, to determine the parts of *V. amygdalina* plants that accumulate more heavy metals. The different parts (stems and leaves) were pulverised in a blender to increase the surface area and dried at a temperature of 65 °C for 24 h in an oven.

Plant digestion for metal analysis

The dried plant samples, 10 g, were sieved and measured appropriately by using the analytical balance. The sample was transferred into a 50 mL conical flask, and 10 mL of 65% HNO_3 was poured into the conical flask, heated for 2 h at 140 °C until a clear solution was achieved. The solution was concentrated to the 5 mL, allowed to cool and filtered using Whatman filter paper 125 mm mesh size. The solution was made up to the 50 mL with distilled water and kept in a 120 mL plastic container, stored in a refrigerator until analysis by inductively coupled plasma-optical emission spectroscopy (ICP-OES) (Talbot & Weiss 1994), which was analysed in triplicates.

Gas chromatography–mass spectrometry analysis

To analyse the effect of different concentrations of Cu on the secondary metabolites, gas chromatography–mass spectrometry (GC-MS) was used. Gas chromatography–mass spectrometry was programmed as follows: injector port temperature was set to 250 °C, interface temperature set as 250 °C and source kept at 200 °C. The oven temperature programmed as a variable (70 °C for 2 min, 150 °C at 8 °C/min, up to 260 °C at 10 °C/min), split ratio set as 1:50 and the injector used was the splitless mode. The DB – 5 MS Nonpolar column (0.25 mm OD × 0.25 μm ID × 30 m length) procured from Agilent Co., United States, was used. Helium was used as the carrier gas at 1 mL/min. The MS was set to scan from 50 Da to 650 Da. The source was maintained at 200 °C and < 40 motor vacuum pressure. The ionisation energy was 70 eV. The MS

contains an inbuilt pre-filter, which reduced the neutral particles. The data system has two inbuilt libraries for searching and matching the spectrum. NIST4 and WILEY9 each contain more than 5 million references. Only those compounds with spectral fit values equal to or greater than 700 were considered positive identification (Martinez et al. 2004).

Deoxyribonucleic acid extraction

Hundred milligram of the fresh plant of *V. amygdalina* (stem and leaves) was weighed and rinsed with distilled water and blended in a mortal with 200 μL phosphate buffer saline (PBS). The mixture was later transferred to a 1.5 mL tube and centrifuged at high speed for 3 min to collect pellets in line with the protocol (Gumińska et al. 2018). The DNA was obtained and sent for the ISSR amplification of DNA and gel electrophoresis to compare the DNA bands of both the control and the spiked sample.

Ethical consideration

This article followed all ethical standards for research without direct contact with human or animal subjects.

Results

Copper concentration in the soil and *Vernonia amygdalina*'s stem and leaves

Unspiked plant and soil (Control)

The concentration of Cu in soil and stem bark samples showed a relatively small amount. The soil and the stem of *V. amygdalina* samples used for the experiment revealed that the soil sample contained 5.00 mg/L, whilst the stem had 2.20 mg/L.

Copper content after spiking: Table 1 shows Cu absorption levels by the soil, stem bark and the leaf after 41 days of *V. amygdalina* cultivation. The leaf sample absorbed an average of 251 mg/L of Cu for the three spiked categories of concentrations, after 41 days of cultivation. Whilst the stem had 100 mg/L, soil had an average of 1308 mg/L for the three spiked categories of concentrations. There was increase in concentration of the extracts as the concentration of the spiked Cu solution increased. The result showed that the leaf sample accumulated more Cu than the stem bark. In addition to the metal uptake by the plant, it was observed that there was poor root development and the leaves showed yellowish coloration and poor branch development.

