academicJournals

Vol. 7(7), pp. 68-75, July, 2015
DOI: 10.5897/JTEHS2015.0331
Article Number: 5C4601B54810
ISSN 2006-9820

http://www.academicjournals.org/JTEHS

Journal of Toxicology and Environmental Health Sciences

*Full Length Research Paper*

# Evaluation of five medicinal plants at gas flaring site, using foliar photomicrography

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Received 27 January 2015; Accepted 21 July 2015

**Gas flaring is a recognized polluting agent in the environment, affecting all forms of life. The gases and particulate matters (dust, mist and fog) follow the same diffusive pathway with carbon IV oxide into the plant leaf, where they react with enzymes, hormones, and other biochemical molecules, resulting in the deformation of organelles with adverse consequences. In this study, foliar photomicrography analysis of five medicinal plants (*Carica papaya, Chromoleana odorata, Ricinus communis, Manihot esculenta and Euphorbia sp.)* from the flare site and non-flare site was used to evaluate the impact of gas flaring in foliar micro-structure. Results showed the occurrence of structural deformities evidenced in distorted veinal arrangement, necrosis, chlorosis, stomata and guard cell destruction. The plants studied have ethno-medicinal and nutritional values in the study area. The study showed that foliar photomicrography could be used as component of vegetation studies in the environmental impact assessment, auditing, and evaluation of any anthropogenic activity.**

**Key words:** Gas flaring, phyto-therapeutics, phyto-potency, pollution.

## INTRODUCTION

Exploration, exploitation, transportation and utilization of petroleum products involve interference with the natural environmental set up. The interference, depending on the intensity and length of time may result to temporary or permanent alteration, distortion and destruction of the environment with associated hazards to the beneficiaries of the environment. This phenomenon is called environmental pollution, a destabilizing of the ecosystem by introduction of foreign bodies, or increasing the concentration of the existing natural components, beyond the limit of environmental self-restoration, and carrying capacity (Otuu et al., 2014).

Gas flaring is one of the unwanted outcomes of petroleum activities because of the inherent dangers attributed to the flared gases (Ayoola, 2011). Globally, the problem of gas flaring in destroying the ecosystem of an impacted area poses a great challenge to the sustainability of environment and lives there in. In Nigeria, the magnitude and diversity of gas flaring has recently been forced into the mass consciousness, especially among the communities living in the coastal belt whose fragile mangrove ecosystem is frequently assaulted.

Anatomical and physiological changes in plant roots,

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shoots, and leaves may be indicative of adverse environmental impacts on plants exposed to gas flaring or environmental pollution (Ogbonna et al., 2013). The enhanced accumulation of phenolics and lignin is considered to be one of the most common reactions of plants to stress (Wild and Schmitt, 1995). Reduction in stomatal closure under metal stress is not uncommon. The changes in the inner anticlinal walls of the guard cells have been shown to be induced by Pb stress (Ahmad et al., 2005). The decrease of the stomatal size may be an avoidance mechanism against the inhibitory effect of a pollutant on physiological activities such as photosynthesis (Irina, 2009).

The object of this study is to use the changes in foliar photomicrograph in plants as indices of gas flaring - induced foliar microscopic changes, when compared with the same medicinal plants from non-gas flaring site within the same geographical location. The following plants were studied: *Ricinus communis, Carica papaya, Chromoleana odorata, Manihot esculenta and Euphorbia sp.* These plants have both nutritive and ethnomedicinal importance in Etche community, as with all other tropical communities (Sofowora, 2008; Trease and Evans, 2009). *R. communis* plant has many uses in medicine and other applications. In laboratory rats, alcoholic extract of the leaf was shown to protect the liver from damage by certain poisons (Joshi et al., 2004; Sabina et al., 2009). *C. papaya* is both useful as a fruit and as an ethno-medicate. Its uses in ethno-medicine include treatment of malaria, when the leaves are ground and made into tea, and topical application in the treatment of cuts, rashes, stings and burns. *C. odorata* leaf when crushed and applied topically is used as a blood-clotting agent, thereby making it an important agent in the treatment of wounds, skin abrasions and cuts. *M. esculenta* is an important staple food in the Tropics. It is also believed to be a strong ethno-medicate. The root has been promoted as a treatment for bladder and prostate cancer (Dailymail.co.uk). Some *Eurphorbia spp.* are used in the treatment of ulcers, cancers, tumors, warts, and as antiherpetics (Betancur-Galvis et al., 2002).

