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Morphological Variations in the Accessions of *Colocasia esculenta* (L.) Schott. and *Xanthosoma maffafa* (L.) Schott. Exposed to Sodium Thiosulphate and Graphite Powder Treatments in Nigeria

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Authors' contributions

This work was carried out in collaboration between all the authors. Author FOA carried out the field and laboratory works, performed the statistical analyses, managed the analyses of the work, literature searches and wrote the first draft of the manuscript. Author JOO designed the study and supervised the field work. Author GOA managed the protocol and determined the concentration of the chemicals applied as treatments in the field work. All the authors read and approved the final manuscript.

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ABSTRACT

Aim: To investigate the morphological variations in the accessions of *Colocasia esculenta* and *Xanthosoma maffafa* exposed to two oilfield chemicals.
Study Design: The Randomized Complete Block Design was used for this study.
Place and Duration of Study: The Ecological Research Centre of the University of Port Harcourt, Nigeria between April 2015 and June 2016.
Materials and Methods: Graded quantities of 5, 10, 20 and 40 mg/kg of sodium thiosulphate and graphite powder were applied to each accession while the control experiment lacked the oil-field chemical additives.
Results: Observations indicated that the differences in plant height, leaf area, girth and yield between treatments were not significant at 5%, but the differences in plant height, leaf area and yield between accessions were significantly different at *P*=.05. The differences in girth among accessions were however, not significant at *P*=.05. Accessions treated with graphite powder had higher values of mean height, leaf area and girth compared to accessions treated with sodium thiosulphate. Accessions treated with sodium thiosulphate had higher mean yield than those treated with graphite powder. Other morphological variations observed as a result of the chemical treatments included malformed leaves, different number of leaves per stand, stem colour difference and plant death.
Conclusions: Cocoyam accessions have been established to be poor environmental bioindicator. Further studies were encouraged in order to obtain a clearer understanding of the potentials of the chemicals employed in the present study to cause other invisible genotoxic effects on the test plants.

Keywords: Oilfield chemicals; aroids; environment; plant height; leaf area.

1. INTRODUCTION

The Araceae comprises a family of herbaceous monocotyledons aroids composed of 110 genera and 2000 species [1], and varies predominantly from tropical to subtropical in distribution. In Nigeria, the widely cultivated Aroids are *Colocasia* (taro) and *Xanthosoma* (tannia). *Colocasia* is believed to have originated from South-east Asia, from where it spread to Africa and America, while *Xanthosoma* originated from tropical America and spread across West Africa and other parts of Africa [2]. *C. esculenta* and *X. maffafa* commonly called cocoyam in Nigeria has been described by Chukwu and Nwosu [3], as Nigeria's "giant crop" among root and tuber crops of economic importance in the country; hence Azeez and Madukwe [4], suggested that cocoyam is a crop of promising economic value. However, cocoyam has been marginalized over the years and has received minimal attention from agricultural, nutritional, industrial researchers and other stakeholders of interest [1]. Williams and Haq [5], suggested that the crop's supposed association with the poor may be the reason why conventional agricultural research has not paid much attention to it. However, recent reports on structural [6], and cytological [7] studies are providing vital information that could enhance further understanding, exploitation and improvement of the local Nigerian aroids.

Cocoyam cultivation is predominantly in backyards and farms located remotely around or near sites of oil exploration and production. For this reason, the crops are frequently exposed to pollution by oilfield chemicals and associated pollutants.

Oil field chemicals are those chemicals that are in use during oil exploration and production activities. Drilling operations can introduce oil and a wide range of other complex chemical compounds into the environment through drilling fluids and muds. There are several classes of drilling fluids, such as: oil-based, synthetic-based and water-based groups of chemical compounds. Sodium thiosulphate and graphite powder are two important water-based oilfield chemicals used in oil exploration and production activities in Nigeria.