The secondary metabolites in the spiked and unspiked stem and leaves of *V. amygdalina* analysed by GC-MS are shown in Table 2. Compounds were arranged according to their occurrence in different concentrations of preloaded Cu metal. Several changes occur between the constituents of leaf and stem, changes in concentration and crude extracts of plants. On the other hand, Table 3 shows the summary of the major components and their occurrences. 9-Octadecenoic acid, methyl ester, (Z)-hexadecanoic acid, methyl ester, glycerine, levomenthol, methyl stearate, N, N-dimethylaminoethanol, oxime-, methoxy-phenyl- and Silanediol, dimethyl- occurred in at least two of the extracts. Glycerine and levomenthol were more predominant in the leaves samples: 7.14% and 73.83% in the 400 mg/L Cu and unspiked samples, respectively. Methyl stearate was also present in the unspiked samples of the leaves (29.90%) and stem (25.40%), but with inconsistent presence in the Cu-laden extracts.

Deoxyribonucleic acid* profiling of *Vernonia amygdalina: Deoxyribonucleic acid bands of the stem and leaf of *V. amygdalina* from control site showed no band shift, while band shift occurred in the stem and leaf of the Cu spiked *V. amygdalina* (Figures 1 and 2).

TABLE 1: Level of Copper in the soil, stem and leaves of *Vernonia amygdalina* after 41 days of planting.

Spiked in soil samples (mg/L)	Soil after planting for 41 days (mg/L)	Stem after planting for 41 days (mg/L)	Leaf after planting for 41 days (mg/L)
400	395.12 ± 1.22	37.00 ± 0.47	52.00 ± 2.47
1000	1180.00 ± 0.12	50.00 ± 0.24	100.00 ± 1.62
2000	2350.03 ± 0.04	215.03 ± 0.35	350.00 ± 0.31
Control	5.00 ±0.12	4.00 ± 1.25	5.00 ± 1.81

Note: Results represent SEM ± S.D from $n = 3$.

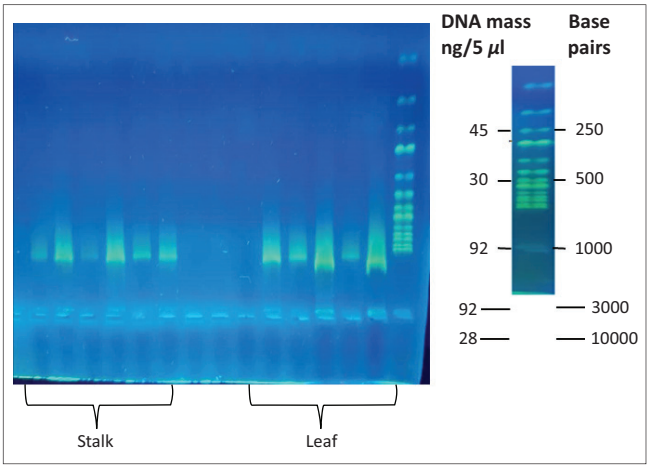

FIGURE 1: Plate 1 showing the deoxyribonucleic acid bands of *Vernonia amygdalina* of control experiment.

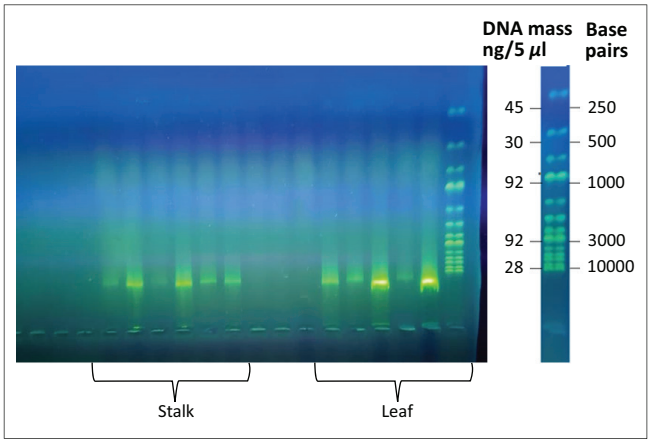

FIGURE 2: Plate 2 showing the deoxyribonucleic acid bands of *Vernonia amygdalina* with heavy metal (Copper) concentration.

