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Bioaccumulation of Heavy Metals in *Cucurbita maxima* Duch. and *Telfairia occidentalis* Hook. F. Grown on Crude Oil Polluted Soil

Okon Okon Godwin

Department of Botany and Ecological Studies, Faculty of Science, University of Uyo, Uyo, Nigeria

Email address
okjunior4zeeb@gmail.com

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Abstract
This research examines the effect of crude oil polluted soil and heavy metal uptake and accumulation in crude commonly consumed vegetables *Cucurbita maxima* and *Telfairia occidentalis* grown and harvested at both crude oil polluted and unpolluted sites and the health implications of consuming these food crops. Heavy metal concentrations of *Cucurbita maxima* and *Telfairia occidentalis* were analyzed. Results showed higher increased values of Cr, Cu, Cd and Zn in both plants when compared to results obtained from the ones planted on unpolluted soils indicating that there is significant difference between both soils. Zn and Cu showed high accumulation in both food crops suggesting that there is a likely possibility of Zn and Cu poisoning if contaminated food crops are ingested. This study revealed that crude oil pollution negatively affected the Physiochemical Properties of the Experimental Soils, increased heavy metal accumulation in *Cucurbita maxima* and *Telfairia occidentalis* which are among the most frequently consumed vegetables in Southern Nigeria, reducing its edibility and quality. These heavy metals even at permissible limits with gradual and steady deposition of such heavy metals in the soils, it may build-up to toxicity level. However, *Cucurbita maxima* and *Telfairia occidentalis* is vulnerable to the effect of oil spill and heavy metals pollution which is common in Southern Nigeria as shown from the present study. Thus, these crops should not be cultivated as a sole crop in areas polluted with crude oil and its associated products as plant parts can serve as sink to heavy metals which are dangerous to human health.

1. Introduction

Crude oil and petroleum product is a major source of energy in Nigeria and the world at large. Oil plays a vital role in shaping the economic and political future of the world. Petroleum industry has created economic boom for Nigeria and at the same time environmental and socio - economic problems [1]. The environmental impact of oil exploration and exploitation is one of the inevitable consequences of economic development and civilization in a technical age [2]. Various activities in crude oil exploration, exploitation, storage and transportation lead to spillage of oil to the environment. Crude oil spills/pollution is a common event in Nigeria and occurs due to a number of causes, including corrosion of pipelines and tankers (accounting for 50% of all spills), sabotage (28%), and oil production operations (21%), with 1% of the spills being accounted for by inadequate or non-functional production equipment [3]. The largest contributor to oil spill, corrosion of pipes and tanks, is the rupturing or leaking of production infrastructures that are described as, "very old and lack regular inspection and maintenance" [3]. The spilled oil pollutes soils and the soils to be less useful for agricultural activities with soil dependent organisms being adversely affected [4]. Oil spillage is the release of a liquid petroleum hydrocarbon into the environment due to human activity [5]. Crude oil spills from vandalized pipelines and pipeline rupture, oil well blow outs, tanker accidents etc. have damaged natural ecosystems in the Niger Delta region in Nigeria and many other places. The quantity of oil spilled during accidents has ranged from a few hundred tons to several hundred thousand tons. This discharge of crude oil on land affects the physicochemical properties of the soil thus causing deleterious effects on plant germination and growth [6].

The physical and chemical properties of the soil undergo major changes, which affects the growth of plants after soil pollution by oil [7]. Some important soil factors which are affected include soil water potential, soil atmosphere, exchangeable Fe and Mn, total nitrogen, available phosphorus and sulphur status in the soil [7].

Many studies have examined the effects of crude oil exploration and production on plants. Physiological defect on plant grown on oil polluted soil as a result of interference with photosynthesis and transpiration probably by blocking the stomata [4, 8]. During oil spills the surface of vegetation is coated [1]. This coating reduces physiological activities of the plant since the stomata are blocked, thus, preventing transpiration and limiting photosynthesis [8].

