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Cuticular Modifications in *Colocasia esculenta* (L.) Schott. and *Xanthosoma maffafa* (L.) Schott. Accessions Exposed to Some Oilfield Chemicals in the Niger Delta, 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 experimental and laboratory processes. 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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Original Research Article

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ABSTRACT

Aim: To evaluate the effects of sodium thiosulphate and graphite powder on the epidermal characters of *Colocasia esculenta* and *Xanthosoma maffafa* accessions.
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.

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Methodology: A total of eight accessions were used for this study, five from *Colocasia esculenta* and three from *Xanthosoma maffafa*. Each accession was planted in a 10 kg soil and planted in 4 different concentrations (5, 10, 20 and 40 mg/kg) of sodium thiosulphate and graphite powder and the unpolluted soil was used as the control experiment. Epidermal sections were obtained using the hand teasing method.
Results: These test plant species were observed to be amphistomatous, and had five types of stomata: brachyparacytic, amphibrachyparachytic, brachyparatetracytic, brachyparahexacytic-monopolar and brachyparahexacytic-dipolar. The brachyparacytic type of stomata was observed to be the basic stomata type. Stomatal complex and contiguous stomata were observed on the abaxial epidermes of some accessions; while the control of NXs 003 was the only accession with contiguous stomata on the adaxial epidermis. The least stomatal index and stomatal frequency were observed in the adaxial epidermes of NCe 001 and NXs 002 while the highest stomatal index was observed in the abaxial epidermis of NCe 003 both treated with graphite powder. Accession NXs 003 had the lowest mean number of epidermal cells of all the accessions. The mean number of epidermal cells on both the adaxial and the abaxial epidermal surfaces were observed to be lowered by sodium thiosulphate treatments. The anticlinal walls were not affected by the treatments as the anticlinal walls were straight for both the control and treated accessions. Statistical analyses showed that the differences in stomatal indices and number of epidermal cells between the adaxial and abaxial epidermes were significant at 5% and these differences between accessions were also significant at 5%.
Conclusion: Different accessions of *C. esculenta* and *X. maffafa* showed sensitivity to environmental change and have unique way of adapting to ecological differences.

Keywords: Adaxial; abaxial; stomata; epidermal cells; sodium thiosulphate; graphite powder.

1. INTRODUCTION

Environmental consciousness has been an issue of discussion by many scientists round the globe in other to ensure the sustainability of the earth. The environment is a life supporting system and comprises the air, water, soil, plants, animals, micro-biota and humans living and interacting with one another. Introduction of any form of pollution in the environment will automatically and detrimentally affect its biophysical (soil property, aquatic ecosystem, biodiversity [livestock, wildlife and plant life]) and socio-economic components (such as health, income), and ultimately diminish the livelihood of the populace [1,2].

Nigeria is one of the world's crude oil producers where oil exploration and drilling activities constantly take place especially in the Niger Delta region; during this operation, as much as 60,000 m^3 of drilling fluids can be discharged into the environment from one production platform alone [3]. When these fluids find their way into the environment the physical, chemical and microbiological properties of the soil are affected [4]. Plants are organisms with complex systems and have over the years, been used as bioassay to study environmental chemicals with mutagenic and carcinogenic potentials [5,6].

Studies show that in polluted conditions, plants develop different morphological, physiological, epidermal and anatomical alterations [7,8,2,9]. However, surveys have shown that cocoyam has received minimal attention from researchers (particularly in Nigeria) towards this area, as there is a dearth of information on the effects of pollution on cocoyam; despite the fact that it is the third most important root crop in Nigeria [10]. It is eaten and grown mainly in the South-east and South-south regions of Nigeria; where majority of oil drilling and exploration activities take place. The study of the effects of oil-related pollutants on cocoyam has become a necessity because cocoyams are cheaper yam substitutes with higher digestibility quality [11] and, provide economic empowerment for youths and women in particular [12].

Sodium thiosulphate and graphite powder are two important oilfield chemicals that are constantly in use during oil exploration and drilling activities; and to the best of the knowledge of the authors, there is little or no literature on the environmental implications of these chemicals on plants. The anatomical effects of the two chemicals on cocoyam have been document [13]. Thus, the interesting results obtained prompted the present study. Therefore, this study is limited to critically evaluate the

tendency of sodium thiosulphate and graphite powder to alter the epidermal characters of *Colocasia esculenta* and *Xanthosoma maffafa* accessions.

2. MATERIALS AND METHODS

The experiment was set up in a Randomized Complete Block Design at the Ecological Research Centre, University of Port Harcourt. Loamy soil obtained from the University was bagged in polythene bags to a weight of 10 kg.

A total of eight accessions were used for this study, five 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); where NCe and NXs stand for Nigeria *Colocasia esculenta* and Nigeria Xanthosoma species respectively. All were identified and collected from the National Root Crops Research Institute (NRCRI), Umudike, Abia State Nigeria.