The flow station of Shell BP, is located at the Etche community - a disperse coastal community, in Etche Local Government, Rivers state, Nigeria. The community is very densely populated, with agriculture and fishing as the main occupation. Like other coastal communities, they rely essentially on subsistent farming of food and cash crops for their nutritional and economic needs. Its mangrove vegetation consists of a variety of plants, some of which are utilized for their medicinal values, in the management of various diseases and ailments.

Since gas flaring has been recognized internationally as one of the major anthropogenic factors implicated in the etiology of phytopathology, there is a need to evaluate the extent the gas flaring from the flow station in Etche community may affect the quality of life of some medicinal plants by causing changes in foliar structure. This is the focus of the study. This involves comparative evaluation of the folia photomicrograph of five (5) medicinal plants from gas flaring and non gas flaring sites in Etche, using the differences in the photo-micrographic structures as indices of flare induced negative impacts. Since plants are major recipients of environmental pollutants, evaluation of foliar microscopic status is an important tool in environmental impact assessment, auditing and evaluation. The relative simplicity of foliar photomicrograph over other methods makes it a better choice.

The effectiveness of any medicinal plants for use in ethno medicine depends on the quality of its biochemical constituents, anatomical and physiological status. When these constituents are compromised by polluting agents, the plants phytopotency will also be compromised, and the plant will lose its phytotherapeutic value as well as become a potential source of introduction of harmful substances to the user system. It was in recognition of the above fact that this study was embarked upon, since evaluation of flare induced changes by a relatively simple method such as foliar photomicrograph would be useful to an environmentalist in site quality assessment, pollution monitoring by an Environmental Health Officer as well as in the evaluation of crude plants for therapeutic uses, because foliar microscopic changes are indications of biochemical negative impacts of anthropogenic activity in vegetation.

## MATERIALS AND METHODS

### Sample collection and treatment

The foliar parts of the following plant samples were randomly collected from the study site 100 meters from the perimeter fencing of Shell flow station: *C. odorata* (L.) R. King & H. Robinson (*Asteraceae*), *C. papaya* L. (*Caricaceae*), *Euphorbia sp. (Euphorbiaceae*), *Manihot esculenta Crantz (Eurphobiaceae*) and *R. communis L. (Euphorbiaceae).* The plants' ages were indeterminate as they have been growing wildly in the site through generations. The plants were thoroughly examined in their habitat and records of their physical features, noted in the field note book. Healthy leaf samples were carefully cut off from their stalk, stored separately in perforated brown envelopes, and taken to the department of Plant Science and Biotechnology, University of Nigeria, Nsukka, for taxonomical identification. The leaf samples were washed in running tap water to remove dust particles, and dried at room temperature in the Anatomy laboratory of the department.

### Foliar photo-micrograph

Using a camel hair brush, nail varnish was applied on 22×22 cm portion of both the adaxial and abaxial surfaces of the leaf and left to dry for 10 min. Subsequent coatings were applied for the second and third times and left to dry for 10 min and 20 min respectively. The samples were then passed through air current for 1 h to ensure maximum dryness. Epidermal strips of the leaf samples were scrapped gently with the aid of forceps and placed on a clean slide, stained with Safranin, washed with alcohol three times and covered

**Table 1.** Photomicrographic features of leaf surface, stomatal type and pore size

| S/N | Species | Leaf surface | Stomatal type | | Pore size (µm) | |
|---|---|---|---|---|---|---|
| | | | Test | Control | Test | Control |
| 1 | *Chromoleana odoranter* | Adaxial | Anisocystic | Actinocytic | 0.1-0.2 | 0.1-0.2 |
| | | Abaxial | Anisocytic | Actinocytic | | |
| 2 | *Carica papaya* | Abaxial | - | Diacytic | - | 0.2 |
| | | Adaxial | - | Diacytic | - | 0.1 |
| 3 | *Euphorbia sp* | Abaxial | Cyclocytic | Anisocytic | 0.3 | 0.2 |
| | | Adaxial | Cyclocytic | Anisocytic | 0.2 | 0.3 |
| 4 | *Manihot esculenta (cassava)* | Adaxial | Diacytic | Cyclocytic | 0.1-0.2 | 0.1 |
| | | Abaxial | Diacytic | Cyclocytic | 0.4-0.6 | 0.2 |
| 5 | *Ricinus communis* | Adaxial | Anisocytic | Cyclocytic | 0.2 | 0.1 |
| | | Abaxial | Anisocytic | Cyclocytic | 0.1 | 0.2 |

with a cover slip before mounting for microscopic examination. The slide was viewed under the light microscope at ×40 magnifications and photomicrographs were taken with Zeiss light microscope with MC'35 Camera for 53 mm film at ×400 magnification.