Cucurbita maxima are a coarse, prostrate or climbing, annual, herbaceous vine, reaching a length of 4 meters or more. Leaves are hispid, rounded, 15 to 30 centimeters in diameter, heart-shaped at the base, shallowly 5-lobed, with finely toothed margins, and often mottled on the upper surface. Flowers are bell-shaped, erect, yellow and about 12 centimeters long; the corolla limb is about as wide, and 5-toothed. Fruit is large, variable in shape, fleshy, with a yellow pulp. Seeds are ovoid or oblong, compressed, and about 1.3 centimeters long [10]. It is often not easy to distinguish *Cucurbita maxima* plants or fruits from the related *Cucurbita moschat*a and *Cucurbita pepo*. The plant habit is similar and the fruit shape and size are variable. Distinction is easiest by observing differences of the fruit stalk, stems and leaves. *Cucurbita maxima* have a soft, rounded fruit stalk not enlarged at the apex, soft, rounded stems and soft, usually unlobed leaves [11]. *Cucurbita maxima* pumpkins are grown in the tropics from the lowlands up to 2000m altitude. Optimum mean daily temperatures for growth are 18 – 27°C. *Cucurbita maxima* are more tolerant to low temperatures than *Cucurbita moschata*, but less tolerant to high temperatures. It is almost insensitive to photoperiod and tolerates some shading. *Cucurbita maxima* are rather drought tolerant, requiring relatively little water, and is sensitive to frost and water logging.

Telfairia occidentalis is member specie of the family Cucurbitaceae commonly consumed in West Africa. It is commonly known in Nigeria as Ugu and Ubong/Mbong by the Ibibios of Akwa Ibom State, Nigeria. It is a prostrate or climbing plant with lobed leaves and twisted tendrils.

Crude oil spillage has a major impact on the ecosystem into which it is released and may constitute ecocide. Immense tracts of agricultural lands have been destroyed. An estimated 5 to 10% of Nigerian agricultural lands have been wiped out either by settlement or oil [3]. It is pathetic that a once rich agricultural environment is being impoverished and made useless by crude oil spillage, thus, this study will aid in understanding the effect of crude oil spillage on crops and the possibility that the crops harvested could contain heavy metals.

2. Material and Methods

2.1. Study Area

This research was carried out in Uyo Local Government Area of Akwa Ibom State, Nigeria. Uyo is a city in South-South Nigeria found between latitude 5.02°N and longitude 7.92°E; it has an average temperature of 25.1-27.8°C and found on latitude 5.02°N and longitude 7.92°E (line 2), an annual rainfall of 3300 mm with the land mass of 95 km^2 and the population of 1,400 thousand persons/km^2. The plants (*Cucurbita maxima* and *Telfairia occidentalis*) used for this research work were identified by a Plant taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria. Samples of the plants used for this research were also deposited at the Department of Botany and Ecological Studies Herbarium.

2.2. Sources and Collection of Samples (Seeds, Soil and Crude Oil)

The matured seeds of *Cucurbita maxima* and *Telfairia occidentalis* were collected from Akwa Ibom State Agricultural Development Project (AKADEP) and local farmers in Uyo. The obtained seeds were pretreated by picking out infected seeds. The viable ones were used for the research. The sandy-loam soil that was used in the research was obtained from the Research garden of the Department of Botany and Ecological Studies, University of Uyo. The soil was sterilized for two hours at 100°C and sieved through a 2mm mesh to remove pebbles. Also, the crude oil was obtained from Nigerian National Petroleum Corporation (NNPC) Uyo, Akwa Ibom State.

2.3. Sterilization of Seeds and Planting

Seeds were surface sterilized with 0.01% mercuric chloride solution for 30 seconds, thoroughly washed several times with sterile distilled water and air-dried [12]. During this treatment, floating seeds were discarded while the good ones were used for the research. Five (5) seeds of *C. maxima* and *Telfairia occidentalis* were sown directly in each perforated polythene bag containing polluted and unpolluted.

2.4. Physicochemical Properties of the Soil

The physicochemical properties of the soils (unpolluted and polluted soil) were determined according to the recommended methods of AOAC, 2005 [13].