The oilfield chemicals used for this study were sodium thiosulphate and graphite powder. Each accession was planted in 4 different concentrations: 5, 10, 20 and 40 mg/kg for each chemical while an unpolluted soil served as the control experiment. The chemical concentrations were 0.5, 1, 2 and 4% w/w in soil on weight basis and were applied by thoroughly mixing each concentration with 400 ml of water. This mixture was used in watering the plants immediately after planting. Further irrigation of the plants was done twice with 200 ml of water as there was sufficient rainfall throughout the duration of this work. Weeding was by hand-picking.

Cuticular assessments of both the treated and the control plants were carried out five months after planting. Epidermal sections were obtained using the hand teasing method [14]. The excised epidermis was then mounted on a clean glass slide and stained with either safaranin or methylene blue for five minutes and washed out with clean water. Filter paper was used to drain the water on the slide; glycerine was dropped on the epidermis, covered with a cover slip and examined under a microscope.

The type of stomata, number of stomata, number of epidermal cells, nature of the anticlinal walls and the shape of the epidermal cells were all visually scored. Good plates were photographed to reveal foliar features of interest. Stomatal indices and frequencies were calculated thus:

$$\text{Stomatal index (\%)} = \frac{S}{S+E} \times \frac{100}{1}$$

$$\text{Stomatal frequency (\%)} = \frac{S}{E} \times \frac{100}{1}$$

where: S = number of stomata; E = number of epidermal cells

All data generated were exposed to two way analysis of variance (ANOVA).

3. RESULTS AND DISCUSSION

3.1 Stomatal Morphology

The shape of a pair of guard cells for both treated and control accessions, was round to oval while the stomatal pore was narrowly elliptic. This agreed with the observation of Osuji and Nwala [14].

3.2 Stomata Types

For the treated and control accessions, the brachyparacytic type were observed as the basic type of stomata but other observed types included: amphibrachyparacytic, brachyparatetracytic, brachyparahexacytic-monopolar and brachyparahexacytic-dipolar stomata (Plate 1). This observation was at variance with the findings of Osuji and Nwala [14] and *Ezeabara* et al. [15]. The former reported brachyparacytic stomata as the type of stomata for NXs 002 and paracytic stomata as the basic type for all the other accessions while the latter reported anomocytic stomata as the stomatal type for the accessions of *Colocasia* they studied. The occurrence of more than one type of stoma on an epidermis of plant species is not new as it was reported by Ugborogho et al. [16]; they discovered eleven types of stomata on the epidermis of *Dioscorea* species. Stomatal complexes were observed on some treated accessions while contiguous stomata were observed on the abaxial epidermis of some treated samples of NXs 003. The occurrence of contiguous stomata has also been reported by authors like Okoli [17], Gill and Nyawuame [18]. Interestingly, NXs 003 control was the only accession with contiguous stomata observed on the adaxial epidermis. Contiguous stomata was also observed on the abaxial epidermis by Ugborogho et al. [19]; they described the

phenomenon as an abnormality and ironically, as being of taxonomic value.

Apart from the brachyparacytic stomata that was the basic stomata type, the mostly occurred type of stomata was brachyparatetracytic occurring 74 times, followed by brachyparahexacytic-monopolar stomata that occurred 35 times, next was the amphibrachyparacytic stomata that occurred 28 times but the brachyparahexacytic-dipolar stomata was the least occurred stomata type as it occurred 2 times: on the abaxial epidermal surfaces of accessions NCe 005 and NXs 003 treated with 20 mg/kg of sodium thiosulphate. Moreover, the brachyparatetracytic stomata occurred mostly in accession NCe 005 (15 times), brachyparahexacytic-monopolar stomata occurred mostly in accession NXs 002 (8 times) while, amphibrachyparacytic stomata occurred mostly in accession NXs 001 (8 times) but the amphibrachyparacytic stomata was totally absent in accession NCe 002 (Table 1). One to four types of these stomatal types were observed to occur on a piece of epidermis (abaxial or adaxial) however, only the abaxial epidermal surface of NCe 005 treated with 20 mg/kg of sodium thiosulphate had all the five types of stomata.

