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# ANATOMICAL CHANGES DUE TO CRUDE OIL POLLUTION AND ITS HEAVY METALS COMPONENT IN THREE *MUCUNA* SPECIES

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## Abstract

The micromorphological response of three *Mucuna* species in crude oil polluted soil was investigated. This experiment was a factorial (3 x 5) arrangement in a completely randomized design. Factors and levels were three species of *Mucuna* (*M. veracruz, M. jaspodea* and *M. ghana*) and crude oil concentrations (volume/weight) in the soil (0 %, 1 %, 2 %, 3 %, and 4%). The *Mucuna* species grown in higher concentrations of crude oil provoked soils (3 - 4 %) exhibited greater sinuosity in their epidermal cell walls than those grown in 0 – 1% oil concentrated soils. The stomatal frequency progressively decreased as the oil concentration increased in soil. Cortical parenchyma cells of stems and roots of the three *Mucuna* species in higher oil polluted soils were flattened tangentially, smaller in size and had reduced intercellular air spaces. Those plants in control soils had large round polygonal cells. Presence of oil droplets on trichomes of leaves was noticed. Also dotted depositions of oil were noticed in the ground tissues, especially around the vascular bundles. The changes in vegetative anatomy of the three *Mucuna* species in response to crude oil pollution and its heavy metal components were discussed as a possible use in phytomonitoring of crude oil pollution.

**Key Words**: Anatomy; Crude Oil; *Mucuna*; Phytomonitoring; Soil.

## Introduction

Crude oil in soil makes the soil condition unsatisfactory for plant growth [1] due to the reduction in the level of available plant nutrient or a rise in toxic levels of certain elements such as iron and zinc [2]. Plants are highly susceptible to oil exposure and this may kill them within a few weeks to several months. Quinones-Aguilar [3] reported that contact with crude oil damages and kills the foliage and some exposed woody tissue. In many species, however not all the perennial tissues are damaged to the point of dying and in some cases adding nutrients to polluted soils may favour the establishing of plants and increase rhizosphere populations [3].

Oil contaminated soils generally causes delayed seed emergence. This could be due to poor wettability and aeration of the soil [4] and loss of seed viability [5]. Delayed seed emergence according to Vwioko and Fashemi, [6], could be attributed to adsorption of the applied oil by the soil which comes in contact with the seeds and penetrate the seed embryos. Crude oil pollution of soils imposes a stressful condition that creates a condition of physiological drought that interferes with water uptake and gaseous exchange. McCown *et al*., [7] had earlier reported that the disruption of soil physical properties by crude oil with anaerobic and hydrophobic condition was found largely responsible for reduction in plant growth.

Omosun *et al*., [8] observed irregular epidermal cell wall shape and its increasing level of sinuosity in *Amaranthus hybridus* growing in crude oil polluted soil. Omosun *et al*., [8] also reported the decrease in the number of stomata due to crude oil pollution. Gill *et al*., [9] had earlier reported that stomata in *Chromolaena odorata* were grossly affected by crude oil which manifested as disruption and reduction in the number of stomata per unit area of the leaf. There are some plant species however that are capable of growing in soils polluted with hydrocarbons they participate in their degradation through the rhizosphere, part of the root

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which favours the growth of several micro-organisms and increases biomass and microbial activity, accelerating degradation [10].

The responses of plants to pollutants provide a simple and cost effective method of monitoring environmental pollutants. However, to use plants as bioindicators, a proper selection of plant characteristics is of vital importance [11]. A number of plant parameters have been used individually for the purpose e.g. visible foliar injury, membrane permeability, ascorbic acid, relative water content, chlorophyll content, leaf extract pH and peroxidase activity [11]. Omosun *et al*., [8] have also suggested the use of changes in the anatomy of a plant due to a pollution effect as a possible phytoindicator of a polluted environment. Usually, for plant effect monitoring, pollution induced changes in individual parameters of plants are quantified and correlated with the level of pollutants.

Considering the growing number of commercially successful applications and the lack of serious environmental concerns both technologies of phytoremediation and phytoindication are gaining acceptance from the scientific community, the general public and regulators. Due to the problems of pollution in agricultural lands of oil producing states of Nigeria, this is an attempt to validate the technology of using plants for biomonitoring of agricultural coastal soils. This will in turn facilitate a quick response for remediation of such soils. The objectives of this study therefore, are investigating the changes in anatomy of these *Mucuna* species grown in different levels of crude oil polluted soils and the possible uses of these changes in anatomy as a phytomonitoring technique of crude oil pollution.