About 1g of sandy loam soil from the research garden was weighed into 300 ml conical flask; 10 ml of nitric acid was added. The mixture was digested at 130°C in fume cupboard until the solution appeared colourless with a slight increase in temperature. After digestion, white fume of $HCLO_4$ appeared and silica became white, this was not taken to dryness. The flask was removed and cooled sufficiently to avoid spattering. Fifty (50 ml) milliliter distilled water was added and the solution was filtered into 100 ml volumetric flask.

The solution was stored in sample bottles and transferred to Aluminum Smelting Company Limited (ALSCON), Ikot Abasi Local Government Area, Akwa Ibom State, Nigeria for determination of physicochemical properties of the soil using Atomic Absorption Spectrophotometer (AAS) AOAC, 2005 [13].

2.5. Samples Preparation for Analysis

The fresh roots and leaves of *Cucurbita maxima* and *Telfairia occidentalis* were air dried for one week and reduce to a coarse powder form; about 700g was macerated with 1000 ml of cold 70% ethanol and shaken intermittently for 72 hours. It was filtered and the filtrate was concentrated (dried) *in-vacuo* at 40°C in a water bath. The extract was weighed and stored in 100 ml beaker and covered with foil paper and stored in the refrigerator at 4°C for use in heavy metal analysis.

2.6. Heavy Metal Analysis

The powdered samples were digested with very strong acids, perchloric acid ($HCLO_4$) and nitric acid (HNO_3) and this is called Wet digestion method. The digested samples would be read using Unicam 939 Atomic Absorption Spectrophotometer.

Wet digestion: 1g of the powdered sample was weighed into a digestion flask, 20 ml of nitric acid was added and 10 ml perchloric acid was also added, a drop of concentrated Sulphuric acid added also. The mixture was allowed to stand for 30 minutes and then digested using digestion chamber in the laboratory until the colour turned white. This showed that the samples were digested. They were allowed to cool. Thirty (30) ml of distilled water were added and were filtered. The filtrate was made up to 50 ml solution with distilled water. The digested solution was stored in a sample bottle ready for the determination of heavy metals using atomic absorption spectrophotometer.

The concentrations of Cadmium (Cd), Chromium (Cr), Copper (Cu), and Zinc (Zn) in the powdered samples were determined according to the recommended methods of AOAC, 2003 [14].

3. Results

3.1. Physicochemical Properties of the Soil

The physicochemical properties of the experimental soil indicated that the sandy loamy garden soil had high C/N ratio, exchangeable potassium, exchangeable acidity, electrical conductivity and particle sizes sand, with a pH of 6.81. Organic matter, total nitrogen, available phosphorus, exchange calcium, silt, clay, organic carbon, exchangeable magnesium, exchange nitrogen, exchange potassium, bases saturation, and effective cation exchange capacity were relatively low (Table 1).

The crude oil polluted soil was high in organic matter, total nitrogen, available phosphorus, exchange calcium, clay, exchange magnesium, organic carbon, exchange nitrogen, effective cation exchange capacity and bases saturation, with a pH of 6.00, while C/N ratio, exchange potassium, exchange acidity, electrical conductivity and particle sizes sand were low (Table 1).

Table 1. *Physiochemical Properties of the Experimental Soils.*

S/No.	PARAMETERS	CONTROL (Garden soil)	POLLUTED SOIL
1	pH	6.81	6.00
2	Organic matter (%)	1.98	3.86
3	Total nitrogen (%)	0.07	0.38
4	Available P. (mg/kg)	36.77	59.00
5	Ex. Ca (cmol. /kg.)	3.25	5.52
6	Silt (%)	4.00	6.00
7	Clay (%)	4.20	8.00
8	Ex. Mg. (cmol. /kg.)	1.30	2.00
9	Organic carbon	1.61	3.09
10	Ex. N. (cmol. /kg.)	0.07	0.09
11	Ex. K. (cmol. /kg.)	0.94	0.33
12	Exchange acidity	3.58	1.76
13	ECEC. (cmol. /kg.)	6.85	9.55
14	Bases saturation	64.81	75.07
15	C/N ratio	19.27	12.89
16	Particle sizes sand (%)	92.04	79.00
17	EC. (gs/m)	0.057	0.0453