## RESULT AND DISCUSSION

Results of foliar epidermal study of *C. papaya, C. odorata, R. communis, Manihot esculentus and Euphobia* sp., are shown in Table 1 and Figures 1 to 5. The adaxial photomicrograph of *C. papaya* in the control show well defined diacytic stomata of size 0.1 µm, with intact guard cells and a network of finely matted veins. The abaxial has a stomata pore of 0.2 µm, intact guard cells and a well laced inter-veinal structure. The test sample recorded no evidence of stomata in both the adaxial and abaxial, and the guard cells were completely destroyed, while the well netted veinal arrangement seen in the control was also lacking. These aberrations were indicative of environmental-induced foliar disruption.

*C. odorata* adaxial features showed well relaxed veinal arrangements in the control, with actinocytic stomata type of pore size 0.1 µm while the abaxial had the same stomata type but of pore size 0.2 µm, with well defined intact guard cells. In the test sample, the guard cells were destroyed, increasing the stomatal pore size to 0.2 µm. The veinal interlocking was destroyed in the abaxial surface, while the stomata type was anisocytic in both surfaces. The adaxial surface showed evidence of necrosis as most of the features seen in the control were bleached in the test. All those deformities were all indicative of foliar disruptive microstructure of plants exposed to polluted environment (Ogbonna et al., 2013). The alteration and destruction of the foliar epidermal structures have negative consequences for the growth, yield and bioavailability of the active excipients necessary for the maintenance of phytopotency and hence, phytotherapeutic applications.

*Euphobia* sp, in Figure 3, revealed a actinocytic stomata type with pore size 0.2 µm, and 0.3 µm for the adaxial and abaxial surfaces, respectively, in the control sample. The interstomatal surface was very distinctive in both the adaxial and abaxial surfaces, with intact guard cells, and continuous veinal arrangement. The above features were contrasted in the test sample with cyclocystic stomata type of adaxial pore size 0.2 µm. The inter-veinal spaces were closed and the abaxial appeared squeezed as if contractile force was applied. Stomata were only scanty, unlike in the control where the stomata were densely distributed. Abrasion, chlorosis and necrosis were very evident in the abaxial features of the test. The photomicrographic revelation was in tandem with field trip visual observation, where incidences of unsightly verigation, stunted growth and folialysis abound. These observations could only be attributed to environmental pollution resulting from gas flaring.

It is a recognized fact most of the gases associated with gas flaring produce acid in reaction with water both in the atmosphere (humidity) and in the leaf. The chlorotic, verigated, and necrotized features as observed, were the result of acid rain. Besides, the particulate matters in the air often contain metallic substances, some of which form basic oxides and hydroxides with the atmospheric moisture, which on deposition on the leaves, together with acid rain constitute $p^H$ stress in the folia integrity, further exacerbating pollution stress.

In Figure 4, the foliar epidermal features of *Manihot spp*, is shown, with cyclocytic stomata type of pore size 0.1 µm in the control, well defined veinal arrangement of continuous interlacing at the adaxial surface. The abaxial