## Materials and Methods

### *Study Area*

The experiment was conducted in an experimental farm behind the Mushroom House, College of Natural and Applied Sciences, Michael Okpara University of Agriculture, Umudike. Umudike is located approximately at latitude $05^0 29^1$ N, longitudes $07^0 32^1$ E. Umudike falls within the rainforest zone of southeastern Nigeria with a mean altitude of 123m. Daily mean temperature ranges from $23^0$ C and $32^0$ C. The field work was carried out between the months of August to November, 2008.

### *Collection of Samples*

The soil used in this study had no previous history of crude oil contamination. The soil sample was obtained from the top soil (0 – 15 cm) collected within the Campus of Michael Okpara University of Agriculture, Umudike. The seeds of the *Mucuna* ascensions used were obtained from the Seed Bank of International Institute of Tropical Agriculture, Ibadan. The crude oil was a Nigerian Bonny light blend obtained from Shell Petroleum Development Company (SPDC) Limited Port-Harcourt, Nigeria.

### *Soil Treatment*

Soil samples collected were homogenized and sieved. The contamination with the crude oil was done by thoroughly mixing with the soil in their respective plastic buckets. Soil of 4 kg was treated with 40 ml, 80 ml, 120 ml and 160 ml of crude oil to obtain 1, 2, 3, and 4% v/w (volume/weight) crude oil contamination. Each treatment including the control (0% v/w) was replicated five times.

### *Anatomical Studies*

Anatomical sections were obtained using the modified method described by Edeoga *et al.,* [12]. Some mature and fresh parts of the leaves, stems and roots of the three *Mucuna* species growing in the different levels of crude oil polluted soils were collected. These plant parts were fixed in FAA (Formalin, acetic acid and alcohol in ratio of 1:1:18 respectively). These plant parts were washed in water and sectioned with a Reichert Rotary Microtome. The sections were first stained with two drops of alcian blue for three minutes. The alcian blue stain is washed off and the sections are counter stained with safranin solution for about two minutes, and then dehydrated with pure xylene at intervals for few seconds. The sections are finally mounted on slides using Canada balsam. A hot plate at $40^o$ C was used to dry the slides.

### *Epidermal peels*

The method of Edeoga *et al*., [13] was used. Epidermal peels were obtained by boiling parts of the fresh leaves (from mature *Mucuna* plant samples growing in the different levels of crude oil polluted soils) in concentrated nitric acid in a water bath for about 2 - 3 minutes. The plant samples were then carefully washed in water and the lower and upper epidermis teased from the mesophyll using dissecting needles and forceps. The epidermal peels were stained with safranin solution for about three minutes and washed off with water before mounting in glycerine.

### *Photomicrography*

Observations and photomicrographs of the slides of the anatomical sections and the epidermis were taken using Leitz Wetzler Ortholux microscope fitted with a Vivitar - V335 camera.

## Results

Changes in the anatomy of the three Mucuna species were observed due to the crude oil polluted soil in which they were grown. These observed changes in anatomy were however uniform across the three species. There were presence of needle shaped trichomes on the lower and upper epidermis of *M. veracruz, M. jaspodea* and *M. ghana*. (Plate 1a). However these needles shaped trichomes were more on the upper epidermis than on the lower epidermis. Droplets of oil were observed in most of these trichomes. This can be seen in Plate 1a in *M. veracruz*.