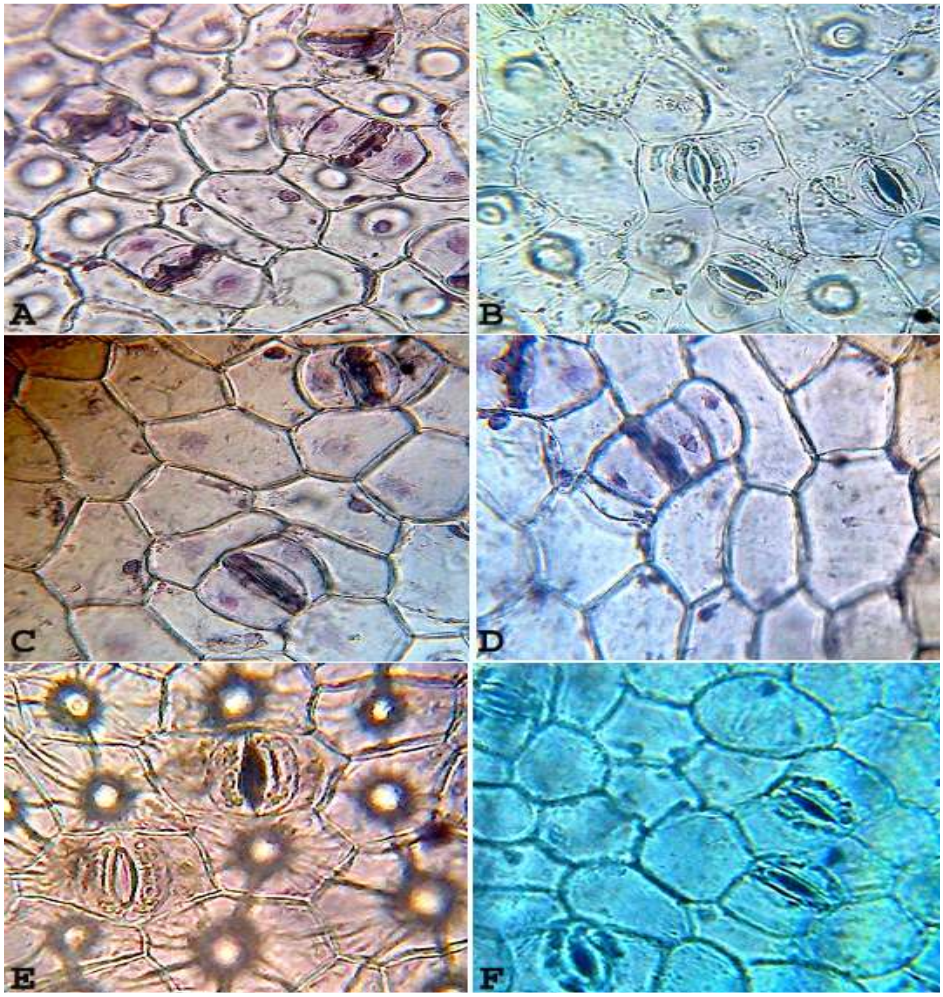

Plate 1. Different types of stomata found in *Xanthosoma* and *Colocasia* cultiars studied. A) Brachyparahexacytic-dipolar stomata [Abaxial epidermis], B) Brachyparacytic stomata [Abaxial epidermis], C) Brachyparahexacytic-monopolar stomata [Adaxial epidermis], D) Amphibrachyparacytic stomata [Adaxial epidermis], E) Brachyparatetracytic stomata [Abaxial epidermis], F) Contiguous stomata [Adaxial epidermis]

Table 1. Different types of stomata present in the epidermes of different accessions treated with different concentrations of oilfield chemicals

Chemical treatment	Conc. (mg/kg)	Ep epidermal surface	NCe 001	NCe 002	NCe 003	NCe 004	NCe 005	NXs 001	NXs 002	NXs 003
Sodium thiosulphate	5	Adaxial	B, BP	B, BP	B, BP	_	B, BP	B, AB, BM	B	B
		Abaxial	B, AB	B, BP	B, BP	_	B	B, AB	B, BP	B
	10	Adaxial	B, BP	B	B, BP	B	B, AB, BP	B,AB,BP,BM	_	B
		Abaxial	B, BP	B	B, BP	B	B, AB, BM, BP	B	_	B, AB
	20	Adaxial	B, BP	B	B, BP	B, BP, BM	B, BP	B, BM	B, AB, BM, BP	B
		Abaxial	B, AB, BP	B, BP	B	B	B, AB, BM, BP, BD	B, AB, BP	B	B,BM, BD
	40	Adaxial	B	B	B	B,AB,BP,BM	B, BP	B, AB	B, BP	B
		Abaxial	B	B	B, BP	B	B	B, BM, BP	B, AB	B
Graphite powder	5	Adaxial	B, BP	B	B, AB, BM	_	B, BM, BP	B, BP, BM	B, BM	B
		Abaxial	B, BP	B, BP, BM	B	_	B, BP, AB	B, BM	B, BM, BP	B
	10	Adaxial	B, BP	B	B, BP, BM	B	B, BM	B, AB	B, AB, BM	B, AB, BM, BP
		Abaxial	B, BP	B	B, BP	B, AB, BP	B, BM, BP	B, BP	B	B
	20	Adaxial	B, BM	B, BP	B, BP	B, BP	B, BM, BP	B, BP, BM	B, BM	B
		Abaxial	B, BP	B, BP	B, BP	B, BP	B, BP	B, AB, BP	B, AB	B, AB
	40	Adaxial	B, BP	B, BP	B	B, BP	B, BP	_	B	B
		Abaxial	B	B, BP, BM	B, BP, BM	B	B, BP	_	B, AB, BM	B, AB
Control		Adaxial	B, BP	B, BP, BM	B, BP	B, BP	B, BP	B, BP	B, BM, BP	B, BM, BP
		Abaxial	B, BP	B, BP	B, BP	B	B, BM, BP	B, AB	B, AB, BM	B, AB,BM, BP