TABLE 2: Gas chromatography–mass spectrometry constituent of unspiked and spiked stem of *Vernonia amygdalina* at different concentrations.

Constituents	Stem				Leaf			
	Unspiked	400	1000	2000	Unspiked	400	1000	2000
(5H)-Furanone	0.00	3.44	0.00	0.00	0.00	0.00	0.00	0.00
(6Z)-Nonen-1-ol	0.00	0.00	1.66	0.00	0.00	0.00	0.00	0.00
(R)-(+)-3-Methylcyclopentanone	0.00	1.12	0.00	0.00	0.00	0.00	0.00	0.00
(s)-(t)-6-Methyl-1-octanol	0.00	0.00	0.00	0.72	0.00	0.00	0.00	21.17
1,2,3,4-Tetrahydro-cyclopenta(b)indole	0.00	0.00	0.00	0.73	0.00	0.00	0.00	4.26
2,5-Dimethyl-1,5-Heptadiene-3,4-diol	0.00	1.64	0.00	0.00	0.00	0.00	0.00	0.00
11-Octadecenoic acid methyl ester	0.00	0.00	19.19	0.00	0.00	0.00	0.00	13.43
1,3-Diphenyl-1-(trimethylsilyloxy)-1-heptene	0.00	0.00	0.39	0.00	0.00	0.00	0.00	0.00
1-Methoxy-2-propanone	0.00	0.00	0.00	0.00	0.00	3.04	0.00	0.00
1-Methyl-1-p-tolylethylamine	0.00	0.49	0.00	0.00	0.00	0.00	0.00	0.00
1-Methoxy-2-methyl-1-propene	0.00	3.71	0.00	0.00	0.00	0.00	0.00	0.00
5-Methyl- 2(3H)-furanone	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2,2-Dimethyl-propyl-2,2-dimethyl-propane-thiosulfate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2,5-Dimethyl-4-(1-pyrrolidinyl)-3(2H)-furanone	0.00	0.36	0.00	0.00	0.00	0.00	0.00	0.00
2,6-Difluoro-3-methylbenzoic acid, hexyl ester	0.00	0.00	0.00	0.00	0.00	0.40	0.00	0.00
2,6-dimethyl-3 amino benzoquinone	0.00	0.00	0.00	0.67	0.00	0.00	0.00	0.00
2-butanol,3- methylbenzoquinone	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.35
2hydroxy-2-Cyclopenten-1-one,	0.00	0.00	0.00	0.00	0.00	2.18	0.00	0.00
2-Hydroxy-gamma-butyrolactone	0.00	5.20	0.00	0.00	0.00	0.00	0.00	0.00
2-Naphthalenamine	0.00	0.00	0.00	1.36	0.00	0.00	0.00	0.00
2-Phenylpropenal	0.00	0.00	0.00	0.36	0.00	0.00	0.00	0.00
2-Pyrrolidinone	0.00	0.00	0.00	2.87	0.00	0.00	0.00	0.00
3-Butylindolizidine	0.00	0.00	0.00	1.70	0.00	0.00	0.00	2.66
2-Methyl-3-hexanol	0.00	0.00	0.00	0.00	0.00	0.00	22.00	0.00
(Z)-3-Nonen-1-ol	0.00	0.00	2.16	0.00	0.00	0.00	0.00	1.37
3,6-Trimethyl-4,5-heptadien-2-ol-3	0.00	0.00	0.00	5.83	0.00	0.00	0.00	0.00
3-Methyl-4-Heptanone	0.00	1.66	0.00	0.00	0.00	0.00	0.00	0.00
