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Leaf epidermal analysis of some plants in the Ishiagu lead–zinc mining area of South Eastern Nigeria

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Abstract

The study examined the leaf epidermis of some plants growing in a lead-zinc mining area of Ishiagu, Nigeria with the aim of evaluating possible impacts of heavy metal stress on leaf structure. Seven dominant plant species, *Adiantum caudatum, Alchornea cordifolia, Centrosema pubescens, Clotalaria retusa, Millettia aboensis, Urena lobata and Vitex doniana* were selected for the study. Analysis of photomicrographs showed clear differences between study site and control samples. In comparison with study site samples, control samples generally had well defined stomata with intact guard cells, and showed no signs of wrinkles or necrosis. This indicates that the study site environment, a heavy metal mining area, had adverse impacts on plant physiological status.

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Introduction

Plants can accumulate heavy metals and other contaminants due to their great ability to adapt to variable chemical properties of the environment and have been described by Kabata – Pendias and Pendias (1984) as intermediate reservoirs through which contaminants from soil and water and air move to man and animals. The main sources of trace elements or heavy metals to plants are soil, atmosphere (through rain and dust) and nutrient solution, from which they are taken up by roots or foliage (Vousta *et al.* 1996). Plant metal load usually corresponds more to surface load than that of soil (Kabata- Pendias and Pendias, 2000).

Heavy metals are abiotic stress factors to plants that affect and change their morphological and biochemical status. These changes are particularly linked to non essential metals such as lead, cadmium and arsenic (Garg and Singla, 2011, Ozdener and Kutbay 2011). These non essential metals directly or indirectly affect physiological processes like photosynthesis, respiration, and water balance and gas exchange (Van Assche and Clijsters 1990). Metals also cause oxidative stress in plants. Oxidative stress is caused by high heavy metal load which stimulates the formation of free radicals and reactive oxygen species (Dietz, *et al.* 1999).

Photosynthesis is inhibited at various levels including carbondioxide fixation, stomata conductance and chlorophyll synthesis (Ernst, 1980, Masarovica, 2002; Shah, 2009). The stomata are important in transpiration and water balance. Plants growing on metal contaminated soils usually show disturbed water balance (Sayed, 1997). Metals reduce plant relative water content and increase stomata resistance. This leads to reduction in stomata pore size or even in stomata closure (Poschenrieder *et al.* 1989). Plant exposure to heavy metals can result to reduction in stomata size and their frequency on both abaxial and adaxial surfaces reduced stomata conductance (Kassim, 2005).

Metals can induce visible stress symptoms in plants including browning of root hairs and tips, reddish brown colouration and death of older leaves and general growth reduction (Andraino, 2001). Lagriffoul (1998) had earlier noted that phytotoxicity in plants is mainly represented by virtual signs such as biomass reduction, chlorosis, necrosis and leaf epinasty.

Plants in the vicinity of metal mining sites are exposed to atmospheric deposition of particulate and gaseous matter generated by mining activities (Lorenz *et al.* 1987). These deposits include gases and particles of toxic metals (Agbaire and Esiefarienrhe 2009). Such plants can therefore serve as biomonitors of environmental stress. In most of these areas mine tailings are indiscriminately dumped (Lar, 2013). Many studies have focused on the heavy metal load of soil and water sources in the Lead-Zinc mining area of Ishiagu (Nwaugo *et al.* 2008, Eze and Chukwu, 2008, Akubugwo *et al.* 2012), to the best of our knowledge information is not available on the effect of Lead – Zinc mining on the leaf of plants in the study area. This study examined leaf epidermis of plants growing in a lead- zinc mining area of Ishiagu to evaluate the effects heavy metals may have on plant physiological status within the mining environment.

Materials and methods

The study area, Ishiagu is situated in Ebonyi State, South Eastern Nigeria. It is between latitudes 5^o 52' to 5^o 60' N and longitudes 7^o 30' to 7^o 37" E. In the study area, mean annual rainfall ranges from 1750mm to 2000mm with double maxima in July and September. Mean relative humidity is 70 percent and mean annual temperature is 27^oC. The study area is located in the tropical rainforest, but due to intensive human modification has been replaced with the so called forest savanna characterized by grasses, shrubs and few trees (Areola, 1983). Studies including those of Nwaugo *et al.* (2008) and Eze and Chukwu (2011) have reported high levels of heavy metals such as lead, zinc, cadmium, arsenic and copper in soil of the study area.

