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Research Article

# ANATOMICAL AND PHYSIOLOGICAL INFLUENCE OF ACIDULATED RAINWATER ON LEAF TISSUES OF TOMATO (*SOLANUM LYCOPERSICUM L.*)

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**Key words:** Anatomy, Chlorophyll, Industrialization, Car emissions and Acid derivative

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**ABSTRACT**

The acidulation of rainwater is connected intimately with industrial development. Rainwater samples collected from industrial and automobile traffic congested Cities of Lagos, Port Harcourt, Kano and Gombe were used in a screen-house experiment. The effect of atmospheric gases $NO_2$, $SO_2$ and $CO_2$ were determined on anatomy of phylloplane and chlorophyll contents. The rainwater samples were administered to the plant for six weeks. One-way analysis of variance approach was used for the statistical analysis followed by Duncan's multiple-range tests. The findings showed that from the month of April through July, the concentrations of atmospheric acid derivatives were significantly ($p<0.05$) decreased. However, the acidulated rainwater induced; alterations on the cuticle, collapsed epidermal cells, formation of lobules of scarred tissue and necrosis. Again, Chlorophyll content (F Tomato=8.4128, 2.5368, 11.411; $p<0.05$) were significantly decreased. The findings showed that both car emissions and heavy industrial activities contributed significantly to the acidity of rainwater. It also demonstrated that acidified rainwater significantly affects the anatomy and physiology of plants studied. It is therefore, important to plant acidophilic trees in the study areas to reduce the effect of acid rain on other vegetable crops.

## INTRODUCTION

Acid rain is an atmospheric precipitation that is abnormally acidic and contains high-level of hydrogen ions (Lal, 2016). This is formed by emissions of Sulfur Dioxide ($SO_2$) and Nitrogen Oxide ($NO_2$) (International Energy Agency, 2016), these atmospheric pollutants dissolve in atmospheric vapour to produce acid rain (Chandra, *et al.*, 2017). When acid is mixed with rainwater, the pH level is reduced below 5.6 (Dondapati, *et al.*, 2013). Urban areas and heavily industrialized areas have experienced problems with acid rain. (Grennfelt, *et al.*, 2020).

It has been suggested that acidulated rain water-causes anomalies in physiological processes including photosynthesis, chlorophyll content, nitrogen metabolism, and production of reactive oxygen species (Martins, *et al.*, 2013). Species diversity declines as a result of water quality degradation (Debnath, *et al.*, 2020).

Anatomically, acid rain is reported to alter the cuticle thickness, deformation of cell and stomatal cells occlusion, removal of trichomes, cellular collapse, and formation of scar tissues (Lebedev, *et al.*, 2019). This study aims to examine the anatomical and physiological elements of resilience in acidulated condition in these urban species. It's also adds to environmental preservation and sustainable development in regions throughout the world that is affected by acid rain and has both scientific and practical ramifications.

### Study Location

The Federal University of Technology Owerri in Imo State served as the study's location. The state can be found in Nigeria's southeast. Between latitudes 5°22′N and 5°19′N and longitudes, 6°58′E and 7°00′E where it is located (NIMET, 2016) (Fig. 1).The region is in a tropical rainforest zone with the wet season from April to October and the dry season from November to March: The local daytime and

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night ranges from 18 hrs and 24 hrs, and the daily average temperature is 19°C to 28°C.

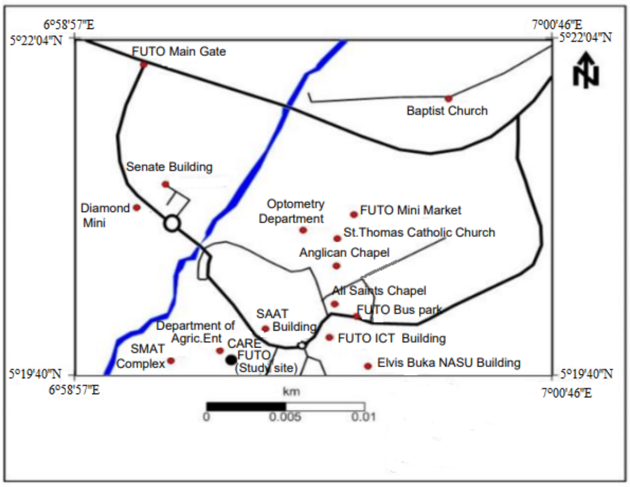


**Fig. 1** Map of federal university of technology owerri showing study site . **Note:** (●) Study area; (/) River; (●) Major structure; (—) Major road; (—) Minor road.