2-(Dimethylamino)-1-phenyl-4-Octanone	0.00	0.00	0.00	0.00	0.00	0.99	0.00	0.00
4-oxopentyl formate	0.00	0.00	0.00	0.61	0.00	0.00	0.00	0.00
Eucalyptol	0.00	0.00	0.00	0.00	0.00	3.76	0.00	0.00
Glutaric acid, ethyl 4-heptyl ester	0.00	0.32	0.00	0.00	0.00	0.00	0.00	0.00
Glutaric acid, ethyl hex-4-yn-3-yl ester	0.00	0.00	0.00	0.00	0.00	0.58	0.00	0.00
Glycerine	0.00	0.00	0.00	10.74	0.00	7.14	47.90	0.00
2-Ethyl-heptanoic acid	0.00	0.00	2.75	0.00	0.00	0.00	0.00	0.00
Hexadecanoic acid, methyl ester	22.10	0.00	21.08	0.00	19.50	0.00	0.00	17.11
4,5-dehydro-isolongifone,	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.00
Levomenthol	0.00	3.70	0.00	1.36	7.50	73.83	0.00	6.72
l-proline,n-vateryl-deodecyl ester	0.00	0.00	0.00	0.08	0.00	0.00	0.00	0.00
Methyl stearate	29.90	0.00	28.32	0.00	25.40	0.00	0.00	23.29
Methyl-4-phenyl-4-propioxy-piperidine	0.00	0.00	0.00	6.54	0.00	0.00	0.00	0.00
N,N-Dimethylaminoethanol	0.00	19.12	0.00	23.58	0.00	0.00	0.00	0.00
N-Benzyl-2-phenethylamine	0.00	0.00	0.00	0.00	0.00	1.61	3.61	0.00
N-notrpropanoic acid	0.00	0.00	0.58	0.00	0.00	0.00	0.00	0.00
Nonadecanoic acid, methyl ester	0.00	0.00	1.85	0.00	0.00	0.00	0.00	0.00
Oxalic acid, dineopentyl ester	0.00	1.77	0.00	0.00	0.00	0.00	0.00	0.00
Methoxy-phenyl-oxime-,	0.00	11.05	0.00	1.84	0.00	0.00	11.60	0.00
Oxirane, (3-methylbutyl)-	0.00	3.13	0.00	0.00	0.00	0.00	0.00	0.00
Oxirane, butylphenyl	0.00	0.00	0.00	3.65	0.00	0.00	0.00	0.00
Methoxy-phenyl-oxirre	0.00	0.00	0.00	3.61	0.00	0.00	0.00	0.00
Tetradecyloxrane	0.00	0.00	0.00	0.00	3.50	0.00	0.00	0.00
Pantolactone	0.00	0.00	0.00	2.87	0.00	0.00	0.00	0.00
3-Bromo-pentane	0.00	0.00	0.00	0.00	0.00	0.00	4.04	0.00
2-Methyl-pentanoic acid,	0.00	0.25	0.00	0.00	0.00	0.00	0.00	0.00
Phenol	1.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2-(2-Furyl)(2-pyrimidylamino)methyl-Phenol	0.00	0.14	0.00	0.00	0.00	0.00	0.00	0.00
2,6-dimethyl-Phenol	0.00	0.00	0.00	0.45	0.00	0.00	0.00	0.00
Phenol,4-(1H-pyrrol-1-yl)	0.00	0.00	0.00	0.00	0.00	0.00	0.25	0.00
Phosphorisocyanatidothioic difluoride	0.00	0.00	0.00	0.00	0.00	0.00	1.25	0.00
3-Methylpropanol,	0.00	0.00	0.00	4.55	0.00	0.00	0.00	0.00
2,5-dimethyl-3-phopyl-pyrazine	0.00	0.00	0.00	1.53	0.00	0.00	0.00	0.00

