EFFECT OF TRAFFIC ON RUDEAL PLANT (*Sida acuta*): A CASE STUDY OF TWO MAJOR HIGH WAYS IN SOUTH WEST, NIGERAIA

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ABSTRACT

Vehicular emission is one of the major sources of pollution of roadside vegetation and soil. Degree of pollution by vehicles depends on the traffic congestions on roads. This study aims at identifying and investigating the metal uptake and leaf anatomy of *Sida acuta* (a diagnostic flora) growing along Akure-Ilesa and Ife-Ibadan roads.

Plants samples were collected at 10 km intervals along Akure-Ilesa and Ife-Ibadan roads, and digested using Aqua regia methods, Metal contents (Cd, Pb, Cr, Zn and Cu) were determined with Atomic Absorption Spectrophotometer (AAS). Data were analysed with ANOVA and t-test and differences tested at $p \leq 0.05$. Anatomical studies were done on the leaves of the plant and compared with same plant collected from Unilorin Zoological garden which serves as the control species.

The study revealed high concentration of Zn (0.457±0.367, 0.344±0.222, 0.262±0.211 and 0.506±0.334, 0.646±0.303, 0.522±0.350 mg/kg) for Akure-Ilesa road, Ife-Ibadan road and the Control sample for the dry and rainy seasons respectively. The metals; Cd, Pb, Cr, Zn and Cu) accumulated more in Akure-Ilesa road than Ife-Ibadan road. The investigated metals were found to be lower in the Control sample than the studied location and they accumulated more metals during the dry season than the rainy season. Anisocytic and paracytic stomatal complex types were found on the adaxial and paracytic stomatal complex type was found on the abaxial surface of the leaves of *Sida acuta* from the Control site. Only anisocytic stomatal complex type was found on the adaxial and only paracytic stomatal complex type was found on the abaxial surfaces of those from the study site. Stomatal size was larger (5.21±1.20 and 6.43±1.25 µm for abaxial and adaxial surface, respectively compared to 1.44±0.13 µm for the Control) and more in the study area than the Control. Glandular and stellate trichome types were observed on the adaxial and abaxial surface of *S. acuta*, respectively in the study area while only glandular trichome type was observed in the control sample.

It was concluded that vehicular emission contributed to the heavy metal load of roadside *Sida acuta* and the plant tend to modify its structures as adaptive strategies to survive the polluted environment.

Key words: vehicular emission, roadside, metal contents, stomatal complex types and *Sida acuta*.

INTRODUCTION

Air pollution is a serious problem in many heavily populated and industrialized areas in the world (Kambezidis *et al.* 1996) in many urban areas of the world, motor vehicle traffic is a major source of air pollution contributing 57% - 75% of total emissions (WHO 2006). In general, metropolitan areas have higher pollution than rural areas (Largerwerff and Specht 1970; Sawidis *et al.* 2001). Air pollutants from motor vehicle effects on the metabolism of roadside plants even before visible symptoms appear (Viskari *et al.* 2000). Urban forests and trees in the urban environment can improve air quality through filtering and uptake of gases and particles (Beckett *et al.* 1998). Therefore, urban trees are of high importance for the inhabitants, but may also be endangered by exposure to pollution. For example, in leaves of a typical urban roadside tree species, *Platanus orientalis* air pollution caused changes in chlorophyll content and peroxidase activities (Alaimo *et al.* 2000; Baycu *et al.* 2006). In various roadside plants exposure to road traffic emissions resulted in changes in foliar anatomy and morphology and caused visible injury (Ghouse *et al.* 1984; Jahan and Iqbal 1992; Pandey and Agrawal 1994; Verma *et al.* 2006; Joshi and Abhishek 2007).

Environmental pollution is always on the increase due to human activities such as agricultural operations, sewage discharge, energy production, smelting, refining, disposal of waste, industrial and vehicular emissions. Emissions of pollutants into the air amounted to the greatest source of heavy metal pollution. Heavy metal pollution of soil enhance plant uptake causing accumulation in plant tissues and eventual phytotoxicity and change in plant community (Zeyed *et al.*, 1998; Gimmler et al., 2002,). Heavy metals such as Pb, Cd, Cu, and Zn have been reported to be released into the atmosphere during different operations of the road transport (Zhang et al., 2012; Sharma and Prasade, 2010).