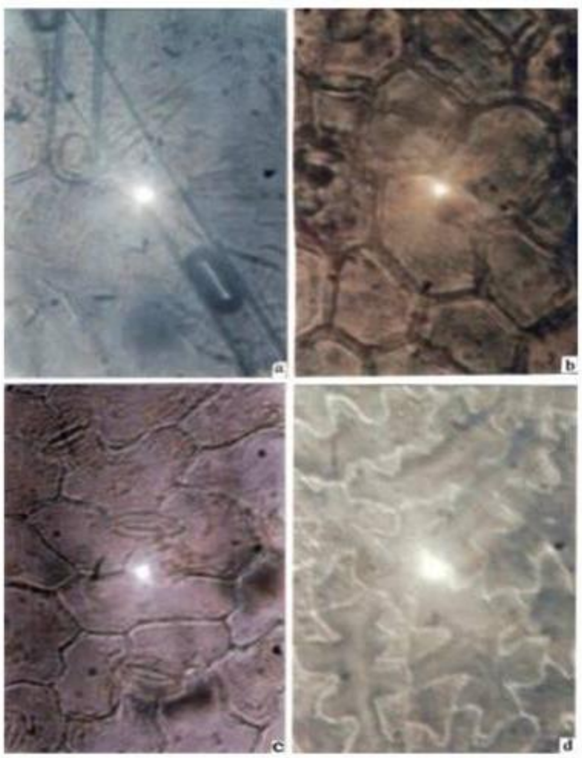

**Fig. 1a:** Presence of an oil droplet in the needle shaped trichome of *M. veracruz* (x400). **Fig. 1b.** Straight cell walls on the upper epidermis of *M. veracruz* grown on 1% crude oil polluted soil (x250). **Fig. 1c:** Slightly curved cell walls of *M. veracruz* (x250). **Fig 1d:** Sinuous epidermal cell walls of upper epidermis of *M. veracruz* grown in 4% oil polluted soil (x100).

On the upper epidermis of the three species of *Mucuna*, the irregularity of the anticlinal cell walls of leaf lamina increased considerably as the level of pollution increased. From Plate 1b - 1c, the level of sinuosity could be seen to increase as contamination increases. The straight walled epidermal cells became slightly curve as observed in upper epidermis of *M. veracruz* grown on 2% crude oil polluted soil (Plate 1c). The curved nature of cell walls increased as the concentration of crude oil in the soil in which they were grown increased. The sinuosity of anticlinal cell wall increased considerably in *Mucuna* species growing 3% and 4% crude oil contaminated soils (Plate 1d). The mean value of stomatal frequency of the leaf epidermis of the *Mucuna* plants grown in control soil surpassed the other plants grown in higher crude oil polluted soil (Table 1).

Table 1: Stomatal frequency of upper and lower epidermis of the three *Mucuna* species in the different crude oil polluted soil investigated.

| Mucuna species | Crude oil polluted soil | *Stomatal frequency | |
|---|---|---|---|
| | | Lower Epidermis | Upper epidermis |
| *M. veracruz* | 0% | 35.1±7.23 | 25.6±2.12 |
| | 1% | 33.0±2.14 | 23.0±3.55 |
| | 2% | 25.6±2.56 | 19.8±2.75 |
| | 3% | 18.5±13.12 | 13.4±1.22 |
| | 4% | 12.8±2.73 | 12.1±2.08 |
| *M. jaspodea* | 0% | 36.7±0.98 | 26.1±4.45 |
| | 1% | 34.4±5.55 | 24.1±2.93 |
| | 2% | 23.8±4.65 | 19.2±2.11 |
| | 3% | 20.1±7.87 | 15.4±19.2 |
| | 4% | 14.3±1.10 | 11.9±0.88 |
| *M. ghana* | 0% | 32.4±2.87 | 24.8±3.55 |
| | 1% | 31.0±2.26 | 22.9±5.23 |
| | 2% | 22.1±18.4 | 18.5±3.43 |
| | 3% | 17.2±4.55 | 12.6±1.47 |
| | 4% | 12.5±0.34 | 9.7±1.89 |

* = mean+S.D.

In the mid-rib anatomy, tissue damage to parenchyma cells were observed (Plate 2a) in *Mucuna* species grown in higher crude oil polluted soils. Distorted parenchyma cells were also observed in the mid-rib anatomy of *M. ghana* (Plate 2c). Tangentially flattened parenchyma cells was also a feature observed in the mid-rib of *Mucuna* species grown in high crude oil polluted soils. This can be seen in *M. ghana* grown in 4% oil polluted soil (Plate 2c). This tangentially flattened parenchyma cells contrast with those Mucuna species grown in control soil (Plate 2b).

Large polygonal shaped parenchyma cells with large intercellular air spaces were seen in the transverse section of stem of *Mucuna* species grown in control, 1% and 2% crude oil polluted soil (3a). In contrast, parenchyma cells of stems of *Mucuna* species of higher concentrated oil polluted soils had small sized parenchyma cells and much reduced intercellular air spaces (3c). The mid rib anatomy also exhibited this characteristic. In the transverse section of the roots, oil contamination also affected the parenchyma cells where large, round to polygonal cells were observed in *Mucuna* species growing in the control soils and the 1% and 2% oil contaminated soil in contrast to the *Mucuna* plants grown in higher crude oil concentrated soils. The breakdown of parenchyma tissues were also observed in the cortical area of the root and stem anatomy (Plate 4a, b and c).