Sample collection

Leaf samples (abaxial and adaxial) of seven dominant plant species (*Adiantum caudatum*, *Alchornea cordifolia*, *Centrosema pubescens*, *Crotalaria retusa*, *Milletia aboensis*, *Urena lobata* and *Vitex doniana*) were randomly collected from the abandoned pit (05.55.695 N and 007.29.909 E) of the mining site. Control samples were collected from Uturu about 20 km from the study area. Samples were immediately sent to the laboratory in a heat proof container for analysis.

Preparation and analysis of Samples

Foliar epidermis of the adaxial (upper surface) and abaxial (lower surface) surfaces of the leaf samples were prepared by impression technique method. The leaf samples were cleaned by washing with water and allowed to dry. Nail varnish was applied using camel hair brush on small portion on both the adaxial and abaxial surface of the leaf samples and left for about 10minutes to dry. A second coating was applied and allowed to dry for the same time. A third coating was then applied and allowed to stay for up to 20minutes. The samples were then passed through air current for 1hour to ensure maximum dryness. Epidermal strips of leaf samples were scrapped gently with the aid of forceps, placed on a clean slide and covered with a cover slip.

The slide was viewed under the light microscope at different magnifications and photomicrographs were taken with Zeiss light microscope with MC'35 Camera for 53mm film at x 100specific magnification. For clearer view, epidermal samples were stained with Safranin and washed three times with alcohol before mounting for microscopic examination.

Results

Results of foliar epidermal analysis of *Adiantum caudatum, Alchornea cordifolia, Centrosema pubescens, Clotaria retusa, Millettia aboensis, Urena lobata and Vitex doniana* are shown on Fig. 1 – .7. The adaxial photomicrograph for *Adiantum caudatum* (Fig. 1) revealed an abrased foliar structure. The undulation in leaf internal structure seen in the control was lost in the study site sample. The study area sample showed a bleached-like internal structure with interveinal necrosis and blotches. The guard cells of the sample from the study area were almost entirely destroyed. In the abaxial photomicrograph, interveinal spaces were very clear in the study area sample in comparison with the control. These features are evidences of contractile stress. In *Alchornea cordifolia* highly remarkable distinction was observed in the control adaxial photomicrograph (Fig. 2). The stomata were well defined with intact guard cells. The veins were well relaxed in the control than in the study area sample. The abaxial stomata and other features also showed clear distinction between the control and study site. The clear venial arrangement in the abaxial control was not seen in the study area. All these suggest environmental stress at the study site.

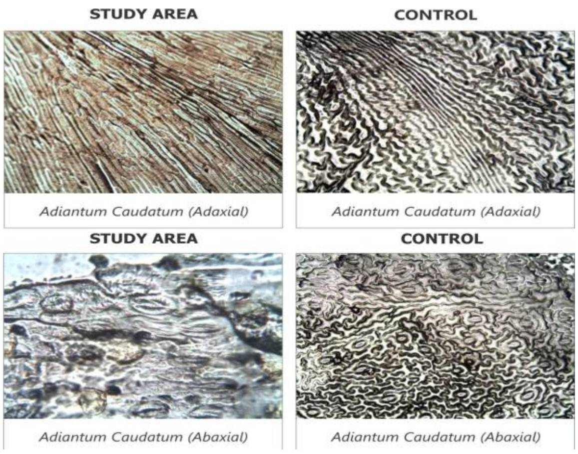

Fig. 1. Photomicrographs of *Adiantum caudatum.*

Foliate photomicrograph of *Centrosema pubescens* revealed alteration of the stomata arrangement in the study area compared with the control (Fig. 3). While stomata arrangement in the control was close that of the study area sample was open. In other words, inter stomata spacing was more pronounced in the study site than in the control, in both the adaxial and abaxial photomicrographs.

The abaxial photomicrograph also showed relative disparity in the stomata and venial arrangement in control and study area. While the control sample showed a distinctive cobweb of veinal structure, the study area sample revealed straited and often discontinued vein arrangement. The stomata are very conspicuous in the control than study site. Both the adaxial and abaxial photomicrographs of *Clotalaria retusa* showed clear differences between the study area and control (Fig. 4). The control showed well defined stomata, unlike at the study area where there were signs of stress. The guard cells in the control were very clear, while at the study area, they have been destroyed. The epidermal and curticular layers were intact in the control leaf, while they were distorted in the study area leaf.