Zhang et al. (2012) reported engine oil consumption as the largest emission for Cd, tyres wear for Zn, and brake wear for Cu and Pb. Cadmium was reported to biomagnified in *Chromolaena odorata* (Emmanuel and Edward, 2010) Ademoroti (1986) noted that organometalics such as tetraethyl lead, an additive to gasoline (petrol), is an important source of lead in automobile exhaust emission. Soil, vegetation and animals including man act as 'sinks' for atmospheric pollutants (Osibanjo and Ajayi, 1980).

This study was carried out on *Sida acuta* representing common plants collected from the study site (Akure-Ilesa road and Ife-Ibadan road) to quantify their heavy metal uptake and to identify some adaptive (anatomical) features which makes them to thrive in that environment compared with those collected from other site that is less polluted.

Materials and Methods:

Study area and sampling

The study was carried out along Akure-Ilesa and Ife-Ibadan roads, south western region of Nigeria. Akure-Ilesa road is not dualised while Ife Ibadan road is dualized; traffic density is a bit high as the two roads lead to some major cities in Nigeria (i.e. Lagos, Abuja, Ekiti State and so on).

Leaf samples of *Sida acuta* were collected during the rainy season (October, 2012) and dry season (March 2013). The collection was done at every 5km intervals along the roadside, the co-ordinate of each sampling location were recorded by the use of a hand-held Global Positioning System (GPS) devise. The plant samples were collected with the aid of knife, and put in separate polyethylene bags and appropriately labeled to avoid mix up and maintain the numbering of the plant samples.

The leaf samples were digested by weighing 2.5g of the plant. Aqua-regia method of digestion was used. 4ml of HNO_3 plus 1ml of HCl was added to the sample. The digestion was carried out on hot plate inside fume cupboard. The digest was allowed to cool to room temperature, then filtered and make up to 25ml of solution.

The samples were analyzed by Atomic Absorption Spectrophotometer (AAS) (GBC A Vanta PM Version 2.02) for metal analysis (Pb, Cr, Cd, Cu and Zn).

Leaf segment of an area of 1cm from each sample was cut and immersed in concentrated solution of nitric acid for 10-20 min. The upper (adaxial) and the lower (abaxial) surfaces were separated with dissecting needle and forceps and rinsed with clean water. Each specimen was stained with 1% aqueous safranin for 3-5 min and excess stain rinsed off in water. The sample was then mounted on glycerin jelly for microscopic observation using an Olympus microscope (Alvin and Boulter, 1974). Shapes of trichomes were kneely observed and noted. Terminologies used for naming of shape of Trichome followed those used by Dilcher (1974) and Metcalfe and Chalk (1950). Frequency of each trichome type was expressed as percentage occurrence of such trichome type based on all occurrences (Metcalfe and Chalk, 1950).

Frequency (%) = Nt/Tt x 100

Where Nt = number of particular type; Tt = total number of trichome types.

Trichome index was determined as number of trichomes divided by number of trichomes plus number of epidermal cell per square millimeter multiplied by 100 (Metcalfe and Chalk, 1950). Using an Olympus microscope at x40 objective in a total of 30 fields of view, the number of subsidiary cells per stoma was counted to determine the frequency of the complex type present in each specimen. Frequency of each complex type was expressed as percentage occurrence of such complex type based on all occurrences (Obiremi and Oladele, 2001). Terminologies for naming stomatal complex types followed those of Dilcher (1974) and Metcalfe and Chalk (1950). Stomatal index (SI) was determined as follows:

SI = S/E + S x 100

Where: SI = Stomatal Index, S = number of stomatal per square millimeter, E = number of ordinary epidermal cell per square millimeter (Salisbury, 1927). The mean stomatal size of a species was determined using this formula:

l x b x K

Where l = length

b = breath

K = Franco's constant (0.78524)

The data generated from this study were analyzed statistically by using Statistical Package for Social Sciences (SPSS). Analysis of Variance was used to test for differences in the concentrations of the heavy metals and the means separated with Duncan Multiple Range Test (DMRT).