## MATERIALS AND METHODS

### Experimental Design and Treatments

**Collection of rainwater sample:** Sampling of rainwater was carried out between April and July, 2022. Sampling was set up at open spaces in five locations (5 replicates) randomly selected to cover the built-up areas of the cities of Lagos, Port Harcourt, Kano and Gombe (control). A plastic container was placed on a 2 m high platform. The sampling container was placed on the platform at the beginning of a rain event. After the rainfall, the container was removed, covered and stored. This was designed to eliminate significant contribution from dry deposition. Collected samples at the internal of two weeks were combined and analysed.

### Determination of PH and Concentrations of $SO_2$, $CO_2$ and $NO_2$ in the Rainwater

**pH test:** Rainwater sample (10 ml) was placed in test tube and 20 mls of bromothymol blue solution was added to each test tube. A glass electrode was dipped into the solution then dipped into the pH meter to test the pH of the water sample (Dinrifo, *et al.*, 2010).

**Sulphur dioxide:** The spectrophotometric (DR2800 N0.290) method was used; whereby 10 mL of water samples were poured into square sample cell, twelve drops of alkaline-cyanide reagent solution were then added to each cell to determine the level of each of the trace metals (Dinrifo, *et al.*, 2010).

**Nitrogen dioxide:** About five drops of water sample were poured in an evaporating dish, then two drops of diphenyl amine were added to sulphuric acid in a conical flask. The content of the flask was poured into the evaporating dish and the solution was heated. Blue colour indicated the presence of nitrate. The absorbance of the mixture was read on a spectrophotometer at 520 nm (Dinrifo, *et al.*, 2010).

**Carbon (IV) dioxide:** Ten milliliters of rainwater sample were measured and four drops of phenolphthalein was added. The colorless solution was then titrated with 0.045 M of sodium carbonate solution. It was stirred gently with glass rod and sodium carbonate was added in bits. A faint pink color that remains for at least 30 seconds indicated the presence of carbon (IV) oxide. (Dinrifo, *et al.*, 2010). The quantity of $CO_2$ was calculated as:

$$CO\ (\mu eq/L) = \frac{NaCO_3 \times N \times 22 \times 1000}{\text{Volume of sample}}$$

Where N=Normality of water, 22=Equivalent rate of $CO_2$.

### Planting Procedure

The experiment was laid in complete randomized design with the treatments (acidulated rain water) from Lagos, Port Harcourt, Kano and Gombe (4 bags for each treatment). Each treatment was replicated 3 times (48 polythene bags) for the whole experiment; five crops of tomato were planted. Three weeks after germination, each bag was sprayed with 500 ml of collected rain water for six weeks.

### Determination of Anatomical Properties

Leaf samples of 3 representative seedlings (n=3) were used for anatomical study. Twenty-four hours after the last rain application, one leaf from each plant was taken. By applying the proper pressure, a leaf sample was fractured from the front and rear, exposing the lower surface's peel (Pham, 2021). The epidermal layer of the leaf's dorsal side was used to collect the leaf peel. A portion of a peel was transferred into a watch glass filled with water and then a few drops of safranine solution were added to stain the leaf peel. After five minutes, the stained peel was transferred to another watch glass containing water to wash off excess stain, and then transferred onto the slide containing a drop of glycerin (Tulay, *et al.*, 2018). The slide was then covered with a cover slip. Using low power microscope (100 X) the micromorphology was observed.

### Extraction and Measurement of Chlorophyll

One gram of a fresh-cut leaf was grounded using a pestle and mortar with 20 ml–40 ml of 80% acetone. The solution was centrifuged at 5000 rpm–10,000 rpm for five minutes. Once the colourless residue was produced, the supernatant was moved to another test tube and the process was repeated. The solution's absorbance was measured at 645 nm and 663 nm wavelengths in comparison to a solvent (acetone) blank. Using spectrophotometer (DR2800 N0.290).