Key:
Ex – Exchange
ECEC – Effective cation exchange capacity
C/N – Carbon/Nitrogen ratio
EC – Electrical conductivity

3.2. Heavy Metal Content of *Cucurbita maxima* Grown on Crude Oil Polluted Soil

Heavy Metal Content of *Cucurbita maxima* Grown on Crude Oil Polluted Soil revealed that zinc and chromium had the highest elemental content with increased crude oil levels, with 50 ml recording the highest accumulation of zinc and chromium when compared to the control (Figure1). Similarly, copper and cadmium showed slight increase with increasing concentration of crude oil (Figure 1).

3.3. The Effect of Crude Oil Pollution Treatment on Heavy Metal Content of *Telfairia occidentalis*

The effect of crude oil pollution on heavy metal content of

Telfairia occidentalis revealed that copper and cadmium had the highest elemental content with increased crude oil levels, with 50 ml recording the highest accumulation of copper and zinc when compared to the control (Figures 2). Similarly, zinc and chromium showed slight increase with increasing concentration of crude oil (Figure 2).

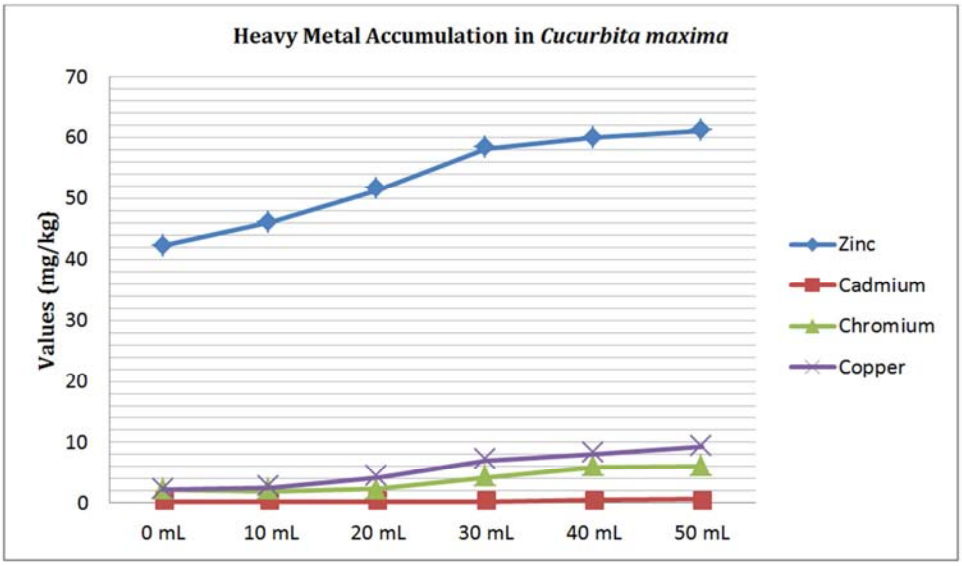

Figure 1. *Heavy Metal Content of Cucurbita maxima Grown on Crude Oil Polluted Soil.*