RESULT AND DISCUSSION

Table 1 shows the mean concentrations of heavy metals in road side *Sida acuta* along Akure-Ilesa road, Ife-Ibadan road and the control sample during 2012 rainy season and 2013 dry season. The mean concentration of Pb was 0.112 mg/kg and 0.087 mg/kg, Cr was 0.188 mg/kg and 0.082 mg/kg, Cd was 0.119 mg/kg and 0.031 mg/kg, Cu was 0.085 mg/kg and 0.036 mg/kg, while Zn was 0.757 mg/kg and 0.344 mg/kg respectively for Akure-Ilesa and Ife-Ibadan road during 2012 rainy season. While the mean concentration of Pb was 0.218 mg/kg and 0.150 mg/kg, Cr was 0.178 mg/kg and 0.159 mg/kg, Cd was 0.132 mg/kg and 0.105 mg/kg, Cu was 0.075 mg/kg and 0.073 mg/kg, while Zn was 0.506 mg/kg and 0.646 mg/kg respectively for Akure-Ilesa and Ife-Ibadan road during 2013 dry season.When the samples were compared with the control sample they were found to be numerically lower but statistical analysis shows that differences existed among the investigated metals, it was found that Pb, Cr, Cd and Cu, were statistically the same but significantly lower than Zn at $P<0.05$.

Table 1: Heavy metal mean concentrations (mg/kg) in road side *Sida acuta* along Akure – Ilesa, Ife – Ibadan and control samples during 2012 rainy Season and 2013 dry Season (n = 10)

Location/Metal	Akure-Ilesa road 2012 rain	Ife-Ibadan road 2012 rain	Control 2012 rain	Akure-Ilesa 2013 dry	Ife-Ibadan 2013 dry	Control 2013 dry
Pb	0.112 ± 0.109^{b}	0.087 ± 0.081^{b}	0.241 ± 0.221^{b}	0.218 ± 0.147^{b}	0.150 ± 0.130^{b}	0.223 ± 0.203^{b}
Cr	0.188 ± 0.066^{b}	0.082 ± 0.070^{b}	0.062 ± 0.044^{b}	0.178 ± 0.062^{b}	0.159 ± 0.097^{b}	0.106 ± 0.011^{b}
Cd	0.119 ± 0.016^{b}	0.031 ± 0.011^{b}	0.018 ± 0.012^{b}	0.132 ± 0.026^{b}	0.105 ± 0.051^{b}	0.075 ± 0.021^{b}
Cu	0.085 ± 0.021^{b}	0.036 ± 0.011^{b}	0.025 ± 0.012^{b}	0.075 ± 0.024^{b}	0.073 ± 0.036^{b}	0.063 ± 0.026^{b}
Zn	0.457 ± 0.367^{a}	0.344 ± 0.223^{a}	0.262 ± 0.211^{a}	0.506 ± 0.334^{a}	0.646 ± 0.303^{a}	0.522 ± 0.350^{a}

Except for vehicle emissions, the concentrations of heavy metals in soil and plants can be influenced by other local factors, like the use of agricultural fertilizers and pesticides, climate and anthropogenic activities. Results of this study reveal high concentrations of the investigated heavy metals. Habashi (1992) and Fuller (1974) reported undesirable and abnormal concentration of lead (Pb) in air, water, soil and vegetation particularly close to heavily plied automobile free-ways. These range of heavy metals are among the wide range of heavy metals found in fossil fuel which are either emitted into the environment as particles during burning or may itself be transported in air and contaminate soil (Yahaya *et al.*, 2010). This is in line with the report of UNEP/GPA (2004) that asserted that combustion and traffic are among the sources of heavy metals into the environment.

Result of this study also revealed high concentration of Zn in the plants, this is an evidence of bioaccumulation or biomagnification of the heavy metals in plants. When soil is polluted with heavy metals, the metals are taken up by plants and as a result accumulate in their tissues (Trusby, 2003). This is in line with the work of Tanee and Albert (2013) that reported increase in metal concentrations in plants along three major roads (Onne-Akpajo, Refinery and Aleto by-pass roads) in Eleme, Rivers State Nigeria.

Zn was found to have the highest concentration in the higher plants in the study area, followed by Pb, Cu was found to have the least concentration in the vegetation of the study area. This is comprehensible since tyre wears released zinc (Kabata-Pendias and Pendias, 1994). In addition, Zn is used in brake linings owing to their heat conducting properties and as such released during mechanical abrasion of vehicles and from engine oil combustion and tyres of motor vehicle (Dolan *et al.*, 2006; El-Gamai, 2000; Hjortenkrans *et al.*, 2007). The high concentration of Zn in this study may also be due to number of vehicles that pass through the studied roads. USEPA (1996) also reported that lubricant oil add Zn to soils closest to major roads in metropolitan areas. Natural occurences such as volcanic eruption, forest fires, dust storms and sea spray also add to the continuos cycling of Zn through nature. It is predictable that these natural discharges of Zn quantify to 5.9 million metric tonnes every year (International Zinc Association (IZA), 2017). Anthropogenic releases of Zn into the environment are estimated to be a portion of the total emissions from the natural cycling of Zn from erosion, sea spray, volcanic eruption etc (IZA, 2017).

