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VARIATIONS IN FOOD CONTENT OF CROPS AS INFLUENCED BY BIO STIMULANTS IN AN OIL CONTAMINATED SOIL

Offor, U.S,
Akonye, L.A
Asouzu. I. A.
Ignatius Ajuru University of Education Port-Harcourt.
Faculty of Plant Science and Biotech University of Port Harcourt.

Abstract

A study was carried out at the University of Port Harcourt botanical garden, to determine the influence of bio stimulants on food content of crops grown at two levels of crude oil contamination. Results showed that bio stimulants (sawdust and chromolaena leaves) enhanced protein and carbohydrate accumulation in the crops. However, chromoleana leaves was more effective than sawdust while the test crops-Z. mays and Vigna unguiculata differed in their accumulation potentials in protein and carbohydrate.

Keywords: Protein and carbohydrate synthesis, biostimulants, oil degradation and plants response.

Introduction:

In Nigeria, the origin of oil spill is synonymous with the discovery of oil in 1956 at Oloibiri in the old River-State (now Bayelsa). Issues over the biological effect of the increasing oil spillage on land and water has mounted from the beginning of oil prospecting in Nigeria (Odu 1982). Records show that the rate of oil spillage has been rising with increasing tempo of petroleum production. Only a single spill was reported in this country in 1970, whereas in 1971, the number escalated to 14 and 105 in 1974 as stated by NEST (1991). Although the crude oil itself used to be the economic mainstay of many countries.

In Nigeria, oil exploration and exploitation has brought about questionable profits and benefits to the nation. This has created awareness on the inhabitants realizing that pollution impact on them directly through effect on food supply, degradation of buildings and other items of cultural heritage

as well as effects on forests, rivers, coastlines and other ecosystems that are familiar (Alloway, 1985). The cost arising from these effects especially depreciation of resources and lost of productivity are very high. Researchers, Okpokwasili and Odukuma (1994) Okpokwasili and Numbia(1995), Isirimah *etal* (1989,) kinako and Zuofa (1991), have also studied large scale contamination on their effects on different biota which includes contamination of habitats, public health and socio-economic hazards. For possible elimination of these effects, it is imperative to clean up pollutants from the environment by applying remedial measures (Ellis, balba and Thaile 1990) which are cost effective in relation to crops that are of most value to mankind. It is on record that in areas where exploration is high, the most cultivated and consumed crops are cereals and legumes.

The use of chromolaena leaves and sawdust as cost effective remedial/bio stimulants have been documented (Offor and Akonye 2006.The choices of Chromolaena leaves and sawdust was due to their ability to contribute to carbon reserve and cache in soil, ability to increase organic matter and absorb oil films, uphold oil for a long time and provide enabling environment for microbial survival, a necessary instrument for contaminants removal and remediation.

Vigna unquiculata is assumed to have more than 22 to 120 species existing in India and Africa. The stems are glabrous and variation in stem habit exist. The leaves are trifoliate usually 3 in number and the inflorescence is an auxiliary racine. About 4 flowers exist in the tip and the flowers are purple or white in colour. The crop is self pollinated but cross pollination is possible particularly when insects are found in that field. Germination is epigeal and the crop is established by seed adapted to a wide range of soil with medium fertility. Rainfall range of 250 – 1000mm per year is ideal for its successful growth and is mostly grown in rotation with crops like millet and sorghum. Inoculation is necessary for seeds while a minimum of two weeding is recommended. The crop may be harvested 3 – 5 months after sowing.

Z. mays, popularly known as maize or corn is an important grain of the world ranking after wheat. It originated from Mexico or Central America (Offor and Ausa 1998). It is a coarse annual grass belonging to the family graminea. The root system consists of Seminal Secondary or coronal or crown and aerial roots. It is usually a warm weather crop grown throughout the year. Its water requirement varies with the type of soil and the season. Rainfall requirement is about 620mm. Irrigation may be required during rainy season whereas the soil moisture falls below the desired level. Maize contains mainly carbohydrate but significant quantities exist in protein, oil and small amounts of minerals. The oil is found in the embryo, composition of maize is approximately 76 – 88% carbohydrate, 6 – 15% protein 4 – 5%,

fat and 1 – 3% mineral. It is prepared and consumed in many ways most of them can be grouped as

- Ground or pounded and boiled
- Ground or pounded and baked or fried
- Boiled whole and roasted whole.