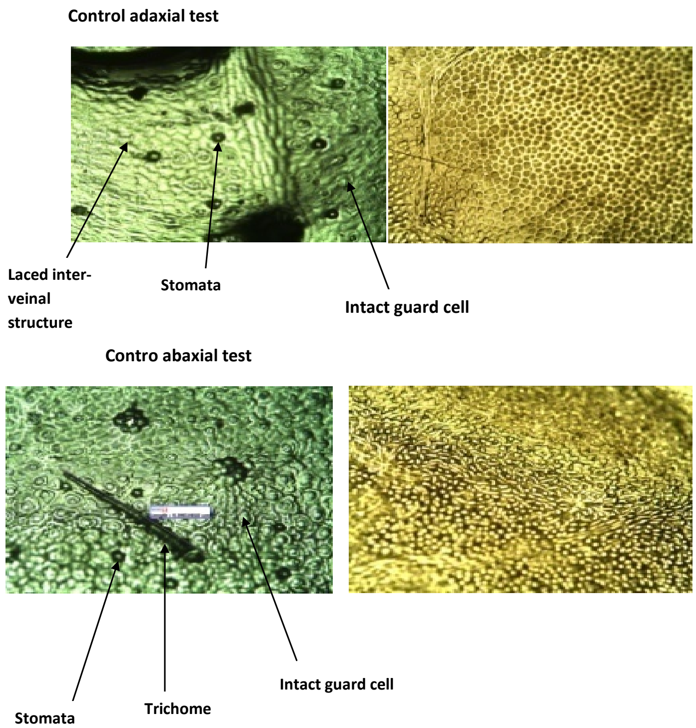


**Figure 1.** Photomicrographs of *C. papaya* showing the changes in the test sample across the adaxial and abaxial parts**.**

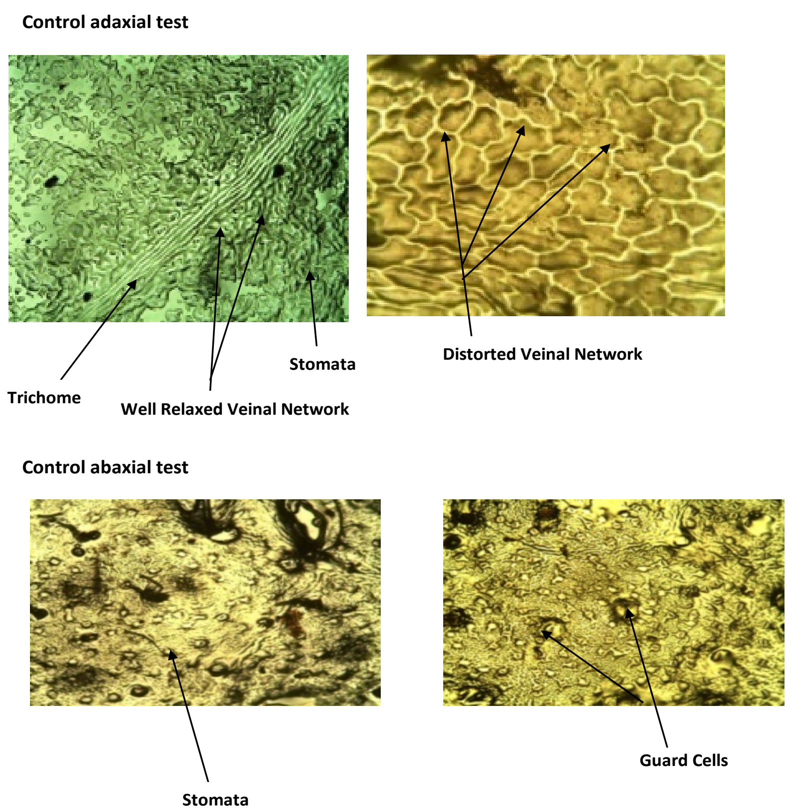


**Figure 2.** Photomicrographs of *chromoleana odoranter* showing the changes in the test samples across the adaxial and abaxial parts.