The higher level of Pb might be from the deposition from automobile exhaust since most petroleum fuel is made up of tetraethyl lead as antiknock (Lenntech Water Treatment and Air Purification, 2004). Large amount of fertilizers are frequently added to soils in intensive farming systems to supply adequate NPK for crop development. The compounds used to provide these fundamentals contain trace quantities of heavy metals (e.g Cd and Pb) as impurities, which after persistent fertilizer application may extensively increase their content in the soil (Jones and Jarvis, 1981). The use of certain phosphate fertilizers ineffectually adds Cd and other potentially toxic elements to the soil such as F, Hg and Pb (Raven *et al.*, 1998) these may also contribute to the high level of Pb in the study area. Moreover, some common pesticides used quite at length in agriculture and horticulture in the past contained considerable concentrations of metals. Lead arsenate was applied in fruit orchards for many years to control some parasitic insects (McLaughlin *et al.*, 2000).

The use of plants as monitors of air pollution has long been accepted since plants often are the initial indicators of air pollution. Individual plant responds differently to different air pollutants. Plants improve the quality of urban life due to their large leaf areas, relative to the ground on which they stand. Depending on structural properties of their surface, they can act as biological absorbers or filters of pollutants (Kapoor and Chittora, 2016). The foliar surface is the most important receptor of atmospheric pollutants where they cause several structural and biochemical changes. The present studies on the plants growing in urban area indicate that auto exhaust pollution brought appreciable changes in the number of epidermal cells and stomata per unit area. It has been observed that the stomatal frequencies decline, resulting in the significant fall in stomatal index (Table 2)

Size of epidermal cells and stomata was increased in polluted population. Since differentiation of stomata mother cells requires division of epidermal cells, therefore, a decrease in the frequency of stomata should be normally accompanied by an increase in the size of epidermal cells. These results were in conformity with the findings of Mishra (1982) which studied the effect of environmental pollution on morphology and leaf epidermis of *Commelona bengalensis*. Results were the same; length and density of trichome increased, while stomatal frequency decreased, in respond to environmental pollution (Table 2).

The epidermal cells of *Sida acuta* on the adaxial and the abaxial surfaces of the samples collected from the study sites and the samples collected from the garden are irregular in shape and their anticlinal wall pattern are wavy (table 3, plate 1 and 2), the stomata complex type of the samples collected from the study sites and the garden on the abaxial surface are paracytic (plate 1), while on the adaxial surface, the stomatal complex type of the samples collected from the study sites are anisocytic (plate 2A and 2B) while the samples collected from the garden shows anisocytic and paracytic stomatal complex type (plate 2C). Anisocytic stomatal complex type was observed on the adaxial surface of the *S. acuta* samples collected from the study area which may also be surviving features of plant growing in polluted environment as both anisocytic and paracytic stomatal complex type were observed in the samples collected from the Control area. Occurrence of subsidiary cells such as anisocytic found prevalent in the leaves of the diagnostic species from the polluted site may be helpful in reducing the impact of blockage of pores, increase transpiration rate, in so doing providing good mechanism for gas exchange situation of stress of plants (Carr and Carr, 1990; Oyeleke *et al.,* 2004).

There is presence of trichome in all the leave samples of *Sida acuta*, the adaxial surface of the samples collected from the garden and the study sites has glandular trichome types (plate 3) while the samples collected from the study sites has stellate trichome types on their abaxial surface (plate 4A and 4B), and the samples collected from the garden have glandular trichome type the abaxial surface (plate 4C). It is important to note that the stellate trichome type that was observed on the abaxial surface of *S. acuta* collected from the study area may be a survival strategy in a polluted environment because it was not observed from the samples collected from the control area. Reports have it that pollution stress change the structure of leaves of plants exposed to air pollution but nonetheless, some are quite resistance to air pollutant actions and grow to maturity with various modifications (Gostin, 2009).