Keys: B= Brachyparacytic, AB= Amphybrachyparacytic, BP= Brachyparatetracytic, BM= Brachyparahexacytic-monopolar, BD= Brachyparahexacytic-dipolar

3.3 Stomata Distribution and Frequency

This study showed that the stomata distribution of cocoyam was amphistomatic but there were more stomata at the abaxial than at the adaxial epidermis except for accessions NCe 001 treated with 5 mg/kg graphite powder (22 stomata on the adaxial and 17 stomata on the abaxial), NCe 002 treated with 5 mg/kg sodium thiosulphate (29 stomata on the adaxial and 27 stomata on the abaxial) and NCe 003 treated with 20 mg/kg sodium thiosulphate (18 stomata on the adaxial and 16 stomata on the abaxial) (Table 2a). The abnormal behaviours of these accessions proved the suggestions of Lata et al. [20] that the degree of sensitivity of plant species depends on its nutritional status and environmental factors. However, the presence of more stomata on the abaxial epidermal surface of cocoyam than at the adaxial surface was in line with the findings of Osuji and Nwala [14]; the presence of more stomata on the abaxial surface helps the plant to conserve moisture and enhance photosynthetic activities since the stomata are organs of transpiration and gaseous exchange. The least stomatal index and stomatal frequency were observed in the adaxial epidermes of NCe 001 treated with 40 mg/kg of graphite powder (Table 2a) and NXs 002 treated with 20 mg/kg of graphite powder (Table 2b); both had stomatal indices of 4.0% and stomatal frequencies of 4.2%. Changes in the number and frequency of stomata have been found to be affected by pollution [7]. However, the highest stomatal index of 23.7% and stomatal frequency of 31.1% was observed in the abaxial epidermis of NCe 003 treated with 40 mg/kg of graphite powder. The presence of more stomata means increased respiratory activities hence, improved energy generation that will accelerate photosynthetic activities of this accession. This suggests that each accession has is unique way of adaptation and expressing sensitivity to different environmental conditions; moreover, differences in stomatal frequency [21] is linked to the polarity determining genes.

Observations showed that on the adaxial epidermes, NCe 003 had the highest mean stomatal index (11.1%) with graphite powder treatments (Fig. 1) while on the abaxial epidermes, NXs 003 had the highest mean stomatal index (20.4%) with graphite powder treatments (Fig. 2). Cumulatively, the mean stomatal indices showed that there were more stomata on the abaxial epidermis than at the adaxial epidermis; also, on the adaxial epidermis, sodium thiosulphate treatments induced (60.1%) higher stomatal index in the accessions than graphite powder treatments (57.8%) but on the abaxial epidermis, graphite powder treatments induced (109.3%) higher stomatal index in the accessions than sodium thiosulphate treatments (105.8%).

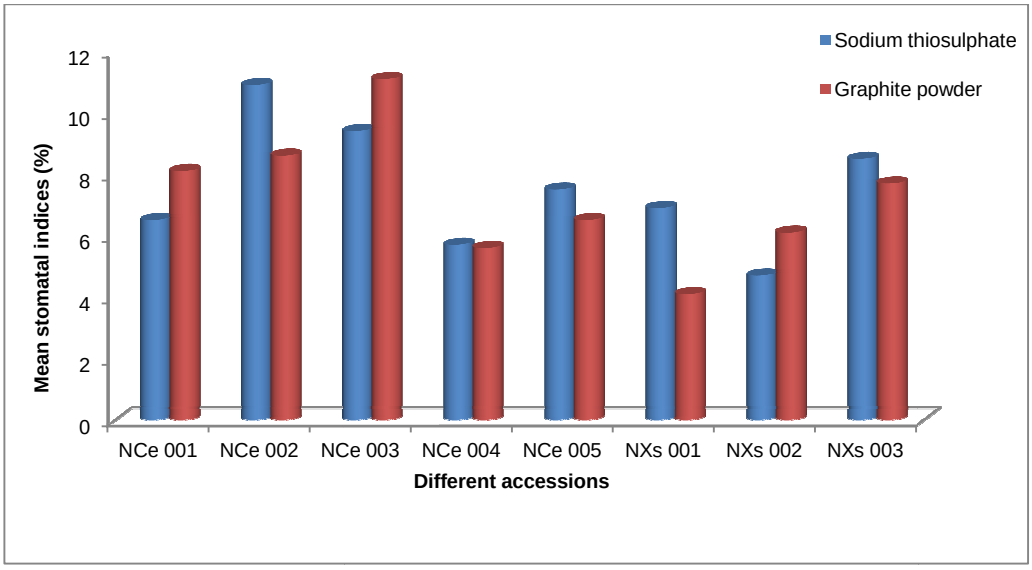

Fig. 1. Effects of sodium thiosulphate and graphite powder on the mean stomatal indices of the adaxial epidermes of different accessions

Table 2a. Stomatal indices and frequencies in the epidermes of different Colocasia accessions treated with different concentrations of oilfield chemicals