In Nigeria it is consumed in two forms in Ogi and Agidi, As livestock feed, stalk, leaves and in mature ears are used as fodder. *Z. mays* has industrial importance for the production of starch, oil and alcohol. The qualities of these crops make it imperative to research on any factor that may hinder its production especially under environmental stress hence this study.

Materials and Methods:

The study was conducted at the botanical garden of the University or-Port-Harcourt. The seeds of *Z. mays* and vigna unquiculata were procured from the Agricultural Development Project, (ADP) Rumuodumaya, Port-Harcourt, in River-State. The crude oil was supplied by Nigerian Agip oil company Ebocha base (bonny type) Port-Harcourt. Chromolaena Leaves and sawdust were obtained from a Local farm along the university axis.

A good' garden soil weighing' approximately 600kg was obtained and use to fill black cellophane bag of equal diameter measuring 50cm and height 45cm leaving a space of 7 .00cm from the top end of the polythene bag to make allowance for crude or addition of amendment and water. Heavy looking seeds which has previously being tested for viability (96%) were first soaked in acohol for 30 seconds to kill seed pathogens and then soaked in water for 24hrs before being sown deeply in the cellophane bag, about 4-5 seeds per bag. 200ml and 400ml of crude oil representing mild and severe pollution were added and thoroughly mixed with the soil using a hand trowel. Sawdust and chromolaena leaves were used as mitigants at the rate of 50g per bag. The chromolaena leaves were previously chopped with knife to ease mixing. The treatments were separated as follows:

>6% pollution (severe)
>6% pollution treated with sawdust
>6% pollution treated with chromolaena leaves
>3% pollution (mild)
>3% pollution treated with chromolaena leaves
>3% pollution treated with sawdust
>control with sawdust
> control with chromoleana leaves
> control

The experiment was constituted in a randomized complete block design (RCBD), each treatment replicated four times.

Cultural details:- After planting, the seedling was thinned to 5 seedlings of equal growth and height making sure they were evenly spaced. The cellophane bags were perforated and separated according to the different pollutant level and mitigation agents.

Determination of carbohydrate content.

The leaf sample was analyzed for carbohydrate content using the cleq Anthrone method 1. 08 of the samples were dissolved in 13m I of 52% (v/v) perchloric acid. The mixture was allowed to stand for 30 minutes and then diluted to 250mI with distilled water. 10mI of thefiltrate of the above solution was pipetted and made up to 100ml with distilled water. 1ml of this was then placed in test tube into which was placed 5ml of 0.1% anthone solution in concentrated· sulphuric acid. The resultant colour was absorbed at 630mml wave length against a standard glucose solution of 1 mg/ml concentration using SP6 pye Unican spectrophotometer.

The concentration of the carbohydrate was calculated by using the formula as state below:

$$Percentage\,carbohydrate = \frac{25 \times absorbance\,of\ Samples}{Weight\,of\ Sample \times absorbance\,of\ s\tan dard\ glu\cos e}$$

ii. Determination of total Nitrogen and crude protein

The leaf sample were analysed for total Nitrogen content using kjedahl methods (Osborne and Vogt 1978). The digestion reagents comprised of a catalyst blend of 1.09 Cu $S0_4$ $5h_20$ and 15.OgN_{a2} $S0_4$ with concentrated Sulphonic acid. Leaf samples were placed in 500ml Kjechahl flask. Then 2. Og of catalyst blend and anti bumping chips were added. 25ml of concentrated sulphuric acid was added to eachsample. The mixture were heated gently (to avoid frothing) in an electro-thermal kjedahl digestion unit. The heating temperature was increased until the charred particles disappeared and the mixture become clear. The digestion was completed after 2 hours, the flask were left to cool and the digests were transferred into 100 ml volumetric flask and were made up to the 100ml mark with distilled water. By steam distillation in the presence of excess alkali (NaOH) free ammonia was librated from the digestible sample. 25ml of 2% boric acid solution ($H3B0_4$) containing 3 drops of methyl red indicator were added to a1O0ml beaker. The beaker was placed under the *tip* of-the condenser from a distillation unit ensuring that the outlet tip was below the surface of the boric acid solution. The condenser was then connected to a cold water supply.

An aliquot of the 2.5ml of the digest was added to the kjedahla flask and to this was added 40ml of NaOH solution. The flask was then quietly connected to the distillation unit in order to avoid loss of ammonia. The

electrical unit was stopped after collecting thrice theoriginal volume by which the colour of the solution had changed from pink to light green.