Table 2: Epidermal cell, Stomata and Trichome features in *Sida acuta*
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No	Taxon	Leaf Surface	Anticlinal Wall Pattern	Shape of Epidermal Cell	Stomatal Complex Type	Stomatal Frequency (%)	Stomatal Size (µm)	Stomatal Index	Trichome Type	Trichome Frequency (%)	Trichome size	Trichome Index
1	*Sida acuta (C)*	Adaxial	Wavy	Irregular	Anisocytic Paracytic	64 36	1.45 ± 0.13^{c}	44.44	GT	100	419.87 ± 589.03^{b}	6.25
2	*Sida acuta(C)*	Abaxial	Wavy	Irregular	Paracytic	100	1.44 ± 0.13^{c}	66.19	G T	100	68.46 ± 9.38^{c}	6.00
3	*Sida acuta (AK)*	Abaxial	Wavy	Irregular	Paracytic	100	5.21 ± 1.20^{b}	75.68	ST	100	468.00 ± 286.52^{b}	16.95
4	*Sida acuta(AK)*	Adaxial	Wavy	Irregular	Anisocytic	100	6.43 ± 1.25^{a}	74.12	GT	100	598.00 ± 367.47^{a}	16.52
5	*Sida acuta (IF)*	Abaxial	Wavy	Irregular	Paracytic	100	6.21 ± 1.21^{a}	74.64	ST	100	24.00 ± 8.49^{d}	16.58
6	*Sida acuta(IF)*	Adaxial	Wavy	Irregular	Anisocytic	100	5.43 ± 1.15^{b}	72.58	GT	100	69.90 ± 11.02^{c}	15.32

Note ST- Stellate trichome, GT- glandular trichome, C- Control, AK- Akure-Ilesa Road, IF- Ife-Ibadan road.

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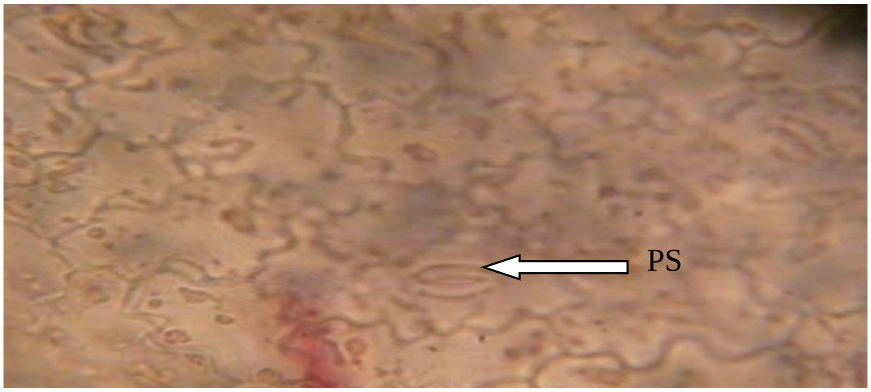
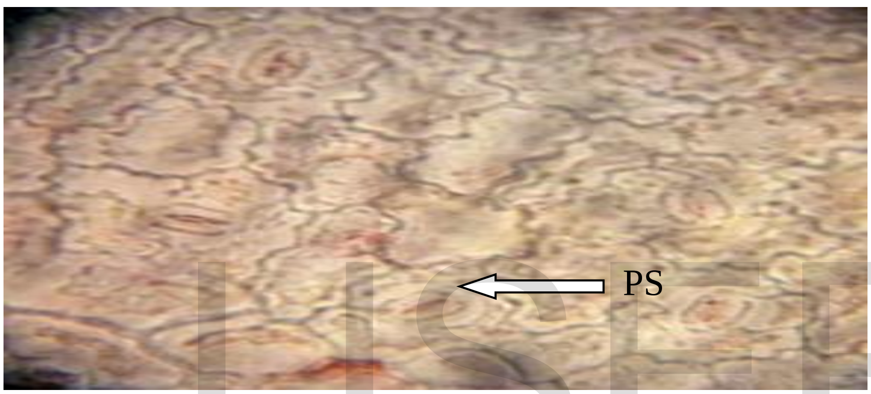