The amount of chlorophyll-a and b in the extract chlorophyll was measured using a spectrophotometer. Chlorophyll absorbance was measured at 645 nm and 663 nm, respectively (Yang, *et al.*, 2011). To get a value that was preferably in the range of 0.2 to 0.8 absorbance units, the extract was diluted with 80% acetone (Harborne, 1987; Gu, *et al.*, 2016). The equation below was used to compute the concentrations of total chlorophyll, chlorophyll-a, and chlorophyll-b in the sample:

Total Chlorophyll: 20.2(A645)+8.02(A663)

Chlorophyll a: 12.7(A663)–2.69(A645)

Chlorophyll b: 22.9(A645)–4.68(A663)

Where: A645=absorbance at a wavelength of 645 nm;

A663=absorbance at a wavelength of 663 nm.

### Statistical Analysis

The one-way Analysis of Variance (ANOVA) was used to analyze the data collected. The R 4.2.1 Statistical Package was used for all analyses. Duncan's multiple-range tests were used to show the differences between means that were statistically significant ($p<0.05$).

## RESULTS

### pH and Concentrations of Acid Derivatives ($SO_2$, $NO_2$ and $CO_2$)

The results of the chemical proportion/concentration of the acid derivatives of the atmospheric acid gases for several months at Ikeja, Lagos State, are shown in Fig.2. An acid derivative ($SO_2$) shows a significant difference at ($p<0.05$) in the month of April, May, June and July. However, $CO_2$ shows no significant difference between the month of April and May; and also, June and July at ($p<0.05$). Also, $NO_2$ did not differ significantly in the month of April, May and June, but differs in the month of July. The pH level did not differ in the month of April and May but differs in the month of June and July at ($p<0.05$).

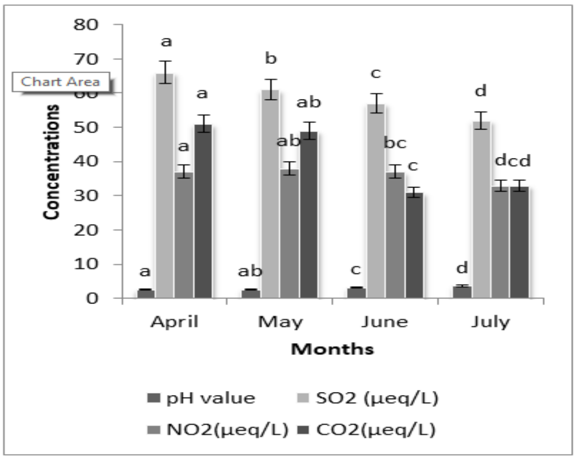


**Fig. 2** Chemical composition of rain water collected from Ikeja, Lagos State. **Note:** (■) pH value; (■) $SO_2$ (µeq/L); (■) $NO_2$ (µeq/L); (■) $CO_2$ (µeq/L).

Fig.3 shows the results of the chemical proportion/concentration of the acid derivative of atmospheric acid gases at Alesa Eleme, Port Harcourt, for various months. The acid derivative ($SO_2$) shows significant difference in all the Months at ($p<0.05$). However, $CO_2$ did not differ in the month of April and May at ($p<0.05$) but differs significantly in the Month of June and July. While, $NO_2$ shows no significant difference in the month of April and May, but differs in the month of June and July at ($p<0.05$). The pH level did not differ in the month of April, May and June but differ in the month of July.

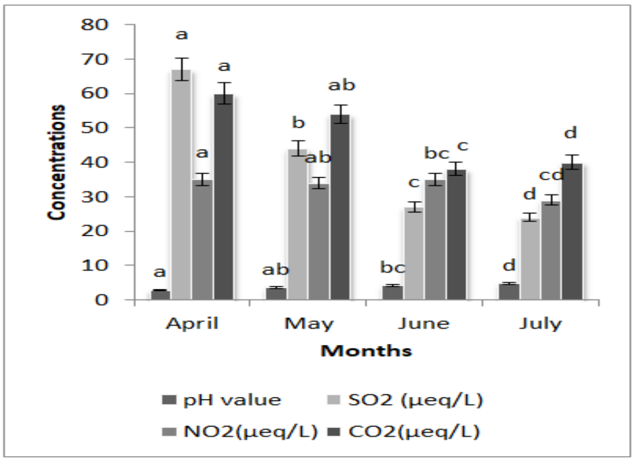


**Fig. 3** Chemical composition of rain water collected from Alesa Eleme, Port Harcourt State. **Note:** (■) pH value; (■) $SO_2$ (µeq/L); (■) $NO_2$ (µeq/L); (■) $CO_2$ (µeq/L).