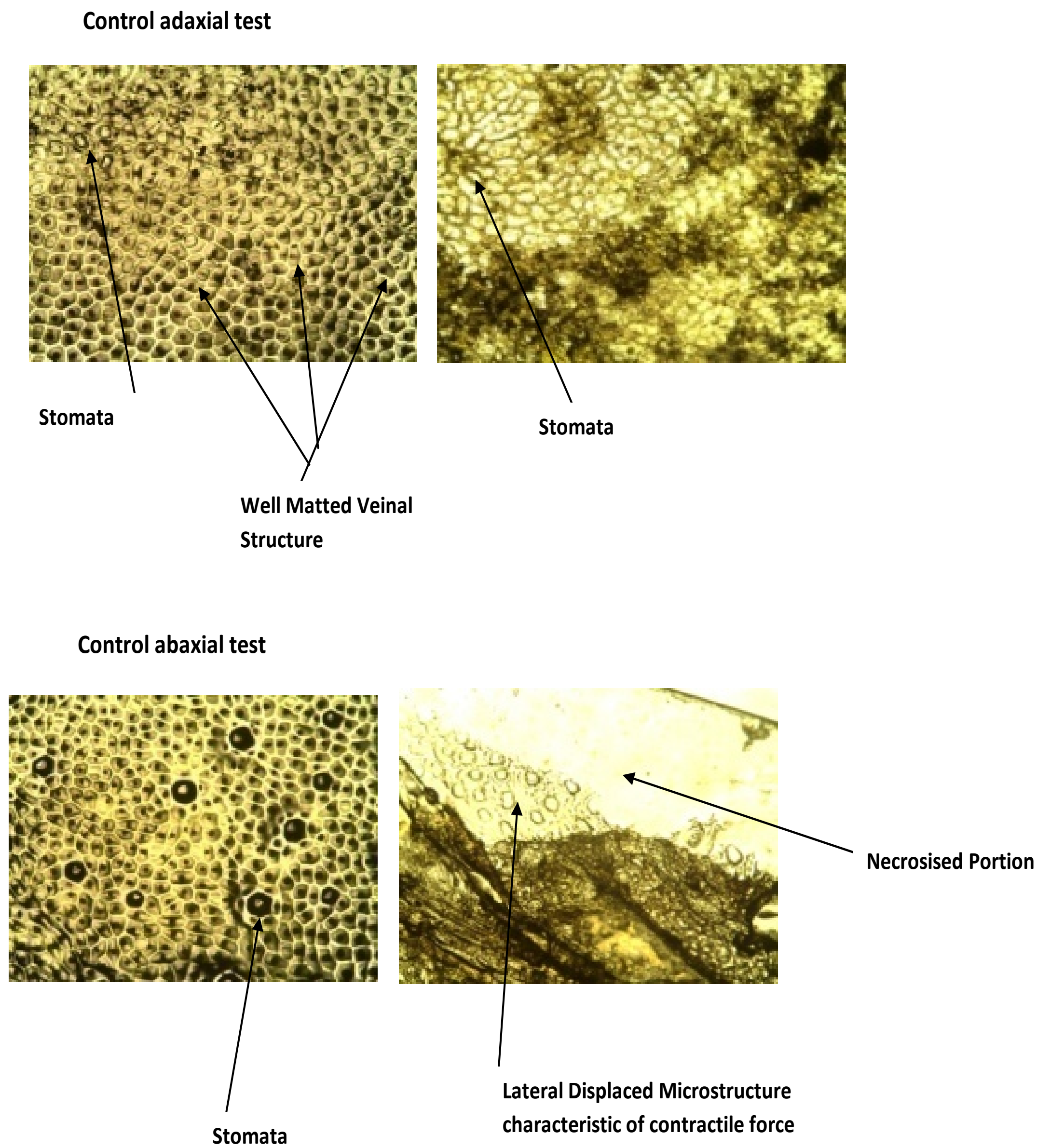


**Figure 3.** Photomicrographs of *Euphorbia sp*. showing the changes in the test samples across the adaxial and abaxial ends.

surface had closely packed internal structure with conspicuous trichomes. Contrarily, this prominent feature was lacking in the test sample, whose interveinal space was pronounced, guard cells destroyed, discontinued veinal structure, all showing evidences of pollution stress. The destruction of the trichomes would expose the leaves to the destructive effects of small herbivores and wind, thereby impacting negatively in foliar physiology, biochemistry and pharmacology. Katiyar and Dubey (2001), reported on the negative impact of sulphur oxide on plant's general physiology and anatomy. Sulphur oxide is a major component of the gas flared particulate matter.

The relative wide disparity in the stomata pore size in the adaxial and abaxial of the test and control could be explained by the destruction of the trichome, guard cells and interveinal arrangement, exposing the finer internal organelles to the destructive effects of the flared constituents. The foliar photomicrograph of *R. communis* revealed cyclocytic stomata type of pore size 0.1µm, on the adaxial, and 0.2 µm on the abaxial, clearly defined guard cells and a cobweb of veinal arrangement. Numerous stomata are seen scattered all over the abaxial, giving the control sample a healthy feature needed for normal plant life. In the test sample, these features are altered. Though the adaxial stomata could