A



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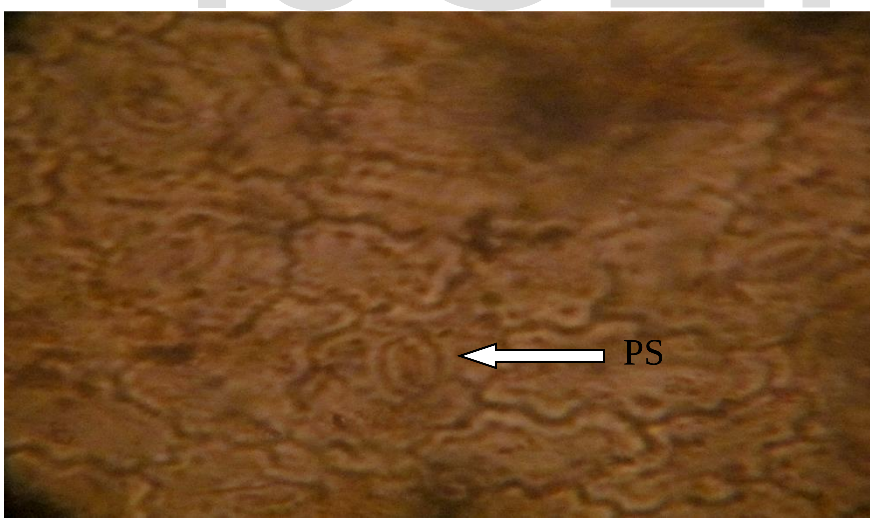

C

Plate 1: Abaxial leaf epidermis of *Sida acuta* showing stomata

PS – Paracytic stomata complex type
(A) – Akure – Ilesa road
(B) – Ife – Ibadan road
(C) – Control

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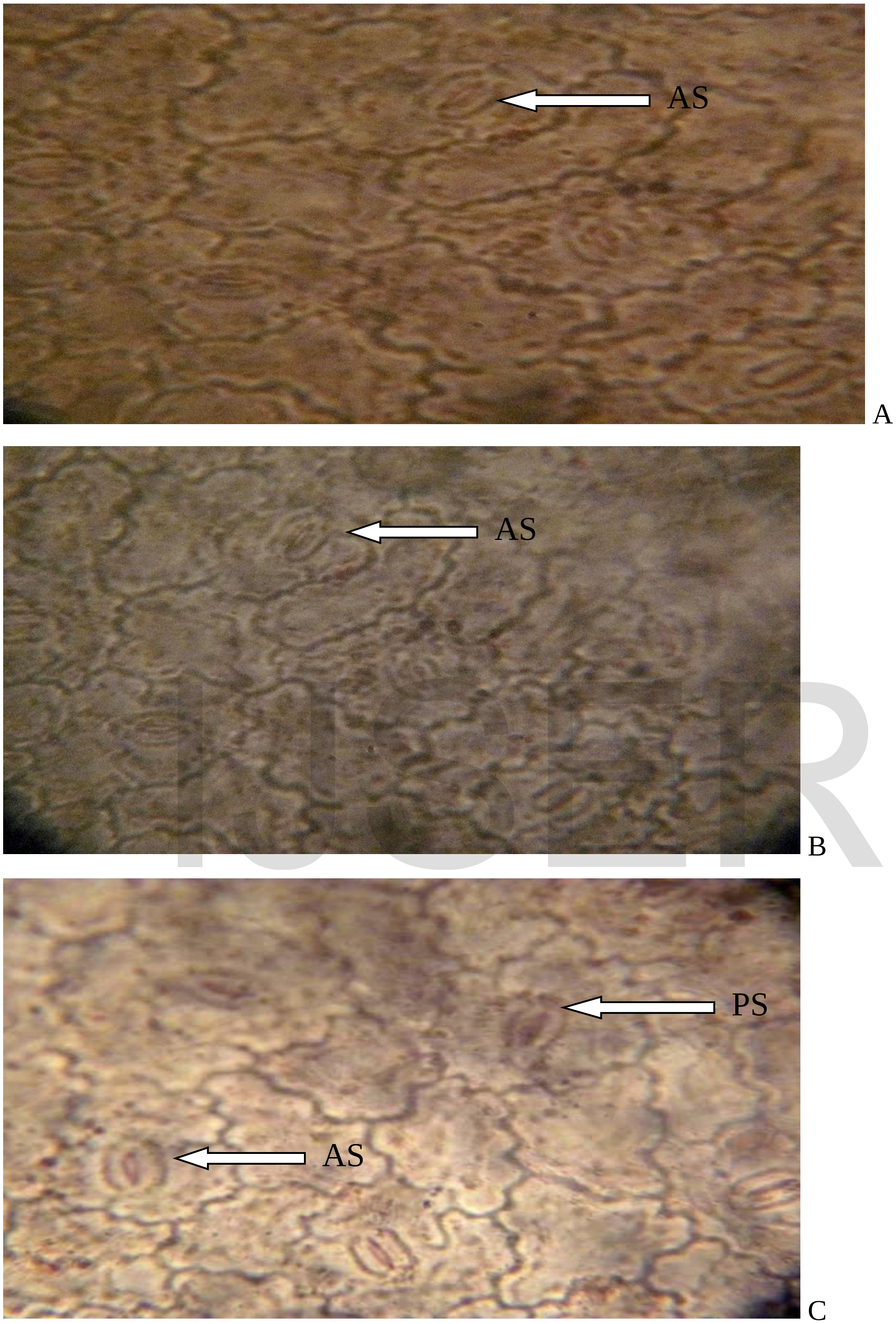