Oil exploration exercises generally have the tendency to pollute the environment through accidental discharges of these chemicals, crude oil spillages and indiscriminate disposal of formation water [8]. As these fluids find their way through the environment, the physical, chemical and microbiological properties of the edaphic environment are affected [9]. Oilfield chemicals are considered to be genotoxic substances because they have the ability to induce xenobiotic responses in the genetic system of some organisms, thereby altering the molecular modes and genetic framework of the organisms. Several chemicals proven to have genotoxic abilities include industrial effluents [10] and petroleum-related substances like oil field waste water [9], crude oil [11], oilfield formation water [12], diesel and gasoline [13], diesel fuel [14]. Hence, the need for proper handling and disposal of oilfield drilling fluid and wastewater has been advocated by many researchers [12].

Sodium thiosulphate is a 'breaker' and acts as a strong oxidizing agent. It is used to depolymerize polysaccharides and break cross links in a way that the viscosity is reduced at a controlled rate thus, enabling the proppant (solid particles used to hold open fracture after conclusion of oil well treatment; have high compressive strength and resistant to dissolution at high temperature and pH) to be deposited in the fracture**.** Graphite powder on the other hand, is a lubricant that offers a means of reducing torque and increases the effective horsepower to the drill bit by reducing friction. Although different genotoxicity assays involving organisms, mammalian cells and other biological entities have been developed, plant bioassay has been proven to have the highest sensitivity [15,16].

Each of the oilfield chemicals of interest has its own peculiar chemistry and induces specific responses in plants. The level of expression of such responses may range from apparent visible deformation to none-phenotypically detectable ones and probably death. It is against this background that the present study was undertaken; to investigate the effects of sodium thiosulphate and graphite powder on the morphology of exposed *C. esculenta* and *X. maffafa accessions*. This study will also examine the ability of cocoyam to serve as an environmental bioindicator.

2. MATERIALS AND METHODS

A total of eight accessions of cocoyam identified and collected from the National Root Crops Research Institute (NRCRI), Umudike were used for this study. Five accessions from *Colocasia esculenta* (NCe 001, NCe 002, NCe 003, NCe 004 and NCe 005) and three from *Xanthosoma maffafa* (NXs 001, NXs 002 and NXs 003). NCe means Nigeria *Colocasia esculenta* and NXs means Nigeria *Xanthosoma* species. Each accession was planted in 4 different concentrations: 5, 10, 20 and 40 mg/kg for each chemical (sodium thiosulphate and graphite powder), while the control experiment lacked the oil-field chemical additives. These chemical concentrations resulted to 0.5, 1, 2 and 4% w/w in soil on weight basis. Each of these accessions was planted in polythene bags containing 10 kg soil and the chemicals were applied by mixing each concentration with 400 ml of water. This mixture was used in watering the plants immediately after planting; the preparations were kept in the open and regularly monitored. Further irrigation of the plants was done twice with 200 ml of water as there was sufficient rainfall throughout the duration of the work. Weeding was by hand-picking. This experiment was set up in a Randomized Complete Block Design at the Ecological Research Centre, University of Port Harcourt.

Observations and assessment of morphological characters of the control and treated plants were carried out five months after planting. Plant heights were obtained by measuring the plants from the soil level to the collar of the uppermost leaf. The leaf areas were determined by measuring the length and width (at the widest point) of each leaf. The product of this was multiplied by a correction factor of 0.75 to cater for leaf shape [16]. The girth of the plants was also measured, while the number of leaves showing malformation, chlorosis and those with different stem colours were visually scored. At maturity, the corms were harvested and weighed. [16].

Data generated were exposed to Microsoft Excel two-way analysis of variance (ANOVA) at P=.05.