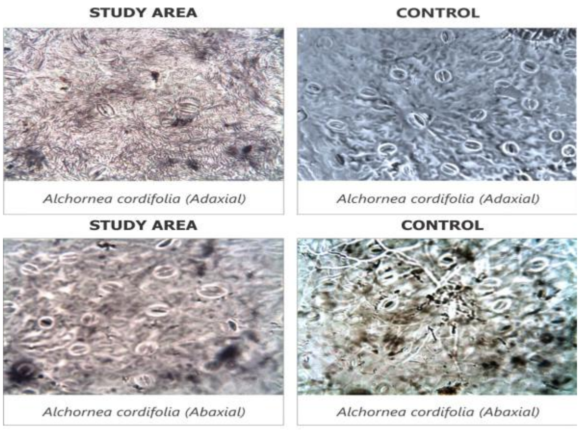

Fig. 2. Photomicrographs of *Alchornea cordifolia*.

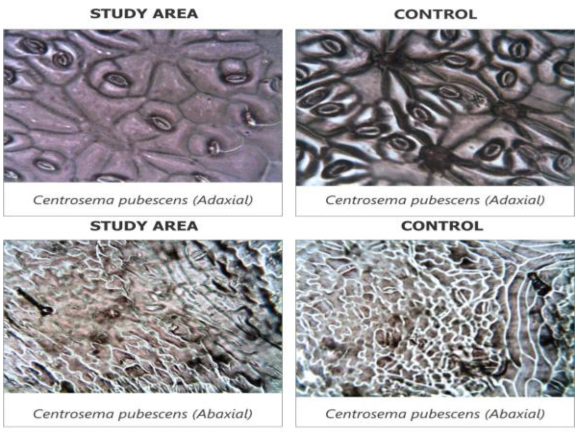

Fig. 3. Photomicrographs of *Centrosema pubescens*.

In the adaxial photomicrograph of the study area sample, the veins were more closely packed and intricately cobwebbed. Interveinal voids were pronounced with traces of particulate matter. The abaxial photomicrograph also revealed remarkable differences in stomatal and venial distribution and arrangement. In the control, veins in the adaxial photomicrograph of *Millettia aboensis* are well matted in comparison with the study sample discontinued veins (Fig. 5). The epidermal layer of the study area sample showed evidences of necrosis and white patches. The lower part of the photomicrograph showed voids with faint stomata presence. The clustered nature of the stomatal arrangement of the leaf in the study area sample also supports the possibility of induced stress. It is also is observed in both the adaxial and abaxial photomicrographs of the study area show destroyed guard cells and veins. In comparison with the control sample the study area sample showed complete destruction of veinal arrangement in *Urena lobata* (Fig. 6). The veins showed discontinuity and small ridges as if they were squeezed together (as is the case with wrinkled leaf). Abaxial control also showed distinctive stomata and guard cells. The limb-like structure of the internal organelles, seen in control is lost in the study area sample. The interlaced venial arrangement of the foliate adaxial photomicrgraph in the control was not seen in the study area sample of *Vitex doniana* (Fig. 7). This suggests environmental stress. The abaxial foliate photo micrograph showed relatively defined stomata in the control than in the study area sample. The arrangement and distribution of the inner organelles are also more orderly in the control then in the study sample.

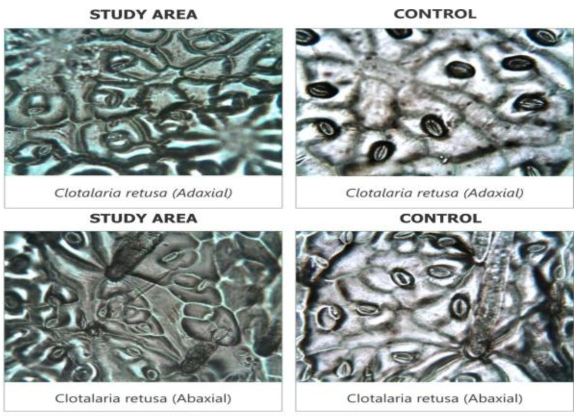

Fig. 4. Photomicrographs of *Clotalaria retusa*.

Discussion

There were clear distinctions between photomicrographs of control and study area samples analysed. In comparison with the study area samples the control samples generally had well defined, unoccluded and more orderly arranged stomata with intact guard cells. Inter stomata spacing was also less in the control with no signs of leaf wrinkles or necrosis. This therefore indicates evidence of stress from mining activities at the study site. Such plants consequently experience physiological and biochemical disturbances including oxidative stress, (Dietz *et al.* 1999) necrosis and general growth reduction, (Andraino, 2001) and stomata reduction and disarrangement (Shah, 2009).