Plate 2: Adaxial leaf epidermis of *Sida acuta* showing stomata

PS – Paracytic stomata complex type, AS – Anisocytic stomata complex type
(A) – Akure – Ilesa road
(B) – Ife – Ibadan road
(C) – Control

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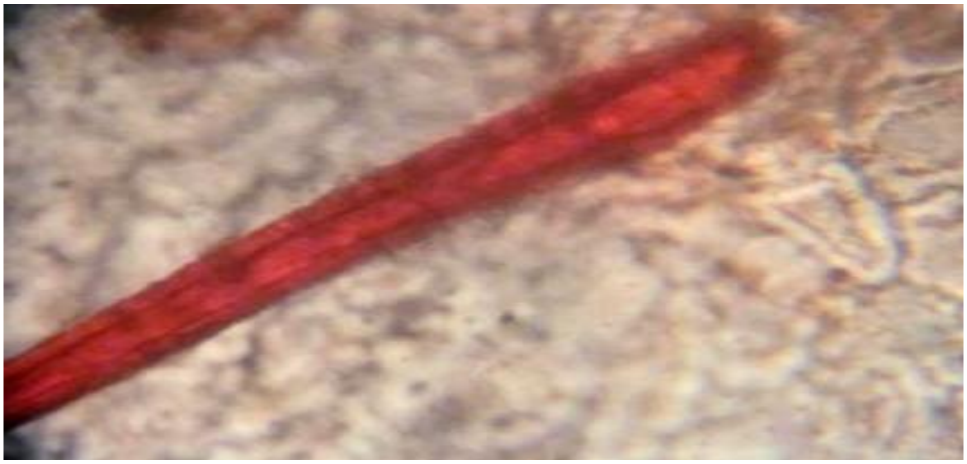
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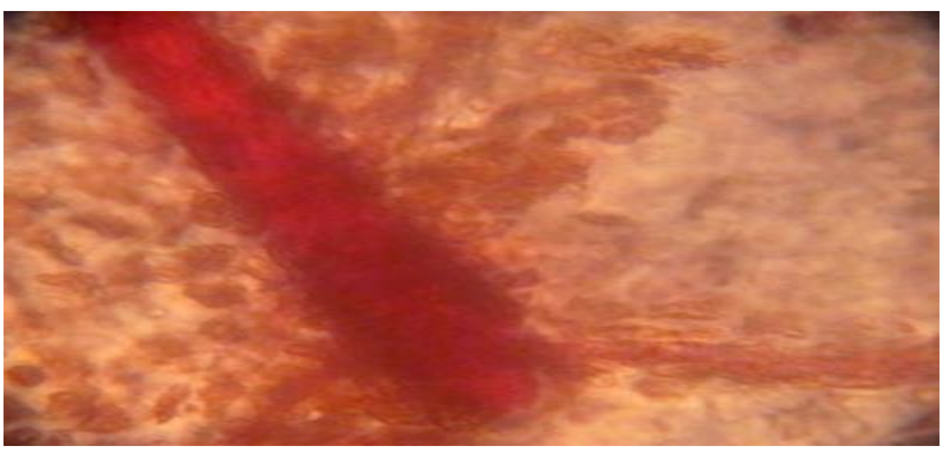
C