3. RESULTS AND DISCUSSION

3.1 Effects on Plant Height

The effects of sodium thiosulphate and graphite powder on the plant heights of all the accessions showed no significant difference at P=.05 across various treatments but there was a significant difference at P=.05 in plant heights between different accessions (Table 2). Apart from the accessions that did not germinate, *Xanthosoma maffafa*, accession NXs 001, reacted poorly to the chemicals, as plant height of 19 cm was observed under 5 mg/kg of sodium thiosulphate

Table 1. Effects of sodium thiosulphate and graphite powder treatments on the plant height (cm) of different accessions

Chemical treatment	Conc. (mg/kg)	NCe 001	NCe 002	NCe 003	NCe 004	NCe 005	NXs 001	NXs 002	NXs 003
Control		29.1	34.0	33.8	28.3	31.0	24.5	26.0	30.2
Sodium thiosulphate	5	35.6	30.9	30.5	_	43.0	19.0	20.0	27.8
	10	33.5	26.0	39.0	44.5	38.5	21.0	_	42.3
	20	35.0	24.0	30.0	32.9	35.8	28.5	23.0	32.0
	40	41.3	35.5	34.4	35.5	32.0	24.0	25.5	30.8
Graphite powder	5	32.0	23.5	30.3	_	32.3	24.0	24.0	33.5
	10	35.0	30.0	44.5	43.0	48.5	27.4	34.3	32.0
	20	35.0	35.9	27.9	42.5	40.0	19.0	40.8	33.0
	40	33.0	36.0	30.5	40.0	27.0	_	27.0	29.4

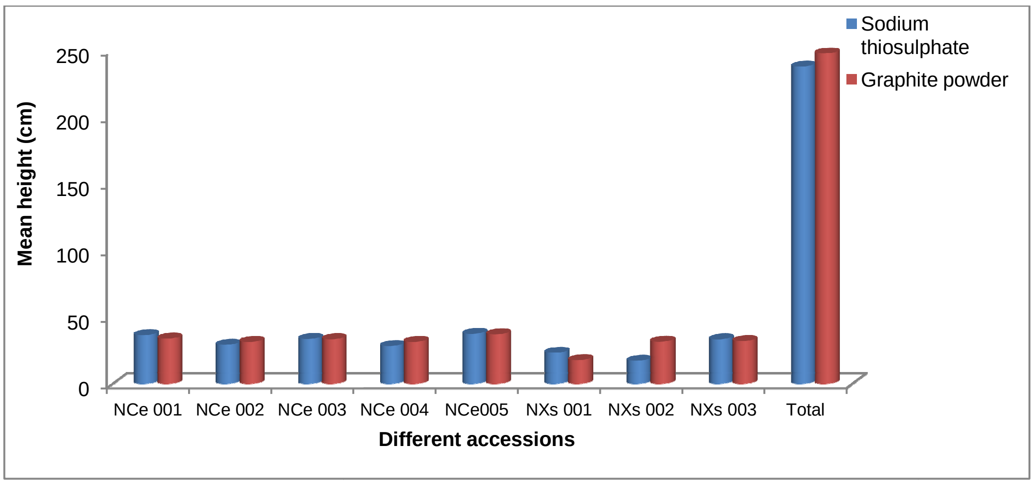

Fig. 1. Effects of sodium thiosulphate and graphite powder on the mean heights of different accessions

and 20 mg/kg of graphite powder treatments (Table 1). However, accession NCe 001 responded well to the chemical treatments; the accession responded well to the two chemical treatments that even at higher concentrations had taller plant than the control. This indicates that the metabolism of different accessions is not the same. This agrees with the findings of Osuji and Nwala [7], who stated that variations within these two species (*C. esulenta* and *X. maffafa*) indicate their unique ecological adaptations to different environmental conditions. Furthermore, each accession reacted differently to different chemicals as the mean height of all the accessions did better in graphite powder treatments than in sodium thiosulphate treatments (Fig. 1). This shows that plants can differentially indicate chemicals that are more toxic to the environment than the other. Njoku et al. [17] reported differential effects of diesel fuel and spent lubricating oil on maize; they attributed the effects to qualitative compositional differences in the various products.