Table 2 continues on the next page →

TABLE 2 (Continues...): Gas chromatography–mass spectrometry constituent of unspiked and spiked stem of *Vernonia amygdalina* at different concentrations.

Constituents	Stem				Leaf			
	Unspiked	400	1000	2000	Unspiked	400	1000	2000
1,2,3,6-tetrahydro-1-methyl-4-phenol pyridine	0.00	0.00	0.00	11.30	0.00	0.00	0.00	0.00
2-methyl-5-phenyl-pyrrole,	0.00	0.42	0.00	0.00	0.00	0.00	0.00	0.00
R(-)3,7-dimethyl-1,6-Octadiene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.67
RS-2,3-hexanediol	0.00	0.00	0.00	0.00	0.00	0.00	1.55	0.00
dimethyl-Silanediol	0.00	28.60	0.00	7.50	0.00	0.00	0.00	0.00
Tetradecanoic acid, 12-methyl-,methyl ester E	2.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Tetradecanoic acid, 12-methyl-,methyl ester S	0.00	0.00	1.43	0.00	0.00	0.00	0.00	0.00
Tetradecanoic acid, 12-methyl-,methyl esterate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.80
Tetradecyne	0.00	0.00	0.00	0.00	5.50	0.00	0.00	0.00
Total	**86.6**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

TABLE 3: Overall constituents of compounds in spiked and unspiked stem and leaves of *Vernonia amygdalina*.

Constituents	Stem				Leaves			
	Unspiked	400	1000	2000	Unspiked	400	1000	2000
6-octadecanoic acid, methyl ester	0.00	0.00	0.00	0.00	0.00	0.00	0.00	21.17
11-octadecenoic acid methyl ester	0.00	0.00	19.19	0.00	0.00	0.00	0.00	0.00
9,12-Octadecanoic acid, methyl ester	0.00	0.00	0.00	0.00	0.00	0.00	0.00	13.43
9-Octadecenoic acid, methyl ester, (Z)-	18.70	0.00	0.00	0.00	6.30	0.00	0.00	0.00
Diethylamine, N-(diisopropylphosphino) methyl-	0.00	0.00	0.00	0.00	0.00	0.00	22.00	0.00
Glycerine	0.00	0.00	0.00	10.74	0.00	7.14	47.90	0.00
Hexadecanoic acid, methyl ester	22.10	0.00	21.08	0.00	19.50	0.00	0.00	17.11
Levomenthol	0.00	3.70	0.00	1.36	7.50	73.83	0.00	6.72
Methyl stearate	29.90	0.00	28.32	0.00	25.40	0.00	0.00	23.29
N,N-Dimethylaminoethanol	0.00	19.12	0.00	23.58	0.00	0.00	0.00	0.00
Oxime-, methoxy-phenyl-_	0.00	11.05	0.00	1.84	0.00	0.00	11.60	0.00
Silanediol, dimethyl-	0.00	28.60	0.00	7.50	0.00	0.00	0.00	0.00

Discussions

Plants are complex organisms because of numerous cells that they contain. In approximation, a single Arabidopsis plant could have approximately 100 million cells per gram fresh weight (Gibeaut et al. 1997). Because of such complexity, several parameters affect the growth and development of plants. Amongst these parameters, environmental, proteomics and metabolomics affect the yield of plant products by as much as 70% (Boyer 1982).

In this study, the concentration of Cu for the soil (5 mg/L) and *V. amygdalina* (2.20 mg/L) in the control site was within the World Health Organization (WHO) permissive levels of between 10 mg/kg and 30 mg/kg in medicinal plants and soil, respectively (WHO 1996, 2005). Therefore, there will be proper growth and development of the plants and such plants cannot pose any danger for human and other animals that may feed on them. However, the negative effects of spiking the soil with graded higher levels of Cu concentration far above the permissive level were obvious in plants growth and development. Akachuku (Akachukwu 2001) had reported that land use for food production can be contaminated with heavy metals, including Cu from natural and anthropogenic sources such as water smelt, irrigation with waste, disposal of solid waste, vehicle exhaust and adjacent industrial activities. In this study, bioaccumulation of Cu by the leaf and stem of *V. amygdalina* showed that it is a good research material for phytoremediation. The concentration of Cu being higher in leaves compared to the concentration found in the stem may be as a result of higher translocation rate in the leaf parts compared to the stem (Quartacci, Cosi & Navari-Izzo 2001). Reports have shown that heavy metals are toxic because they tend to bioaccumulate in plants and animals, bioconcentrate in the food chain and attack specific organs in the body.