Plate 3: Adaxial leaf epidermis of *Sida acuta* showing glandular trichome

(A) – Akure – Ilesa road

(B) – Ife – Ibadan road

(C) – Control

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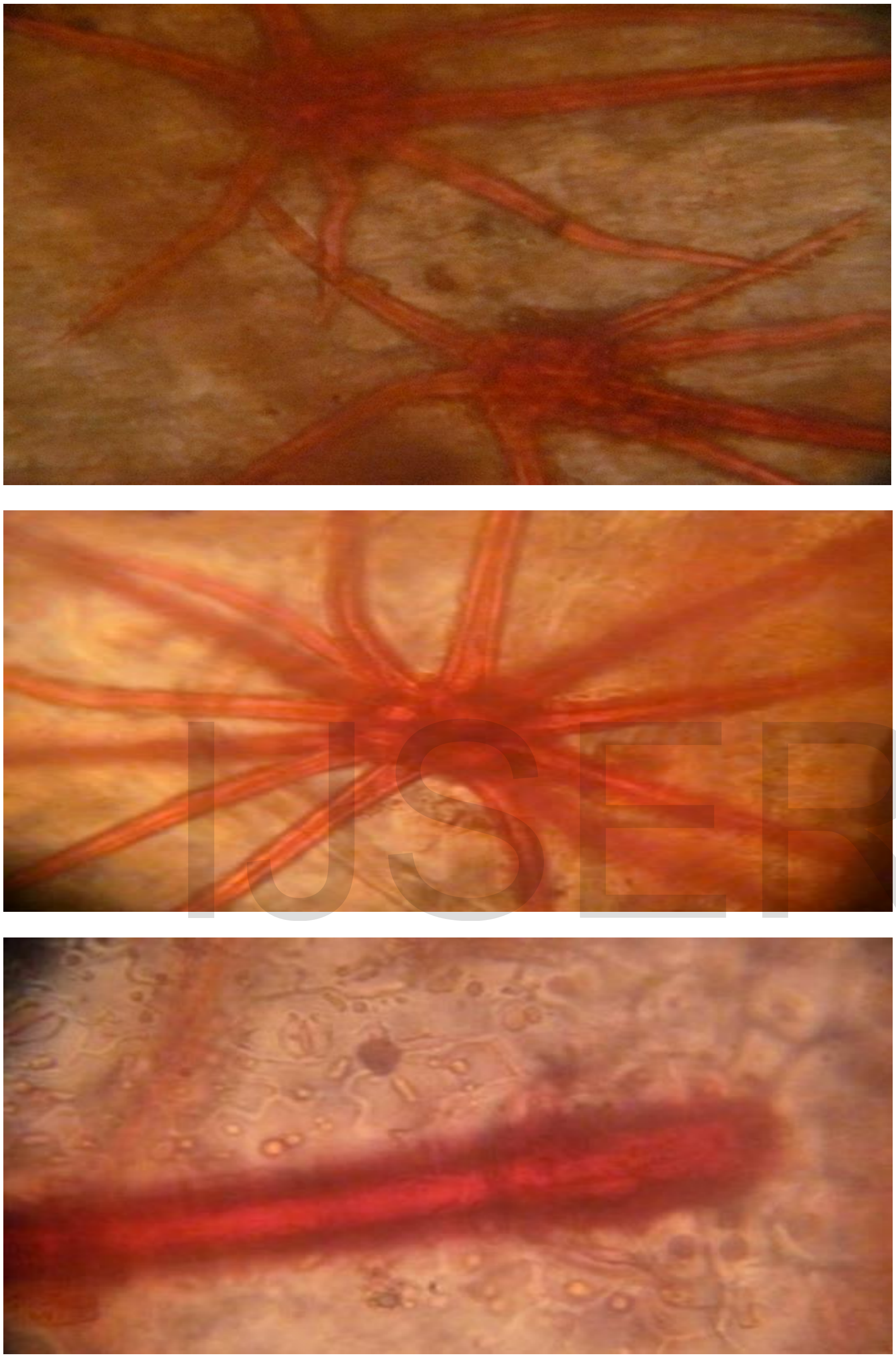

A

B

C

Plate 4: Abaxial leaf epidermis of *Sida acuta* showing stellate and glandular trichome

(A) – Akure – Ilesa road

(B) – Ife – Ibadan road

(C) – Control

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