Table 2. Analysis of variance showing mean square of effects of the treatments on plant height

Source of variation	*df*	*MS*	*F*	*F crit*
Accessions	7	247.45	3.36**	2.18
Treatments	8	117.69	1.61	2.11
Error	56	73.62		
Total	71			

***= indicate significance at P=.05*

3.2 Effects on Leaf Area

The difference in the leaf areas between different accessions was significant at *P*=.05 but this difference between different treatments was not (Table 4). For instance, NCe 002 had the least leaf area (39.2 cm^2) in 20 mg/kg sodium thiosulphate treatment (Table 3) while NXs 002 had the least leaf area (80.8 cm^2) in 5 mg/kg graphite powder treatment. However, NCe 005 had the overall best performance as it responded well to both chemicals and also had the highest

Table 3. Effects of sodium thiosulphate and graphite powder treatments on the leaf area (cm^2) of different accessions

Chemical treatment	Conc. (mg/kg)	NCe 001	NCe 002	NCe 003	NCe 004	NCe 005	NXs 001	NXs 002	NXs 003
Control		113.1	139.5	194.5	160.5	201.5	149.5	121.5	201.7
Sodium thiosulphate	5	154.4	109.3	150.1	_	371.3	76.7	81.9	146.1
	10	149.8	105.0	288.2	329.3	252.4	146.8	_	330.1
	20	109.6	39.2	141.8	125.5	273.3	127.2	100.8	238.6
	40	198.8	191.1	146.1	222.0	188.1	97.7	130.3	189.5
Graphite powder	5	91.1	91.5	157.5	_	195.8	123.1	80.8	254.0
	10	145.4	116.1	293.1	230.6	566.1	141.4	183.0	199.7
	20	133.7	160.1	132.4	273.1	251.4	149.1	280.3	203.1
	40	156.1	141.9	193.5	223.0	163.4	_	163.6	262.0

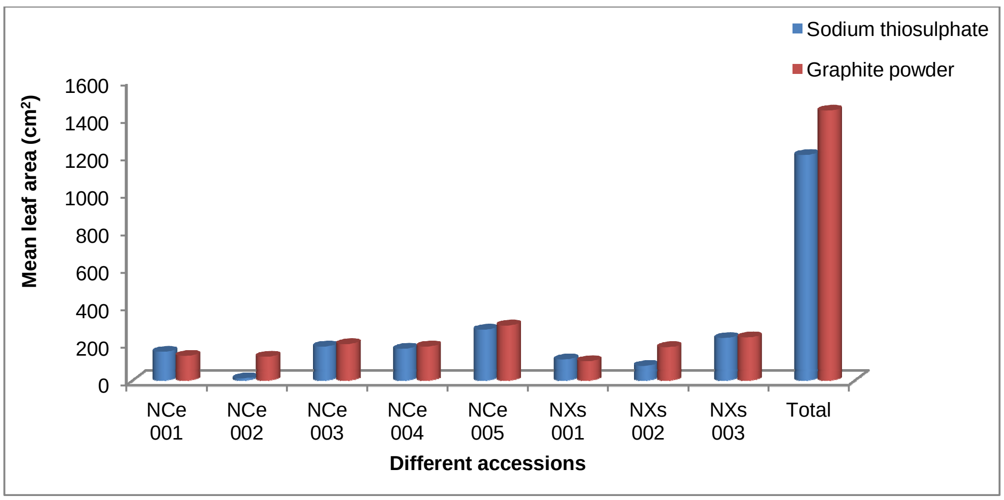

Fig. 2. Effects of sodium thiosulphate and graphite powder on the mean leaf areas of different accessions

leaf area (Fig. 2). It was also observed that NCe 005 treated with graphite powder at 10 mg/kg had a leaf area of 566.1 cm^2. Treatment with sodium thiosulphate at 5 mg/kg produced a leaf area of 371.3 cm^2 while the control had a leaf area of 201.5 cm^2. Anoliefo and Vwioko [18] from studies on *Capsicum annum* using spent lubricating oil, reported enhanced growth at 1% concentration in relation to the control. The leaf area (566.1 cm^2) of NCe 005 treated with 10 mg/kg (1% w/w), showed that graphite powder (a lubricant) acted as a growth enhancer. The mean leaf area of all the accessions performed better in graphite powder than in sodium thiosulphate treatments (Fig. 2).