Fig.4 shows the chemical proportion/concentration of the acid derivative of atmospheric acid gases for several months along Sani Abacha Road in Kano State. An acid derivative $SO_2$ differs significantly in the month of April and July but did not differ in the month of May and June at ($p<0.05$). While, $CO_2$ shows no significant difference in the month of April, May, and June but differs in the month of July. Moreover, $NO_2$ did not differ between the month April and May also between June and July significantly at ($p<0.05$). The pH level differs in the month of April and July but did not differ significantly in the month of May and June.

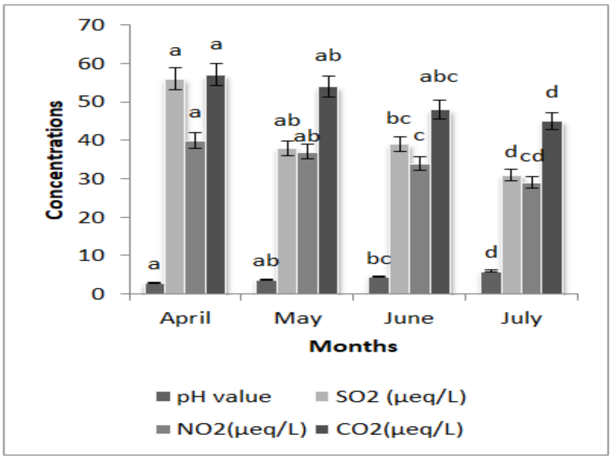


**Fig. 4** Chemical composition of rain water collected at Sani Abacha Road, Kano State. **Note:** (■) pH value; (■) $SO_2$ (µeq/L); (■) $NO_2$ (µeq/L); (■) $CO_2$ (µeq/L).

Fig.5 shows chemical proportion/concentration of the acid derivative of atmospheric acid gases for several months at Kwadon, Gombe State. An acid derivative $SO_2$ differs significantly in all the months at ($p<0.05$). $NO_2$

shows significant difference in the month April and May, and also inJuly a but did not differs in the month of May and June. Moreover, $CO_2$ differs significantly across the months. Furthermore, the pH level shows no significant difference at (p<0.05) across the months.

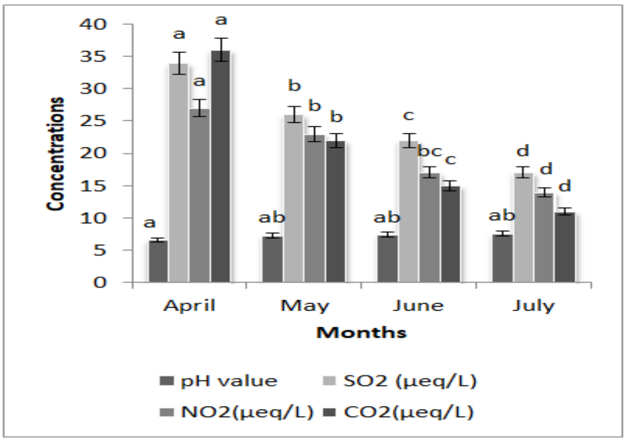


**Fig. 5** Chemical composition of rain water collected from Alesa Eleme, Port Harcourt State. **Note:** (■) pH value; (■) $SO_2$ (µeq/L); (■) NO2 (µeq/L); (■) $CO_2$ (µeq/L).

### Anatomical Damages in Leaves

Fig.6a-6d shows theanatomical damages of the phylloplane. Fig.6a shows complete collapse of epidermal cells on the adaxial surface as well as the buildup of epicuticular waxes in specific intercostal regions. Fig.6b shows lobules of scarred tissue and necrosis. Also, abundant cellular contents are observed throughout the mesophyll which have an intense coloration after staining. Fig.6c shows intact mesophyll with fewer stomatal cells. Fig.6d reveals no damage on the mesophyll with numerous stomatal cells.