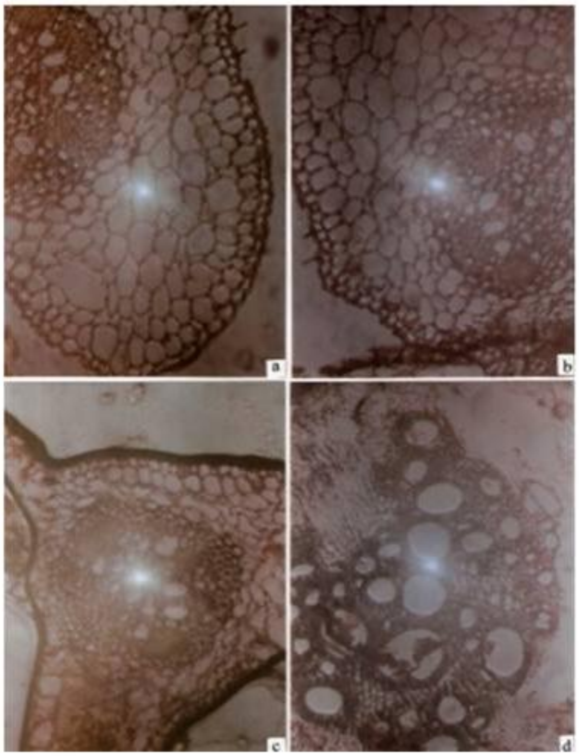

**Fig 2a:** Tissue damage of parenchyma cells of midrib of *M. jaspodea* growing on 3% crude oil polluted soil (x100). **Fig 2b:** T/S of midrib of *M. ghana* on control soil showing normal parenchyma cells (x100). **Fig. 2c:** Distorted and tangentially placed parenchyma cells of midrib of M. ghana on 4% oil polluted soil(x100). **Fig. 2d:** T/S of root of *M. jaspodea* showing clogged vascular tissues grown on 4% crude oil polluted soil (x100).

## Discussion

The presence of oil droplets in the trichomes and clotted depositions in vascular bundles and the ground tissues occurred through plant uptake. Vwioko and Fashemi [6] have reported the presence of dark glossy spots on the stomata of leaves. These dark glossy spots were oil droplets taken up by the plants alongside uptake of water from the soil. The clotted deposition observed in the ground tissues that clog vessels might as well have been heavy metal depositions. Sridhar and Diehl [14] have reported clotted depositions in *Brassica juncea* caused by accumulation of zinc and cadmium in the plant tissues. So the clotted depositions observed in the root and stem anatomy of these *Mucuna* species could also have arisen as a result of the accumulation of heavy metals by these plant parts.

The curved and irregular epidermal cell shape and increasing level of sinuosity of the lower and upper epidermis of these plants as the crude oil concentration increased can be attributed to morphological aberrations. Sharma *et al*., [15] have reported the adverse effect of environmental pollution on plant growth to include morphological aberrations and stomata abnormalities. The degree of sinuosity of epidermal cells is usually caused by the degree of force exerted on the stomata in their course of development [16]. The acute sinuosity observed in the epidermal cell walls of the three *Mucuna* species grown in the 3% and 4% crude oil polluted soil could be as a result of the stress conditions caused by the physiological drought imposed on the plants by the oil pollution.