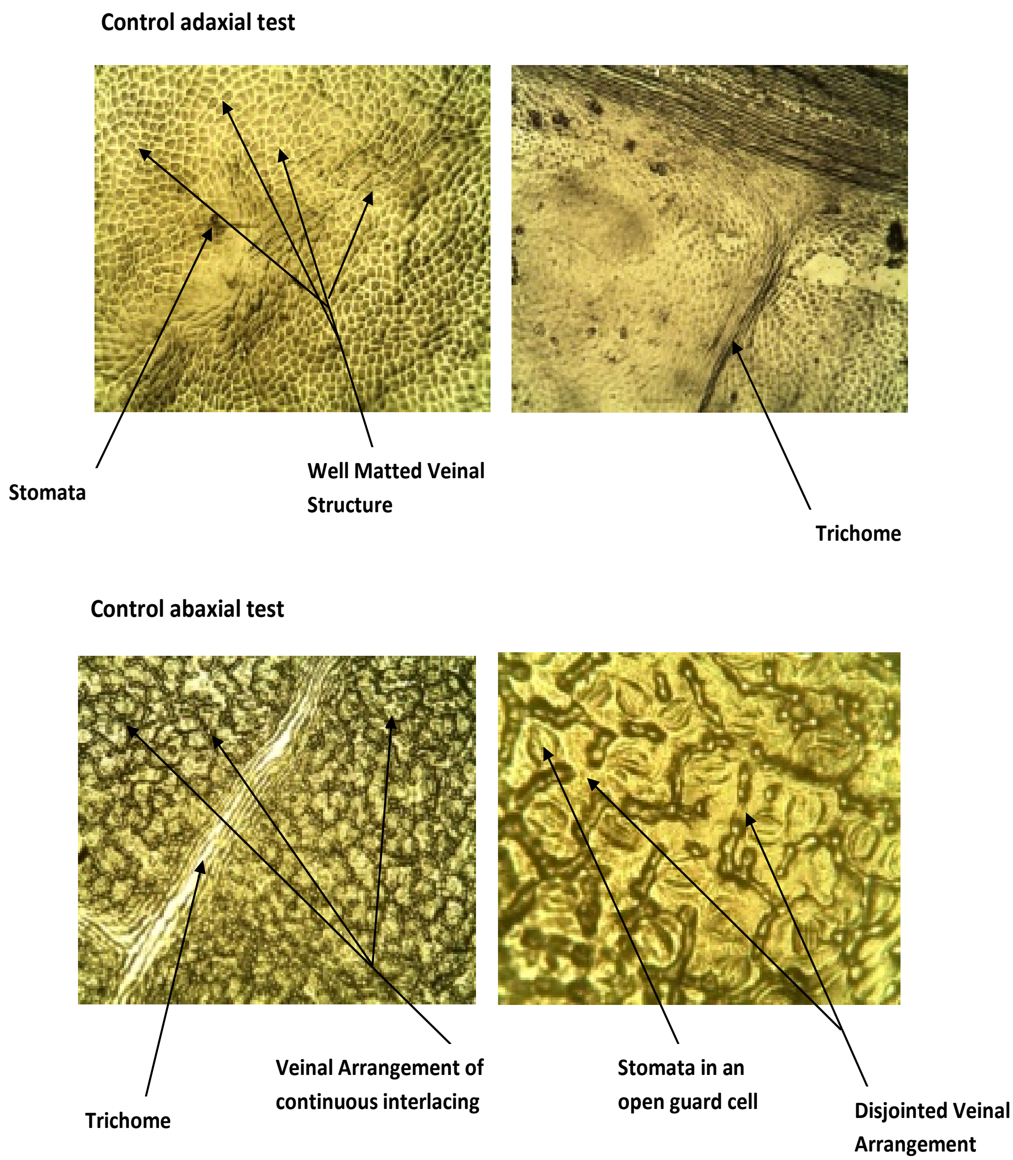


**Figure 4.** Photomicrographs *of Manihot sp.* showing foliar changes in the test samples across the adaxial and abaxial ends.

still be identified, the absence of guard cells was indicative of the destructive effects of the flared gases.

## Conclusion

Gas flaring-induced environmental pollution has negative consequences for the sustainability of medicinal plants, impacting negatively on their phytotherapeutic uses, as well as the socio-economic values. Foliar structural alteration is an indication of adverse physiological and biochemical features of the whole plant, and may be used as bio-indicator of polluted environment. Plants with significant alteration may be considered sensitive to the polluting agent, while those with less significant alterations may be considered as tolerant plants, and hence may be recommended for phyto-remediation of gas flaring polluted environment. The alteration as observed may also be explained as a result of pollutants accumulation in the foliar microstructure. This may have significant health effects. People living around the study area and the neighborhood should be careful when using those plants for food and ethno-medicament.