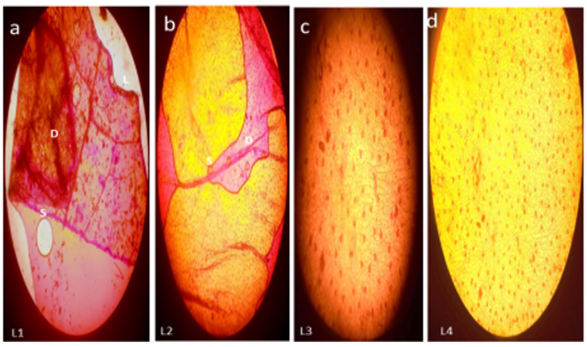


**Fig. 6** Anatomical damageson leaves of *Solanum Lycopersicum*. (a-d) Adaxial view of the epidermis (100 X); D: damage; S: scar; L: lobule.

*L indicate the locations in which the rain water samples were collected. L1- Lagos/Ikeja. L2-Port Harcourt/ Alesa Eleme. L3- Kano/Sani Abacha Road. L4- Gombe/ Kwadon.

### Chlorophyll Contents

Fig.7 shows the Chlorophyll A levels of *S. Lycopersicum.* irrigated with acidulated rainwater from; Lagos (18.45 mg/g) and Port Harcourt (22.40 mg/g) did not differ significantly. Again, that of Port Harcourt (22.40 mg/g) and Kano (25.96 mg/g) showed no difference significantly but that of Lagos and Kano showed significant difference. Moreover, rainwater from Gombe gave Chlorophyll A level of 32.79 mg/g with a significant difference at (p<0.05) from the other treatments. All the plants irrigated with rainwater from; Lagos (32.35 mg/g), Port Harcourt (40.22 mg/g), kano (46.06 mg/g) and Gombe (42.56 mg/g) did not differ significantly except that of Lagos and Kano which showed significant difference (p<0.05) in term of chlorophyll B content. In contrast, there was discernible difference in total chlorophyll at (p<0.05) for tomato plant irrigated with rainwater from Lagos (50.79 mg/g) and Port Harcourt (61.67 mg/g), but there was no significant difference for Kano (72.00 mg/g) and Gombe (75.33 mg/g).

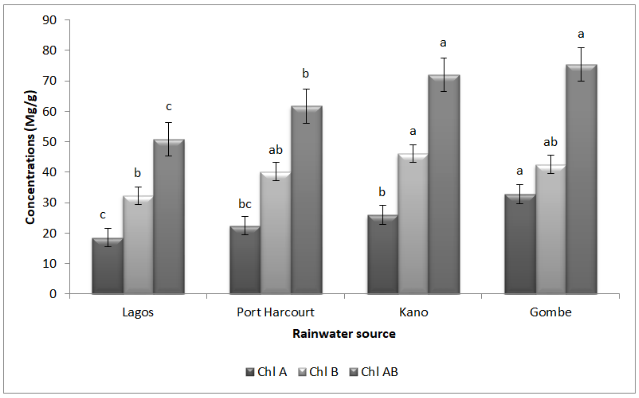


**Fig. 7** Chlorophyll Content of *Solanum lycopersicum*. irrigated with rain water. **Note:** (■) Chl A; (■) Chl B; (■) Chl AB.

## DISCUSSION

In April, the chemical composition of rainwater in Ikeja and Alesa Eleme revealed significant levels of $SO_2$, whereas high levels of $CO_2$ were detected in the Sani Abacha Road, Kano, and Kwadon area of Gombe state (Fig.2). With the exception of Ikeja, where the $NO_2$ level in Lagos increased for the month of May, all areas had a drop in the levels of $SO_2$, $CO_2$, and pH (Fig.3). This was likely caused by the fact that the majority of the accumulated contaminants must have been brought back to the surface by the period's frequent rainfall. However, in June, the $CO_2$ level in Ikeja and Eleme junction had the lowest concentration, whereas Sani Abacha Road, Kano, saw a rise (Fig.4). Additionally, $CO_2$ concentration has increased at Ikeja and Eleme, and in the month of July, levels of $SO_2$, $NO_2$, and pH have decreased (Fig.5).