Chemical treatment	Conc. (mg/kg)	Epidermal surface	NCe 001				NCe 002				NCe 003				NCe 004				NCe 005			
			S	E	S.I	S.F	S	E	S.I	S.F	S	E	S.I	S.F	S	E	S.I	S.F	S	E	S.I	S.F
Sodium thiosulphate	5	Adaxial	8	138	5.5	5.8	29	156	15.7	18.6	15	275	5.2	5.5	_	_	_	_	13	192	6.3	6.8
		Abaxial	16	115	12.2	13.9	27	128	17.4	21.1	36	174	17.1	20.7	_	_	_	_	28	192	12.7	14.6
	10	Adaxial	7	146	4.6	4.8	18	146	11.1	12.3	18	217	7.7	8.3	18	230	7.3	7.8	15	202	6.9	7.4
		Abaxial	17	149	10.2	11.4	46	180	20.4	25.6	29	163	15.1	17.8	31	240	11.4	12.9	26	200	11.5	13.0
	20	Adaxial	12	158	7.1	7.6	16	138	10.4	11.6	18	141	11.3	12.8	19	214	8.2	8.9	18	158	10.2	11.4
		Abaxial	22	132	14.3	16.7	28	132	17.5	21.2	16	233	6.4	6.9	35	262	11.8	13.4	32	168	16.0	19.1
	40	Adaxial	12	124	8.8	9.7	12	183	6.2	6.6	29	190	13.2	15.3	18	231	7.2	7.8	13	189	6.4	6.9
		Abaxial	22	106	17.2	20.8	26	113	18.7	23.0	35	234	13.0	15.1	31	185	14.4	16.8	39	224	14.8	17.4
Graphite powder	5	Adaxial	22	150	12.8	14.7	16	150	9.6	10.7	21	180	10.5	11.7	_	_	_	_	16	215	6.9	7.4
		Abaxial	17	118	12.6	14.4	27	147	15.5	18.4	35	202	14.8	17.3	_	_	_	_	32	168	16.0	19.1
	10	Adaxial	14	143	8.9	9.8	15	149	9.2	10.1	25	193	11.5	13.1	14	199	6.6	7.0	15	239	5.9	6.3
		Abaxial	23	145	13.7	15.9	30	161	15.7	18.6	31	189	14.1	16.4	25	231	9.8	10.8	36	244	12.9	14.8
	20	Adaxial	11	153	6.7	7.2	16	184	8.0	8.7	23	211	9.8	10.9	24	260	8.5	9.2	13	196	6.2	6.6
		Abaxial	20	137	12.7	14.6	18	156	10.4	11.5	39	236	14.2	16.5	49	287	14.6	17.1	22	185	10.6	11.9
	40	Adaxial	7	167	4.0	4.2	14	167	7.7	8.4	31	218	12.5	14.2	18	230	7.3	7.8	16	215	6.9	7.4
		Abaxial	18	155	10.4	11.6	19	146	11.5	13.0	42	135	23.7	31.1	33	210	13.6	15.7	33	192	14.7	17.2
Control		Adaxial	21	125	14.4	16.8	18	161	10.1	11.2	27	221	10.9	12.2	23	180	11.3	12.8	12	171	6.6	7.0
		Abaxial	22	99	18.2	22.2	29	119	19.6	24.4	47	169	21.8	27.8	31	182	14.6	17.0	26	173	13.1	15.0

Table 2b. Stomatal indices and frequencies in the epidermes of different Xanthosoma accessions treated with different concentrations of oilfield chemicals

Chemical treatment	Conc. (mg/kg)	Epidermal surface	NXs 001				NXs 002				NXs 003			
			S	E	S.I	S.F	S	E	S.I	S.F	S	E	S.I	S.F
Sodium thiosulphate	5	Adaxial	10	93	9.7	10.8	9	161	5.3	5.6	7	107	6.1	6.5
		Abaxial	14	117	10.7	12.1	17	106	13.8	16.0	22	99	18.2	22.2
	10	Adaxial	7	143	4.7	4.9	_	_	_	_	11	91	10.8	12.1
		Abaxial	10	102	8.9	9.8	_	_	_	_	20	90	18.2	22.2
	20	Adaxial	12	128	8.6	9.4	15	248	5.7	6.1	9	110	7.6	8.2
		Abaxial	14	138	9.2	10.2	42	245	14.6	17.1	20	98	17.1	20.4
	40	Adaxial	7	142	4.7	4.9	17	205	7.7	8.3	8	77	9.4	10.4
		Abaxial	15	152	9.1	9.9	32	186	14.7	17.2	22	115	16.1	19.1
Graphite powder	5	Adaxial	9	167	5.1	5.4	14	218	6.0	6.4	7	79	8.1	8.9
		Abaxial	24	114	17.4	21.1	25	147	14.5	17.0	21	78	21.2	26.9
	10	Adaxial	9	135	6.3	6.7	16	207	7.2	7.7	7	92	7.1	7.6
		Abaxial	11	148	6.9	7.4	41	204	16.7	20.1	20	81	19.8	24.7
	20	Adaxial	8	156	4.9	5.1	8	190	4.0	4.2	9	97	8.5	9.3
		Abaxial	25	175	12.5	14.3	24	218	9.9	11.0	26	89	22.6	29.2
	40	Adaxial	_	_	_	_	10	132	7.0	7.6	8	105	7.1	7.6
		Abaxial	_	_	_	_	16	86	15.7	18.6	24	110	17.9	21.8
Control		Adaxial	9	153	5.6	5.9	13	230	5.4	5.7	22	221	9.1	10.1
		Abaxial	16	149	9.7	10.7	51	238	17.7	121.4	29	212	12.0	13.7