The influence of heavy metals on phytochemicals has been reported. The finding of Prasad et al. (2011), which analysed the effect of heavy metals on the essential oil yield of sweet basil (*Ocimum basilicum* L.) revealed that increase in Cu, Cd, Mo and Zn concentrations decreases the linalool and increases 1–8 cineole content in the essential oil of sweet basil.

In the present study, several changes in the concentration of Cu resulted in the preponderance of masking of some phytochemicals. In the leaf sample, levomenthol yield was 73.83% at 400 mg/L of Cu concentration, Glycerine (47.90%) at 1000 mg/L and methyl stearate (23.29%) at 2000 mg/L to mention a few. These compounds and others identified in the analysis are growth regulators of plants during stress and have been figured in the control of *V. amygdalina* growth and development as observed in the research. It is therefore not

surprising how the plant was able to survive the presence of high concentration of Cu in its environment. The fact that all the experimental plants did not contain the same concentration might be as a result of their different morphological features and physiological composition. The mechanisms by which the various constituents in the *V. amygdalina* were increased or decreased by the application of Cu metals could be related to the uptake of essential and nonessential or phytotoxic metals as opined by Prasad et al. (2011). In addition, several enzymatic activities are usually responsible for the interconversion of acetyl-Co A to several phytochemicals such as terpenoids, alkaloids, flavonoids and phenolic compounds. Hence, changes in phytochemicals observed in this study could be alluded to biotransformation of the enzymatic pathway by the presence of heavy metals, inhibition of enzymes, inactivation of biomolecules and oxidative stress (Fryzova et al. 2017).

In this study, the DNA bands of stem and leaves of *V. amygdalina* in the control experiment, which displayed no band shift, indicated healthy growth and development in the plants (Nicolia et al. 2013). In the Cu-spiked experiment, as the concentration of heavy metals increased (400 ppm, 1000 ppm and 2000 ppm), the bands of the DNA reduced, both in the stem and the leaves, and this indicated that the DNA of the *V. amygdalina* stalk and leaves was being affected by the concentration of the heavy metal (Cu). This could be as a result of the fact that copper bonds to the DNA or the plant molecule, thereby preventing the formation of chromosomes, and may inhibit DNA synthesis and hinder cell division (Prasad & Strzalka 1999). Quartacci et al. (2001) also reported that plants grown in the presence of high levels of copper normally show reduced biomass and chlorotic symptoms. This is in line with this study at the collection of plants for laboratory test.

Conclusion

The results of this study represent an obvious role of Cu in the alteration of the DNA and secondary metabolites in *V. amygdalina*. The deposit of the Cu metal increases as the spiked concentrations increased, with the leaf sample accumulating more of the metals. The effect on phytochemicals is revealed in quantity and quality of the phytochemicals present before and after spiking. In addition, the reduction in the DNA bands also shows the negative impact of Cu at elevated concentrations. However, the mechanism of translocation and hormonal changes in this plant are still subjected to further studies.

Acknowledgements

Competing interests

The authors have declared that no competing interests exist.

Authors' contributions

A.A. conceptualised the idea, designed the project and corrected the manuscript; B.J. performed the experiment and prepared manuscript; O.N.A. interpreted data, corrected manuscript and supervised the research; A.O. supervised the student, interpreted data and corrected manuscript.

Funding information

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Data availability statement

Experimental data are available for future referencing from the corresponding author.

Disclaimer

The views and opinions expressed in this article are those of the author(s) and do not necessarily reflect the official policy or position of any affiliated agency of the authors.

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