The volume collected was titrated against 0.1 NHCL in a conical flask until the first appearance of permanent pink colour was observed, The total nitrogen present was calculated as follows:

$$\text{Percentages Nitrogen} = \frac{T.V \times 1.4 \times 50 \times 100}{0.1 \times 20 \times 100}$$

Where T.V = Nitrogen volume (Titre)

1.4 = Nitrogen equivalent of the molarity of theHCL used in the titrametric analysis.

50 = Total dilution of the sample volume

0.1 = Dry weight of the sample

0.2 - Volume of *the* aliquot used in *the* analysis

100 = conversion factor from gram to milligram.

iii. Total protein

This was calculated by multiplying the value of protein nitrogen by 6.25 (since nitrogen constitute 16% of protein)

Data analysis: Data collected were subjected to analysis of variance and Duncan multiple range text (DMRT) was employed to separate means according to the procedure of statistical analysis system SAS (1991) the standard error bars (SE) is at 5% probability ($P<0.05$)

RESULTS AND DISCUSSION.

The result on the crude protein (total nitrogen) total protein and carbohydrate contents of the crops- *vigna unquiculata a*nd *Z. Mays* as influenced by biostimulants in a crude oil contaminated soil are presented in Table1.

From the result, the total Nitrogen content of *vigna unquiculata* in all treatment were significant with addition of chromolaena leaves in all level of pollution Application of sawdust also enhance total nitrogen accumulation especially at mild oil concentration. The control showed significant increase in total Nitrogen accumulation compared to pollution without remediation.

With *Z. mays*, the total Nitrogen improved significantly with presence of biostimulants. At 6% pollution, application of chromolaene leaves gave higher significantly value than saw dust. The result in mild (3%) oil concentration showed no significant difference with pollution without amendments. The result further indicates a higher accumulation of total Nitrogen in *vigna unquiculata* than *Z. mays*.

In total protein, similar trend as in crude protein (total Nitrogen) was obtained in all treatments with the crops. The result in carbohydrate content

shows that addition of biostimulants (chromolaens leaves and sawdust) improves its contents in *vigna unquiculata.* Presence of chromolaena leaves equally gave significant value at severe and mild oil concentration and control treatments. least carbohydrate content of the crops was observed at severe pollution untreated.

With *Z. mays,* the result indicates corresponding significant increase in carbohydrate content with presence of biostimulants at various level of treatments. Treatment with chromolaena leaves at severe oil concentration showed significant increase in carbohydrate content than at mild oil concentration. However, at control plus sawdust, carbohydrate content in *Z. mays* increased significantly compared to 3% pollution with chromolaena leaves. In all, the results with chromolaena leaves exhibit a higher significant increase in carbohydrate content in *Z. mays* than *vigna unquiculata*.

Carbohydrate is the first product of photosynthesis which means that any distortion in the photosynthesis process will definitely affect the accumulation of carbohydrate and other classes of food (protein and lipids).

Addition of biostimulants to the polluted soil enhanced carbohydrate accumulation with time in *Z. mays* and *vigna unquiculata*. The extent of carbohydrate enhancement depends on the type of biostimulant used.

The improvement is expected since variations exist in the leaf chlorophyll content of the crops used in the study and the nature of the biostimulants too. Highest carbohydrate content was observed in all treatments with addition of biostimulants than control and polluted soil untreated. This might be due to some hydrocarbon molecules still found in these treatment which are later incorporated into the plant tissues interfering with carbohydrate accumulation in the plants. (Dennenman and Robberse, 1990, Chen etal 1998).

The decline in carbohydrate content in treatment at severe and mild pollution with our amendments is expected since high amount of oil traceable within these treatments may have been toxic to chlorophyll synthesis. The toxic nature of crude oil on enzymic, photo- hormonal and biochemical processes leading to the synthesis of carbohydrate, protein and lipid have been reported. (Baker and Brooks 1989). Also as a result of low nitrogen content (i.e reduction in chlorophyll content) induced photosynthesis. The resulting slow down of photosynthesis causes a nitrogen deficient plant to lack the machinery for synthesis of necessary carbohydrates and carbon skeletons for all manner of organic synthesis.