3.3 Effects on Girth

The summary of the effects of these chemicals on the plant girth was captured in Table 5. The chemicals had no significant difference at *P*=.05 on the girth either between or within accessions. This type of observation on girth was also reported by Okonokhua et al. [16] with spent engine oil on *Zea mays* and by Adu et al. [19] with spent and unused engine oil on *Vigna unguiculata*. However, it was observed that NCe 005 reacted well to both chemicals and also produced the highest girth of 10.8 cm in 10 mg/kg of graphite powder. Observations also showed that the girth of all accessions did better in graphite powder treatments than in sodium thiosulphate treatments (Fig. 3). This also depicts plants as powerful biomonitoring agents.

Table 4. Analysis of variance showing mean square of effects of the treatments on leaf area

Source of variation	*df*	*MS*	*F*	*F crit*
Accessions	7	29076.59	5.48**	2.18
Treatments	8	9873.87	1.86	2.11
Error	56	5309.3		
Total	71			

***= indicate significance at P=.05*

Table 5. Effects of sodium thiosulphate and graphite powder treatments on the girth (cm) of different accessions

Chemical treatment	Conc. (mg/kg)	NCe 001	NCe 002	NCe 003	NCe 004	NCe 005	NXs 001	NXs 002	NXs 003
Control		5.7	6.2	7.2	5.1	5.3	5.7	5.0	6.2
Sodium thiosulphate	5	5.7	4.4	5.7	_	7.5	3.5	5.5	4.1
	10	5.2	4.7	6.2	8.1	7.5	4.3	_	7.3
	20	5.0	4.0	4.1	4.5	6.3	5.6	4.5	5.5
	40	8.5	5.5	5.0	6.3	7.0	6.0	5.9	5.0
Graphite powder	5	5.5	3.4	4.2	_	5.5	4.8	4.9	6.5
	10	4.8	5.0	8.6	7.0	10.8	5.8	7.0	6.0
	20	5.7	5.9	4.8	7.5	5.8	5.3	10.5	5.1
	40	5.0	4.8	4.9	7.0	5.5	_	7.4	6.0

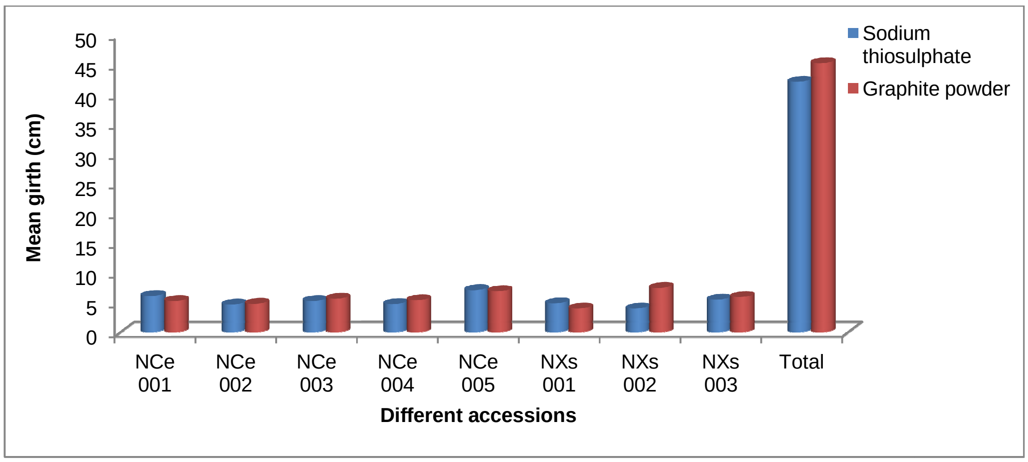

Fig. 3. Effects of sodium thiosulphate and graphite powder on mean girth of different accessions