Keys: S= Number of stomata, E= Number of epidermal cells, S.I= Stomatal index (%), S.F= Stomatal frequency (%)

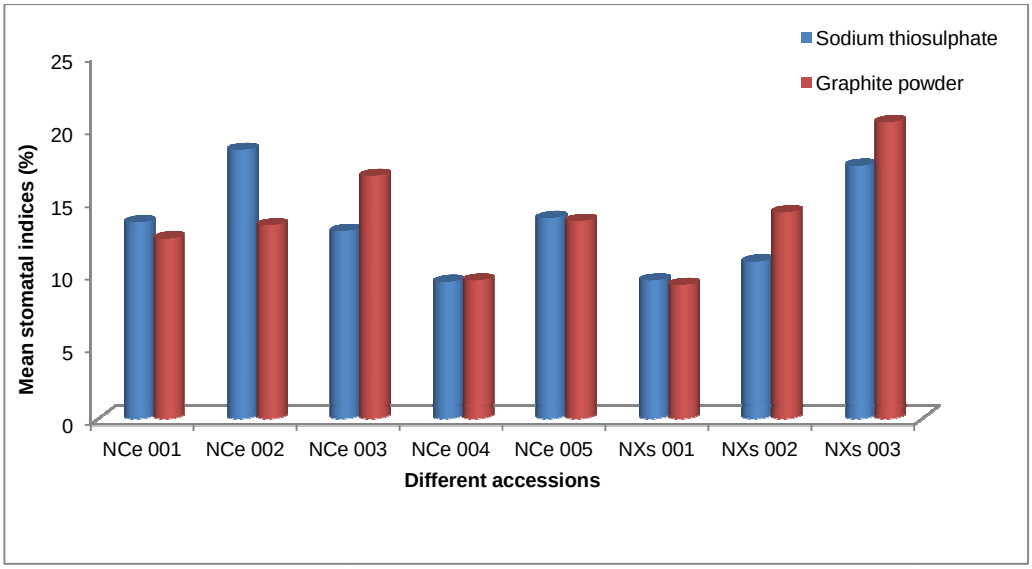

Fig. 2. Effects of sodium thiosulphate and graphite powder on the mean stomatal indices of the abaxial epidermes of different accession

Statistical evidence showed that the difference in stomatal index between the adaxial and abaxial epidermis was significant at 5%; the difference in stomatal index between accessions was significant at *P*=.05 but the difference in stomatal index between treatments was not significant at *P*=.05.

3.4 Epidermal Surface

The epidermal surfaces were glabrous without trichome; the presence of papillae was observed mostly in the abaxial epidermal surfaces of all the accessions. Osuji and Nwala [14] also observed the presence of papillae; De Oliveira et al. [22] reported the presence of papillae on the abaxial epidermis of *Manihot esculenta* and suggest that papillae act as lenses that converge solar energy for chlorophyllian tissues in plants.

3.5 Epidermal Cells

Sodium thiosulphate and graphite powder treatments did not have much effect on the shapes of the epidermal cells; as both the treated and control accessions were mostly quadrilateral to decagonal in shape, except for the control of NXs 003 that had some isodiametric and circular shaped epidermal cells (Plate 2). The number of subsidiary cells was mostly 5-7 for NCe 001, 6-7 for NCe 002, 5-8 for NCe 003 and NCe 005, 6-8 for NCe 004, 4-6 for NXs 003 and 5-6 for NXs 001 and NXs 002 (Table 3). The anticlinal walls were straight in all treated and control accessions. Ajah and Obute [3], stated that the nature of the anticlinal wall is dependent on the force exerted on the stomata in the course of development. Since the anticlinal walls of the epidermal cells were all straight, it means that these oilfield chemicals did not exert pressure on the stomata as to distort the nature of the anticlinal walls.