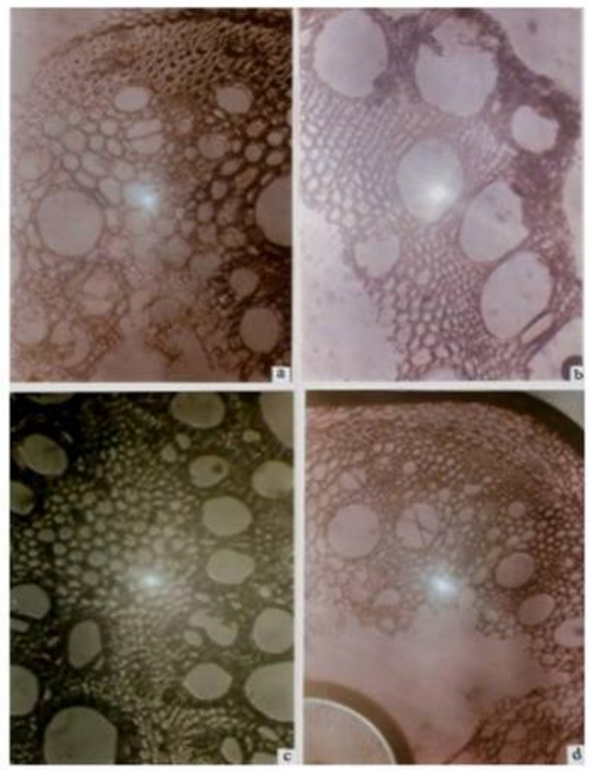

**Fig. 3a:** T/S of stem of *M. jaspodea* growing on 1% polluted soil with large parenchyma cells and intercellular air spaces (x100). **Fig. 3b:** T/S of stem of *M. veracruz* growing on 4% crude oil polluted soil showing oil depositions on outer cortical cells. **Fig. 3c:** T/S of *M. ghana* on 4% crude oil polluted soil with reduced parenchyma cells and intercellular air spaces (x100). **Fig. 3d:** Thick walled parenchyma cells of *M. veracruz* growing on 4% polluted soil (100)

The decrease, in the stomata frequency in the epidermis as the level of pollution increased, had earlier been reported in other plants affected by crude oil or its derivatives [6,8,9]. Stomata play a pivotal role in controlling the balance between water loss and biomass production. The reduction in stomatal frequency as concentration of crude oil increased in the soil could be an adaptation mechanism to reduce water loss through transpiration.

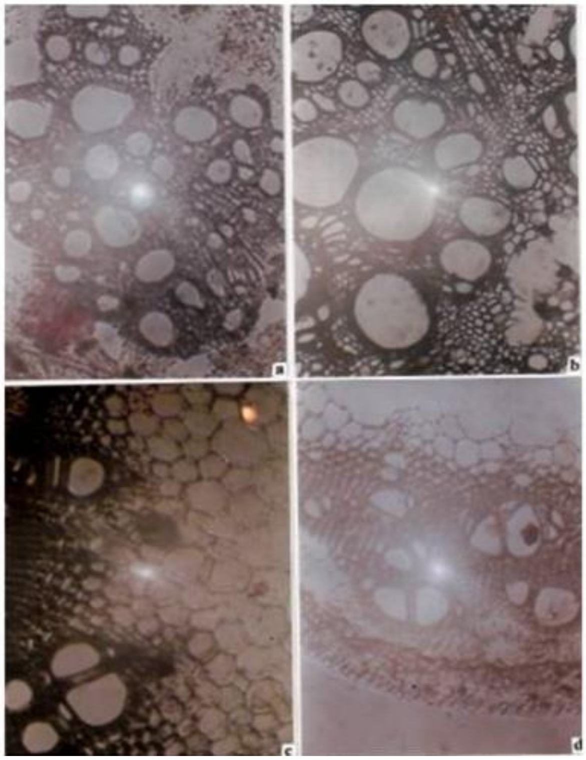

**Fig. 4a and 4b:** Tissue breakdown evident on the root of *M. jaspodea* and *M. veracruz* grown on 2% and 4% crude oil polluted soils respectively. **Fig. 4c:** T/S of stem of *M. ghana* showing normal parenchyma cells (x400). **Fig. 4d:** T/S of of stem showing tissue

The reduction in cell size, the increase in cell shape irregularity and increased thickness of cell wall of the parenchyma tissues as oil concentration increased could be as a result of a condition of physiological drought. This condition caused parenchyma cells to become smaller and have thicker cell walls as a drought avoidance mechanism to reduce water loss. The tangentially placed parenchyma cells could also be attributed to this same stress condition due to pressure on the cell walls during their course of growth and development.

This is a phytotoxic response of these plants because of their contact with crude oil. Sridhar and Diehl [14 have reported parenchyma tissue breakdown as a result of phytotoxic response due to the accumulation of zinc and cadmium by *Brassicaa juncea*. Chang *et al*., [17] stated that a positive confirmation of metal toxicity includes that the plant have sustained injury, a harmful metal have accumulated in the plant and the observed abnormalities are not due to other disorders of plant growth. These three factors were observed in the investigated *Mucuna* species and therefore confirm the harmful effect of heavy metals and their accumulation in these plants.

These visible anatomical changes observed in these *Mucuna* species as a response to crude oil pollution can be employed as a phytomonitoring technique for oil pollution and its heavy metal component. Singh *et al*., [11] proposed that for biological effect monitoring, pollution induced changes in individual parameters of plants should be quantified and correlated with the level of pollutants. From the present study, it can be observed that responses or changes in the *Mucuna* species anatomy increased as the oil pollution level increased.

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