3.4 Effects on Yield

The effects of the treatments on the weight of yield of different accessions were captured in Table 7. The effects of sodium thiosulphate and graphite powder on the yield showed no significant difference between treatments but a significant difference at *P*=.05 between the different accessions (Table 6). *Colocasia esculenta* accessions were observed to have better yield than *Xanthosoma maffafa* accessions. The result also showed that NCe 001, NCe 003 and NCe 005 did well with the treatments. A mean yield of 287.5 g was recorded in NCe 001 treated with sodium thiosulphate (Fig. 4) while NCe 005 gave a mean yield of 230 g in graphite powder treatments. It was also observed that NCe 005 produced the highest yield (500 g) in 10 mg/kg of graphite powder while the control yielded 100 g. The accession, NCe 004, yielded 420 g in 10 mg/kg of sodium thiosulphate while the control yielded 75 g. The lowest yield (20 g) was recorded by NXs 002 in 5 mg/kg of graphite powder and the control had 25 g.

Table 6. Analysis of variance showing mean square of effects of the treatments on yield

Source of variation	*df*	*MS*	*F*	*F crit*
Accessions	7	32683.33	3.99**	2.18
Treatments	8	13451.65	1.65	2.18
Error	56	8174.96		
Total	71			

***= indicate significance at P=.05*

Generally, all the accessions treated with sodium thiosulphate produced higher mean yield than those treated with graphite powder (Fig. 4). The use of chemical substances for improved plant growth and yield is well documented in literature [20,21]. Similar results were obtained by Mensah et al. [22] with colchicine and sodium azide treatment on sesame seeds. They observed that the yield of sesame was higher with these two chemicals than in the control. It can therefore be deduced that sodium thiosulphate and graphite powder worked as enhancer (fertilizer effect) to induce higher yields in cocoyam. This in itself is worrisome because these chemicals may be incorporated in the corms and cormels (yields; food source) of these plants and this can pose serious health issues for humans in the long run.

3.5 Effects on Leaf Surface

Sodium thiosulphate treatments (20 and 40 mg/kg) induced malformed leaves on some accessions (Plate 1). However, the new leaves that emerged when these malformed leaves fell off were observed to be normal and not malformed. It was also observed that these malformed leaves were all induced in *Xanthosoma* species alone. Malformed leaves have been attributed to stress and change in enzymatic activities of the soil microbes [23]. Adu et al. [19] reckoned that malformed leaves interfere with proper photosynthetic activities in plants, thereby causing low yield. This explains the low yields observed in NXs 001 and NXs 002.

Table 7. Effects of sodium thiosulphate and graphite powder treatments on the weight of yield (g) of different accessions

Chemical treatment	Conc. (mg/kg)	NCe 001	NCe 002	NCe 003	NCe 004	NCe 005	NXs 001	NXs 002	NXs 003
Control		150	150	100	75	100	50	25	200
Sodium	5	200	110	120	0	250	40	25	120
thiosulphate	10	250	60	400	420	250	75	0	300
	20	300	55	150	125	300	100	75	120
	40	400	50	100	70	50	50	100	175
Graphite	5	200	110	220	0	185	150	20	175
powder	10	200	150	325	100	500	100	25	65
	20	100	200	150	250	190	125	100	200
	40	175	155	100	150	45	0	100	25

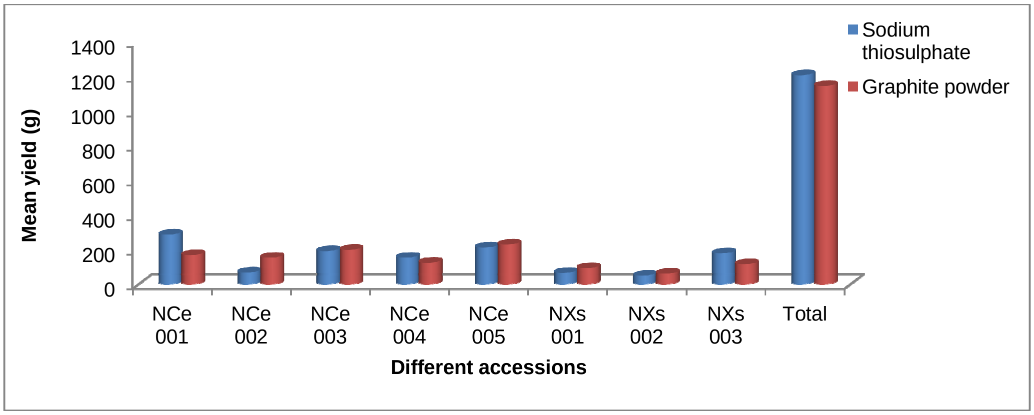

Fig. 4. Effects of sodium thiosulphate and graphite powder on the mean yield of different accessions