Since foliar micro-structural alteration in plants is the resultant effect of anthropogenic activities, foliar photomicrography, owing to its simplicity is therefore, the most advisable technique in measuring and assessing stress levels in plants, and may be employed by such

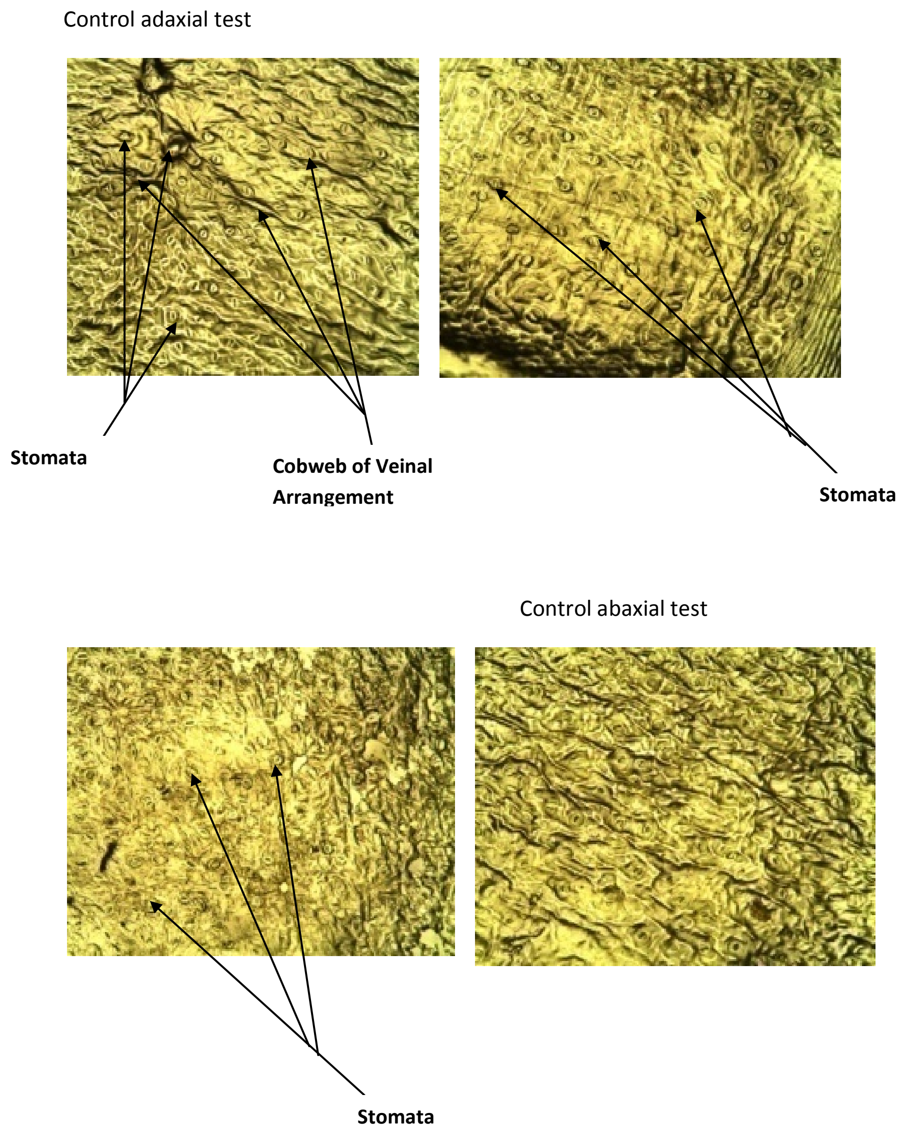


**Figure 5.** Photomicrographs of *R. communis* showing foliar changes in the test samples across the adaxial and abaxial ends.

diverse disciplines and practices as ecology, botany, and other ecological studies as well as in evaluating environmental impact, and auditing, of any anthropogenic activity with potentials for generating environmental pollutants.

## Conflicts of interest

The authors have none to declare.

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