According to the report of Burns, Most of the sources of acidic rain are the result of emissions from industrial activities, vehicles, and pollution formed from man's anthropogenic activities(Burns, *et al.*, 2016). However, these produced more air pollutants than naturally occurring sources of acidity, including sulfur, nitrogen, and carbon oxides (Chandra, *et al.*, 2017). The fluctuation of $CO_2$ in cities with heavy industrial and vehicular activity reflects carbon emissions that add to the buildup of $CO_2$ in the atmosphere. Rainwater naturally contains acid due to atmospheric carbon dioxide dissolving in it (Carr,*et al* ., 2006). The levels of $CO_2$ could potentially be a result of the high volume of traffic. The majority of the pollution load is typically present in the first rush of rainwater that falls at the start of rainfall. The biggest contributor to these phenomena is the pollution buildup and deposition that occurs during dry seasons (Hunt R. 1990).

On leaves irrigated with acidulated rain water from Ikeja, the surface collapsed and epicuticular wax collected in some intercostal areas, the cuticle changed and the epidermal cells on the adaxial collapsed due to the acidulated rain water (Fig.6a). When plants were irrigated with acidulated rainwater from Eleme, the cellular contents increased and the pin-colored staining of the mesophyll became more obvious. In addition, the damaged cells create scar tissue that gets quite black, separating it from the healthier tissue (Tulay, *et al.*, 2018). The tissue eventually engulfed the lobule, separating it from the rest of the leaf as it assumed an oval shape (Fig.6b). The results of Veronica, Sant' Anna-Santos, Da Silva agree with these findings (Veronica, *et al.*, 2020; Sant' Anna-Santos, *et al.*,2006;Da Silva, *et al.*, 2005).

In this study, chlorophyll levels dropped as acidity increased (Fig.7). The control (Plants irrigated with acidulated rain water from Kwadon/Gombe) exhibits substantially more chlorophyll than other treatments. Reduced leaf area was the cause of the decrease in photosynthesis and chlorophyll concentrations (Gu,*et al.*, 2016). Chlorophyll synthesis and chloroplast development were also negatively affected when exposed to acid rain. As a result, the amount of leaf chlorophyll is a crucial marker of direct foliage damage and has a direct impact on plant productivity, which is significantly decreased by acid rain. This has traditionally been explained by stating that because acid rain causes an imbalance in H+ ions in leaf cells, the drop-in chlorophyll concentration may indicate their destruction. This suggests that physiological harm may exist even in the absence of morphological or anatomical damage. Similar results have been reported by Vina, Kausar, Shaukat, Long (Vina, *et al.*,2020; Kausar, *et al.*, 2019; Shaukat, *et al.*, 2018; Long, *et al.*, 2017).

## CONCLUSION

The study established the benchmark for rainwater collected in Nigerian cities with high vehicular traffic and industrial activities. The actual information from the field reveals some intriguing insights: it demonstrates that emissions from vehicles as well as intensive industrial operations contributed more to the acidity of rainwater. It also revealed that rainwater that has been acidified has a significant impact on plants' morphology, anatomy, and physiology.

Anatomically, applying acidulated rainwater treatments to plants causes greatest harm to the epidermal cells, dissolving the cuticle, altering the permeability of the leaves, and causing chlorosis and necrosis. White scars, stunted growth, lesions, inhibition of leaf development, curving of leaves, withering of leaves, leaf abscission, and even death of plants are indicators of acid-poisoned plants in plants treated with acidulated rainwater, demonstrating that morphological damage led to anatomical harm. The anatomical damage was observed in rain water collected from industrial areas such as Ikeja of Lagos State and Alesa Eleme of River State. Even at Sani Abacha Road where apparent damage was not present, the rainwater decreased the amount of chlorophyll a and b in both plant species, suggesting that physiological damage resulted from anatomical damage. It is therefore, important to plant acidophilic trees in the study areas to reduce the effect of acid rain on other vegetable crops.

## REFERENCES

Lal N. 2016. Effects of acid rain on plant growth and development. e-Journal of Science & Technology. 11(5).

International energy agency. 2016. Weo-2016 Special report energy and air pollution. international energy agency: Paris, France. 266.

Chandra MK, Mainpuri V and Pradesh U. 2017. Acid rain-the major causes of pollution: its causes, effect. Int J Appl Chem. 13(1): 53-58.

Dondapati N, Reddy SK and Recharla KP. 2013. A novel method for detecting acid rain patterns of so2 and no2 using ph in image processing. InternationalConference on Green Computing, Communication and Conservation of Energy (ICGCE). 17–22.