Plate 1. Malformed leaves: A) NXs 001 in 20 mg/kg of sodium thiosulphate, B) NXs 002 in 20 mg/kg of sodium thiosulphate, C) NXs 002 in 40 mg/kg of sodium thiosulphate, D) Control leaf of *Xanthosoma sp*

Table 8. Effects of sodium thiosulphate and graphite powder treatments on the stem colour of different accessions

Chemical treatment	Conc. (mg/kg)	NCe 001	NCe 002	NCe 003	NCe 004	NCe 005	NXs 001	NXs 002	NXs 003
Control		GB	G	B	G	G	G	GB	G
Sodium	5	GB	G, B	B	_	G	G	GB	G
thiosulphate	10	GB	G	G, B	G	G	G	_	G
	20	GB	G	B	G	G	G	GB	G
	40	GB	B	B	G	G	G	GB	G
Graphite	5	GB	G, B	B	_	G	G	GB	G
powder	10	GB	G	G	G	G	G	GB	G
	20	GB	G, B	G, B	G	G	G	GB	G
	40	GB	G	G, GB	G, B	G	_	GB	G

(KEYS: G= Green, B= Brown, GB= Greenish-brown)

Table 9. Effects of sodium thiosulphate and graphite powder treatments on the number of leaves per stand of different accessions

Chemical treatment	Conc. (mg/kg)	NCe 001	NCe 002	NCe 003	NCe 004	NCe 005	NXs 001	NXs 002	NXs 003
Control		4	4	4	3	3	4	4	3
Sodium	5	4	3	3	_	4	3	5	3
thiosulphate	10	5	3	4	4	3	3	_	4
	20	4	3	3	2	3	3	4	3
	40	6	4	3	3	2	3	5	4
Graphite	5	6	3	3	_	3	4	5	5
powder	10	4	2	4	3	4	4	7	3
	20	3	4	3	4	4	4	6	3
	40	3	3	3	3	3	_	5	4

3.6 Effects on other Morphological Features

Change in stem colour was observed in NCe 002, NCe 003 and NCe 004 (Table 8); this has been linked to reduced chlorophyll and nutrient immobilization [19]. Modifications in the number of leaves per plant stand vary between accessions (Table 9); this trend may be attributed to the inability of the seedlings to absorb water due to the change in the physical and chemical structure of the soil [24,25].

Plant deaths were also observed in some accessions: NCe 004 in 5 mg/kg sodium thiosulphate and 5 mg/kg graphite powder, NXs 001 in 40 mg/kg graphite powder and NXs 002 in 10 mg/kg sodium thiosulphate. These accessions did not germinate at all; which meant that they were killed by these chemicals. Adu et al. [19] surmised that plant deaths are caused by the destruction of microbes present in the soil due to insufficient aeration following a decrease in the air pore by pollutants.

4. CONCLUSION

This study has shown that cocoyam is an unreliable/poor environmental bioindicator because accessions treated with these oilfield chemicals most times have more vegetative vigour than the controls; therefore, soil assessment and monitoring should be carried out on plots of land where cocoyam would be planted. It is also pertinent to carry out biochemical analysis on these cocoyam species especially on their corms and cormels so as to determine if there is an incorporation of these chemicals in this organ. Cytological studies should also be carried out on these accessions in order to assess any genotoxic effects which may not be visible. Protection of the environment is paramount to ensuring the sustainability of the earth thus; careful handling of these chemicals is advocated.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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