In accession NCe 003 control, the number of epidermal cells in the abaxial epidermis was lower than the number of cells in the adaxial epidermis but this phenomenon was not so in some treated accession as the number of epidermal cells in the abaxial epidermis was found to be higher than that of the adaxial epidermis. This trend was also found in NCe 002 and NCe 001. In NCe 004, the treatments increased the number of the epidermal cells in both epidermes when compared with the control. Increase in the number of epidermal cells due to pollution in not new as it has been observed in *Cassia siamea* [23], in *Nyctanthese arbortristis*, *Quisqualis indica* and *Terminalia arjuna* [24] and in *Pongamia pinnata* [8]. The control sample of accession NCe 005 had the least number of epidermal cells when compared to all the treated accessions, except for the accession treated with 20 mg/kg of sodium thiosulphate that had 158 cells on the adaxial and 168 cells on the abaxial as against 171 cells and 173 cells respectively for the control. In NXs 002

accession, the number of epidermal cells in the control sample was more than the number of epidermal cells in the treated samples except for the accession treated with 20 mg/kg of sodium thiosulphate that had 248 cells on the adaxial and 243 cells on the abaxial epidermes as against the 230 and 238 cells respectively for the control. However, it was observed that the treatments reduced the number of epidermal cells on both epidermes in accession NXs 003 when compared to the control (Table 2b). Different reactions of the accessions to the chemicals indeed confirm the theory of Osuji and Nwala [14], that these species (*C. esculenta and X. maffafa*) have unique ways of responding to different ecological conditions.

Cumulatively, the accession with the highest mean number of epidermal cells on the adaxial epidermis was NCe 005 with graphite powder treatments (Fig. 3) while the accession with the highest mean number of epidermal cells on the abaxial epidermis was NCe 003 with sodium thiosulphate treatments (Fig. 4). It was observed that graphite powder treatments induced higher number of epidermal cells in the accessions than sodium thiosulphate treatments for both epidermes.

Table 3. Number of subsidiary cells present in the epidermes of different accessions treated with different concentrations of oilfield chemicals

Chemical treatment	Conc. (mg/kg)	Epidermal surface	NCe 001	NCe 002	NCe 003	NCe 004	NCe 005	NXs 001	NXs 002	NXs 003
	5	Adaxial	7	5-8	8-10	_	6-9	4-6	5-6	4-5
Sodium		Abaxial	5-7	6-7	5-8	_	4-7	4-7	5-6	4-7
thiosulphate	10	Adaxial	6-8	7	5-8	6-8	5-8	6-7	_	4-5
		Abaxial	6-7	6-7	5-7	4-6	5-8	5-6	_	4-5
	20	Adaxial	5-8	6-9	7-8	5-8	5-7	5-7	4-6	4-5
		Abaxial	5-7	6-8	6-7	6-8	5-7	5-6	5-6	4-6
	40	Adaxial	6-8	6-9	5-8	5-8	6-8	5-6	5-6	4-5
		Abaxial	5-7	6-7	5-8	5-8	6-7	4-6	5-6	4-5
Graphite	5	Adaxial	5-6	6-7	5-7	_	7-8	5-6	5-7	4-5
powder		Abaxial	4-7	6-7	5-7	_	5-7	4-8	5-6	5-6
	10	Adaxial	5-8	5-7	5-7	6-7	5-9	5-6	5-6	4-6
		Abaxial	4-8	6-7	5-7	5-7	5-7	5-6	4-6	4-5
	20	Adaxial	5-7	5-7	6-8	6-9	6-8	5-7	5-6	5-6
		Abaxial	5-6	6-8	5-7	6-8	6-9	4-7	4-6	4
	40	Adaxial	5-7	6-7	5-9	7-9	6-8	_	5-6	4-5
		Abaxial	5-6	5-7	5-7	6-7	5-8	_	4-6	4-5
Control		Adaxial	6-8	6-7	6-8	6-9	6	5-7	5-7	5-8
		Abaxial	5-8	5-7	5-8	6-8	6-8	5-6	5-6	6-7

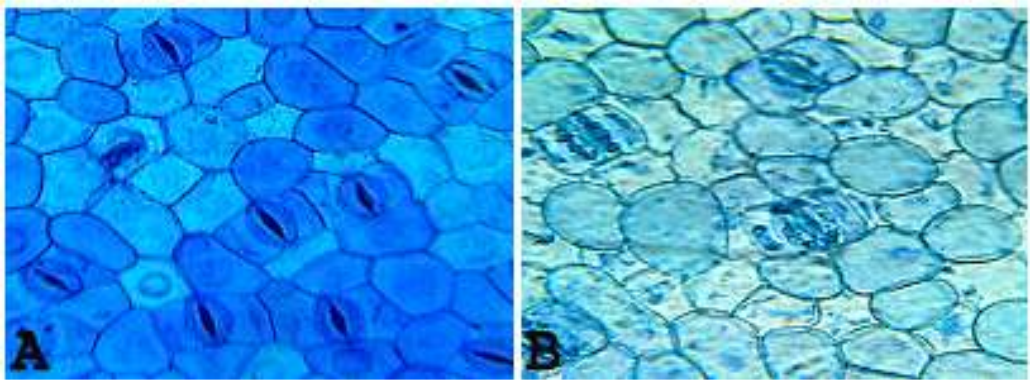

Plate 2. Isodiametric and circular shaped epidermal cells of NXs 003 control [A] abaxial epidermis [B] adaxial epidermis