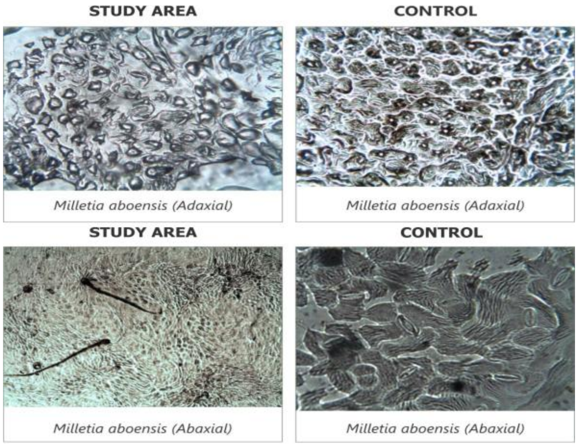

Fig. 5. Photomicrographs of *Millettia aboensis*.

These observations are consistent with the findings of Garg and Singlar (2011) and Ozdener and Kutbay (2011). Saha and Padhy (2011) reported morphological distortions in leaves of *Shorea robusta* and *Madhuca indica* in the vicinity of mining sites. The findings for stomata pore diameter supports the observations made on the photomicrographs of the various leaf samples. In general, mean stomata pore diameter was higher at the control for both abaxial and adaxial surfaces. Reduction in stomata size can have severe effects on many processes such as transpiration, photosynthesis as a result of stomata closure (Werszko- Chmielewska and Chwil, 2005).

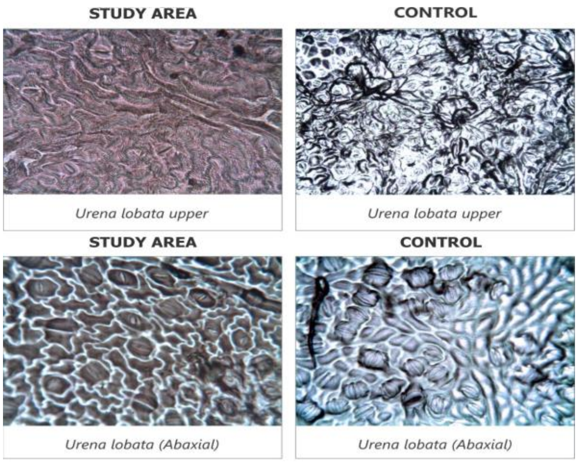

Fig. 6. Photomicrographs of *Urena lobata*.

In excavation and extractive activities, pollutants such as particulate matter, gaseous emission and vaporized trace elements, are usually generated. Some of these pollutants, especially, the gaseous pollutants (SOx, NOx, CO_2, H_2S, NH_3) react with the water droplets, depending on the relative humidity of the environment, to form acids, which eventually fall back to the plants as acid rain. The acid rain attacks the leaf blade, mid rib and veins of the leaf as well as interferes in the normal biochemical status of the leaf. The leaf so affected will manifest symptoms as observed in adaxial photomicrograph (wrinkled vein, interveinal necrosis, epidermal distortion, interstomatal spacing and masked stomata pore.

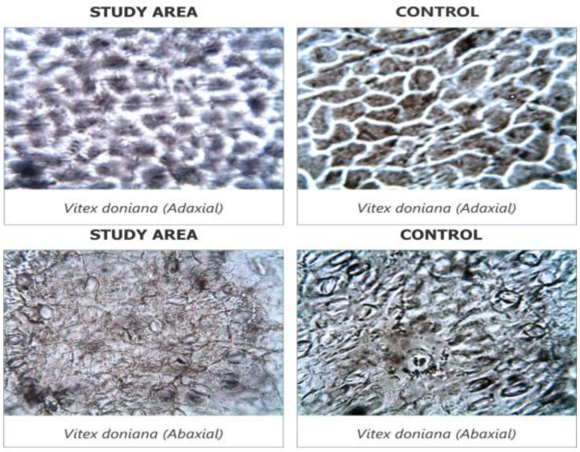

Fig. 7. Photomicrographs of *Vitex doniana*.

Further, some of the plants studied (*Alchornea cordifolia, Adiantum caudatum, Millettia aboensis, Urena lobata* and *Vitex doniana*) have medicinal values (Burkhill, 1994; Foncho *et al.* 2009; Onaegbe *et al.* 2010; Owolabi, 2011 and Pan *et al.* 2011). Their ethno medicinal and phytotherapeutic importance may be limited by heavy metal contamination of these plants. There is is also the added danger of exposing man and animals to the metal intoxication of dietary origin.

Conclusion

Heavy metal induced stress can limit phytotherapeutic and medicinal uses of these plants and their presence in plants increase the possibility of human ingestion of toxic metals through the food chain. This will have severe health effects. Inhabitants of the study area and environs should therefore be wary of using plants that are found in mining site.

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