Grennfelt P, Engleryd A, Forsius M, Hov, Rodhe H and Cowling E. 2020. Acid rain and air pollution: 50 years of progress in environmental science and policy. Ambio. 49: 849–864.

Martins N, Gonçalves S and Romano A. 2013. Metabolism and aluminum accumulation in Plantago almogravensis and P. algarbiensis in response to low pH and aluminum stress. Biol Plant. 57:325–331. doi: 012-027.

Debnath B, Li M, Liu S, Pan T, Ma C and Qiu D. 2020. Melatonin-mediate acid rain stresses tolerance mechanism through alteration of transcriptional factors and secondary metabolites gene expression in tomato. Ecotoxicol Environ Saf. 200(9): 110720.

Lebedev VG, Krutovsky KV and Shestibratov KA. 2019. Effect of phosphinothricin on transgenic downy birch (betula pubescensehrh. Containing bar or gs1 genes. Forests.10(12): 1067.

Nigeria Meteorological Agency (NIMET). 2016. Drought and flood monitoring in South East Bulletin.

Dinrifo RR, Babatunde SOE, Bankole YO and Demu QA. 2010. Physico-chemical properties of rainwater collected from some industrial areas of lagos state, nigeria. European Journal of Scientific Research. 41(3): 383-390.

Pham TT, Do TN, Tran MT, Bui NK and Le TS. 2021. Assessment of acid rain development in hoa binh in the period of 2000–2014. J Sci Earth Environ Sci. 32: 117–124.

Tulay AA, Adnan A and Cengiz Y. 2018. Effects of chromium on anatomical characteristics of bread wheat (Triticum aestivumL.).Journal of International Environmental Application & Science. 13(1): 27-32.

Yang M, Huang SX, Fang, SZ and Huang XL. 2011. Response of seedling growth of four Eucalyptus clones to acid and aluminum stress. Plant Nutr Fert Sci. 17 :195–201.

Harborne JB. 1987. Phytochemical methods. 2nd (ed), Chapman hall, London. 214-219.

Gu DD, Wang WZ, Hu JD, Zhang XM, Wang JB and Wang BS. 2016. Nondestructive determination of total chlorophyll content in maize using three-wavelength diffuse reflectance. Journal of Applied Spectroscopy. 83: 541–547.

Burns DA, Aherne J, Gay DA and Lehmann C. 2016. Acid rain and its environmental effects: Recent scientific advances. Atmospheric Environment.146:1-4.

Carr GM and Neary PJ .2006. Water quality for ecosystem and human health. United nations environment programme global environment monitoring system (gems)/water programme.

Hunt R. 1990. Basic growth analysis: plant growth analysis for beginners. Springer Dordrecht. 41.

Veronica SR, Rosas U and Estela Z. 2020. Does acid rain alter the leaf anatomy and photosynthetic pigment in urban trees?. Plants (Basel). 09(7): 863.

Sant' Anna-Santos BF, Silva LC, Azevedo A and Deque-Brasil R. 2006. Effects of simulated acid rain on photosynthesis, chlorophyll fluorescence and antioxidative enzymes in cucumis sativus L. Photosynthetica. 40: 331-335.

Da Silva L, Alves A, Da Silva E and Oliva M. 2005. Effects of simulated acid rain on the growth of five Brazilian tree species and anatomy of the most sensitive species (Joannesia princeps). Aust J Bot. 53:789–796.

Vina N, Diah R, Didik I and Maryani . 2020. The effect of low ph on physiological characters in vegetatif phase of kalimantan local swamp rice (Oryza sativa L.). AIP Conference Proceedings 2260: 030019.

Kausar S, Hussain A and Khan A. 2019. Response of simulated acid rain on morphological, biochemical, and leaf characteristics of wheat. Trends in Biosciences.3(1):34-36.

Shaukat SS, and Khan MA. 2018. Growth and physiological responses of tomato (lycopersicon esculentum mill.) to simulated acid rain. Pakistan Journal of Botany. 40(6):2427-2435.

Long A, Zhang J, Yang l, Ye X, Lai N, Tan L, Lin D and Chen L. 2017. Effects of low ph on photosynthesis, related physiological parameters, and nutrient profiles of citrus. Journal Frontiers in Plant Science. 8:185.