Among the crops. *Z. mays* exhibits higher potential in carbohydrate accumulation than *vigna unquiculata.* The explanation of these differences should be that *Z. mays* is able to cope with environmental stress caused by crude oil pollution such as water, oxygen and nutrient shortages and development of more efficient ways of utilizing factors for better

performance in carbohydrate synthesis, this could be due to its genetic composition in that they posses genes that made them to adapt to stressful condition and carbohydrate synthesis than *vigna unquiculata.*

The crude protein and total protein revealed similar results as in the carbohydrate content in relation to amendments. Improvement in protein content of *vigna unquiculata* and *Z. mays* was observed with time. The increment is expected due to the addition of the bio-stimulants. This is possible because protein contain approximately 18% nitrogen and a constituent of amino acid, nucleotide and co-enzymes. Nitrogen plays an important role in protein synthesis thus, it is clear that conditions that will favour those processes will enhance protein accumulation. (Hamson *etal*1977).

Chromolaena leaves experience the highest level of protein enhancement at severe and mild oil pollution than sawdust. The cause might be traceable to its biochemical and physiological constituent which contain Nitrogen in its soluble form which are easily absorbed by plants and incorporated into the body tissues. Result also shows that variations exist among crops – *Z. mays* and *Vigna Unquiculata* in their accumulation of protein for each treatment option. In many of the treatment option, it was observed that *Vigna unquiculata* accumulate more protein than *Z. mays*. This can also be attributed to its physiological and genetic constitution in which *vigna unquiculata* has the ability to effectively synthesis other nutrients from primary product under stressful conditions (Stewart and Lee 1974). This study recommends that biostimulants are effective in promoting synthesis of protein and carbohydrates in legumes and cereals grown under environmental stress.

Table 1: Effects of treatments on food content of crop (a) *Vigna unquiculata (b) Zea mays*

	Vigna unguiculata			*Zea mays*		
Treatments	Crude Protein (Total N_2	TP (mg/mg dry wt)	CHO (mg glucose g^{-1})	Crude Protein (Total N_2	TP (mg/mg dry wt)	CHO (mg glucose g^{-1})
6% pollution Untreated	0.17 ± 0.01^{d}	1.06 ± 0.08^{d}	0.13 ± 0.02^{bgi}	0.14 ± 0.03^{ed}	0.52 ± 0.03^{d}	0.14 ± 0.03^{ed}
6% pollution + sawdust	0.19 ± 0.02^{bcd}	1.20 ± 0.10^{bdac}	0.14 ± 0.02^{bc}	0.18 ± 0.08^{cb}	0.53 ± 0.04^{d}	0.18 ± 0.05^{cb}
6% pollution + chromolaena	0.23 ± 0.02^{a}	1.36 ± 0.09^{a}	0.16 ± 0.03^{b}	0.21±0.19a	0.78 ± 0.08^{bc}	0.21 ± 0.04^{a}
3% pollution untreated	0.19 ± 0.01^{bcd}	1.17 ± 0.08^{bdc}	0.07 ± 0.01^{c}	0.19 ± 0.19^{b}	0.73 ± 0.08^{bc}	0.19 ± 0.19^{b}
3% + pollution + sawdust	0.21 ± 0.01^{ba}	1.29 ± 0.12^{bae}	0.15 ± 0.02^{bc}	0.17 ± 0.02^{cb}	0.63 ± 0.05^{c}	0.17 ± 0.02^{cb}
3% pollution + chromolaena	0.21 ± 0.02^{ba}	1.34 ± 0.07^{ba}	0.16 ± 0.04^{b}	0.15 ± 0.02^{cb}	0.79 ± 0.05^{bc}	0.15 ± 0.02^{cb}
Control	0.20 ± 0.02^{bc}	1.13 ± 0.07^{dc}	0.14 ± 0.02^{bc}	0.17 ± 0.02^{cb}	0.83 ± 0.06^{a}	0.17 ± 0.01^{cb}
Control + sawdust	0.17 ± 0.02^{cd}	1.08 ± 0.11^{d}	0.16 ± 0.02^{b}	0.16 ± 0.02^{cb}	0.81 ± 0.05^{bc}	0.16 ± 0.02^{cd}
Control + chromolaena	0.21 ± 0.01^{ba}	1.29 ± 0.07^{bac}	0.25 ± 0.05^{a}	0.12 ± 0.01^{e}	0.93 ± 0.09^{a}	0.12 ± 0.01^{e}

Note = CP = Crude protein, (Total N_2), TP = Total protein, CHO = Carbohydrate.
* Within columns mean ± SEM with different superscripts are significant different at $P<0.05$.

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