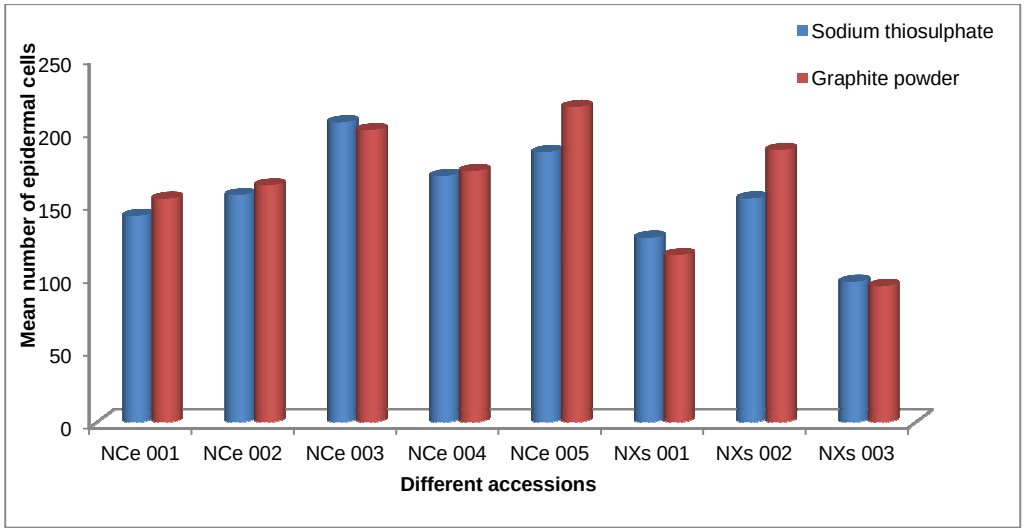

Fig. 3. Effects of sodium thiosulphate and graphite powder on the mean number of epidermal cells of the adaxial epidermes in different accessions

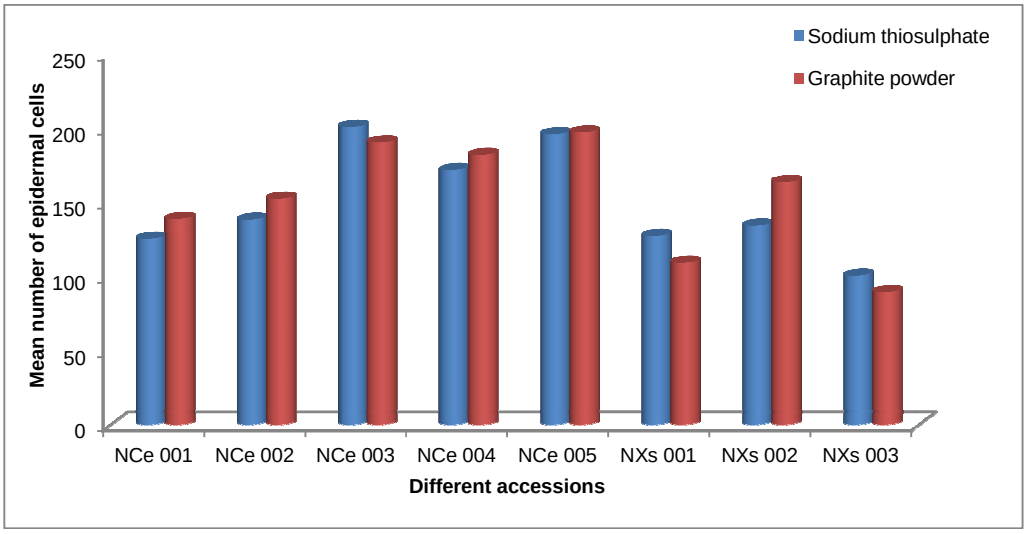

Fig. 4. Effects of sodium thiosulphate and graphite powder on the mean number of epidermal cells of the abaxial epidermes in different accessions

Statistical evidence however showed that the difference in the number of epidermal cells between the adaxial and the abaxial epidermis was significant at 5%; the difference in the number of epidermal cell between treatments was not significant at *P*=.05 but the difference in the number of epidermal cells between accessions was also significant at *P*=.05.

4. CONCLUSION

It has been established that different accessions of *Colocasia esculenta* and *Xanthosoma maffafa* has more than one type of stomata on their epidermes and the chemical additives further induced different types of stomata other than the ones in their respective controls. This study also reported for the first time the presence of contiguous stomata in this species. Moreover, these two oilfield chemicals (sodium thiosulphate and graphite powder) have demonstrated their ability to generally alter the epidermal and stomatal morphology of these plant species even in small quantities. Other assays of assessing the debilitating effects of environmental chemicals (morphological, cytological, physico-

chemical analyses and DNA fingerprinting) also have to be employed in this species so that the extent of damage by these oilfield chemicals can fully be evaluated. However, proper handling of these oilfield chemicals is strongly advocated in order to ensure the safety of plants, animals, humans and the environment as a whole.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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