Key: mg/kg = milligrams per kilograms
mL = milliliter

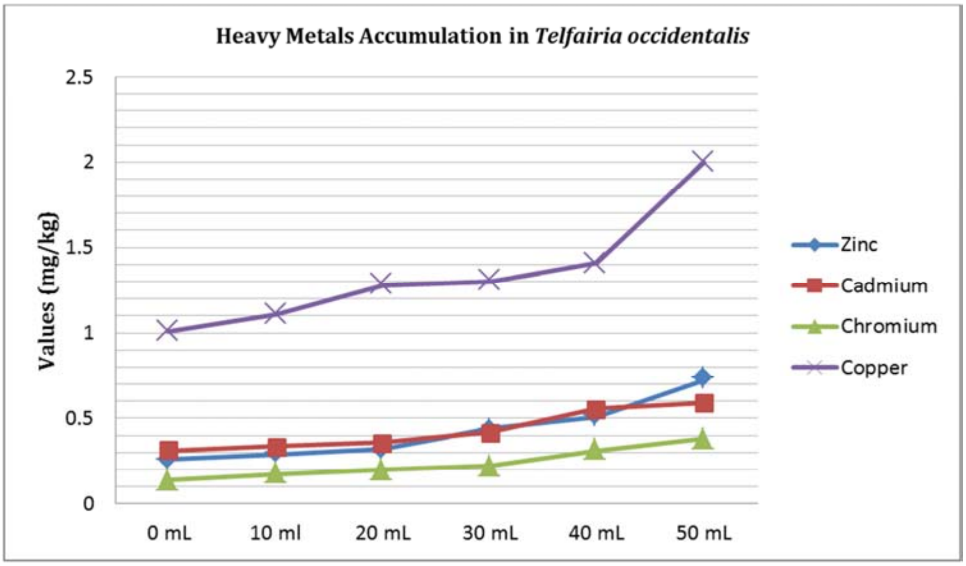

Figure 2. *The Effect of Crude Oil Pollution Treatment on Heavy Metal Content of Telfairia occidentalis.*

Key: mg/kg = milligrams per kilograms
mL = milliliter

4. Discussion

The effect of crude oil pollution on heavy metal accumulation of *Cucurbita maxima* revealed increasing concentrations of Zinc, cadmium, copper and chromium with increased crude oil pollution levels, with zinc showing the highest concentration. This observation agrees with the findings of Agbogidi *et al.* [15] who reported the accumulation of iron, zinc, cadmium, manganese, lead and cadmium contents in maize (*Zea mays* L.) grown in soil contaminated with crude oil which led to poor growth and yield reduction. Lead and cadmium prevents mineral uptake by either synergistic or antagonistic reactions leading to poor growth [16]. Similarly, Ogbuehi *et al.* [17] who reported the accumulation of zinc, lead and nickel on seeds and leaves of groundnut *(Arachis hypogea)* grown on spent engine oil polluted soil. Also, Ogbuehi *et al*. [18] Ogbuehi *et al.* [19] and Dushenkove *et al.* [20] reported separately that certain plants including cassava can extract heavy metals from crude oil polluted soils. Ekundayo *et al*. [9] studied the effect of crude oil spillage on growth, productivity and nutrient uptake of maize (*Zea mays* L.) The results showed that in crude oil polluted soils, germination was delayed and the germination percentage was significantly affected by oil pollution.

Chukwuemeka *et al*. [21] reported the accumulation of Nickel, Vanadium, Cadmium, Lead, Manganese, Iron, Cobalt and Zinc in *Telfairia occidentalis*. Osu *et al*. [22] corroborates this fact by stating that *Vernonia amydalin*, *Telfairia occidentalis* and *Amaranthus* are good accumulators of heavy metals. Heavy metals found in food crops, have a potential hazard to man through the dietary pathway [23]. Furthermore, Benson *et al.* [24] reported that the poor growth of crops in higher levels of oil treatment was due primarily to the toxic effects of heavy metals on mineral uptake. The amounts of some of these metals in soil samples/vegetables studied here are below permissible limits (Table 2) given by Food and Agriculture Organization [25, 26] but with gradual and steady deposition of such heavy metals in the soils, it may build-up to toxicity level.

Table 2. *FAO/WHO Permissible standards of Heavy Metals used in this Study.*

Heavy Metals	WHO/FAO Standards
Zinc	6.0
Cadmium	0.1
Chromium	0.1
Copper	0.1/0.2

Key:
FAO = Food and Agriculture Organization
WHO = World Health Organization

5. Conclusion

The results from this study revealed that crude oil pollution negatively affected the Physiochemical Properties of the Experimental Soils, increased heavy metal uptake of *Cucurbita maxima* and *Telfairia occidentalis* which are among the most frequently consumed vegetables in Nigeria, reducing its edibility and quality. From this study, However, *Cucurbita maxima* and *Telfairia occidentalis* is vulnerable to the effect of crude oil spill and heavy metals as shown from the present study. These crops should not be cultivated as a sole crop in areas polluted with crude oil and its associated products as plant parts can serve as sink to heavy metals which are dangerous to human health.

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