



Spatial Distributions of Anthropogenic Induced Gaseous - Particulate Pollutants and Non-Enzymatic Abiotic Stress Responses of *Citrius sinensis* Leaves

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Authors' contributions

This work was carried out in collaboration among all authors. Author EUD designed the study and wrote the first draft of the manuscript. Author EMD performed the statistical analysis and wrote the protocol. Authors AEE and NEE managed the analyses of the study. Author AIU managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To determine the spatial distribution pattern of anthropogenic induced gaseous and particulate pollutants in air samples around major roads within Uyo Metropolis, Southern Nigeria. Also, to assess the response in terms of changes in concentrations of some non-enzymatic biochemical markers of *Citrius sinensis* leaves (orange) collected from these locations.

Study Design: Air samples from six major heavy traffic roads and a control site within Uyo Metropolis was collected. *Citrius sinensis* leaves were also collected from each major roads and Control to determine possible variations in concentrations of biochemical markers contents.

Place and Duration of Study: Department of Chemistry, University of Uyo, Uyo, between September 2019 and January 2020.

Methodology: Adsorption train techniques involving the adsorption of pollutant gases from air samples in a particular location into appropriate reagent solutions was adopted for this study. Estimation of the concentrations of SO₂, NO₂ and CO was done by calorimetric techniques. Concentrations of total phenolic, proline, malonaldehyde (MDA), cellulose, chlorophyll and ascorbic acid contents in *Citrius sinensis* leaves were determined using UV/Visible spectrophotometer.

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Results: Air samples collected from major roads with heavy traffic and extraneous local automobile emissions recorded higher levels of all the gaseous and particulates pollutants analyzed than Control. Leaves of *C. sinensis* exposed to higher concentrations of these pollutants showed a higher accumulation of all the biochemical markers studied except chlorophyll, which was higher in leaves from the control site.

Conclusion: Anthropogenic activities directly increases the spatial distribution pattern and concentrations of gaseous and particulate pollutants (PM₁₀ and PM_{2.5}). Also, *C. sinensis* leaves from major roads within Uyo Metropolis showed different responses in terms of accumulation of these biochemical markers.

Keywords: Spatial distribution; anthropogenic; non-enzymatic; biochemical markers; gaseous-particulate pollutants.

1. INTRODUCTION

Several activities; both natural and anthropogenic have contributed to the release of substances (pollutants) into the atmosphere. These pollutants including gases, particulate matter, trace metals and others continue to accumulate in the atmosphere undergoing several transformations with other air constituents causing tremendous effects to both living and non-living things around such air environment. The concentrations of most of these air pollutants usually have a relationship with the population of people occupying a particular location, activities they predominantly involve in (commercial and non-commercial), and many other factors related to man. According to the Clean Air Act (CAA) of 1970, six toxic pollutants namely CO, Pb, NO₂, O₃, SO₂ and particulate matters were identified as common air contaminants and their concentrations in air usually determine the air quality indices (AQI) of a particular air environment. Increased concentration of gaseous and particulate pollutants in air at a particular location and time has been demonstrated to cause poor air quality of any area and researchers around the world continue to use air quality index measurement as a yard stick for understanding the local air quality with the consideration that the concentrations of these pollutants change on regular basis [1].

Plants usually response to changes in the atmosphere (troposphere) whenever it is stressed as a result of increased concentration of gaseous and particulate pollutants, leading to noticeable and varying changes in their physiological, biochemical and other cellular formulations [2]. Air environment that are largely stressed by multiple air pollutants are usually characterized by changes in plant physiological processes (mineral nutrition, photosynthesis, transportation, respiration and even plant growth)

and biochemical markers including total phenolic, proline, malonaldehyde (MDA), cellulose, chlorophyll and ascorbic acid contents [3]. These observable changes especially in biochemical compositions of plant parenchyma tissues such as leaves, roots, stems, and flower as responses to different environmental stresses have become a useable tool by scientists and researchers in assessing and monitoring any environment whether air, water or land [4].

The term 'bio-markers' refer to changes in biological responses in plant as molecular, cellular, physiological and behavioral which correlates to toxic environmental chemical exposure [5]. Examples of these biomarkers includes, but not limited to phenol, ascorbic acid, proline, chlorophyll, relative water content, malonaldehydes, cellulose, flavonoids etc. Plants can be used as an environmental biomarkers to assess, monitor and evaluate the environmental quality in terms of pollution status, not only that, but also help in developing certain toxicity mechanisms in different plant species in an ecosystem [6]. Several authors have researched and reported on variations in concentration of some important biochemical markers of different plant parts and tissues under different environmental conditions of pollution stresses including heat, salts, flood, waste dumpsites, trace metals and industrial activities [7-14]. Therefore, this work focuses on assessing the space distribution of some gaseous (CO, NO₂, VOC, SO₂) and particulate pollutants (PM₁₀, PM_{2.5}) in air samples around six major heavy traffic roads within Uyo Metropolis, Akwa Ibom State, Southern Nigeria. Also, the study seeks to examine the causative impacts in terms of changes in contents of some important plants' biochemical markers including phenols, proline, chlorophyll, ascorbic acid, cellulose and malonaldehydes (MDA) in *C. sinensis* leaves sourced around these six major roads. The

choice of the studied locations was predicated upon certain features like; traffic density, human populations, nature of activities and others, while *C.sinesis* plant was chosen because it was common to all the studied locations. The results of this study shall help in understanding the air quality state of these major roads, and also provide additional data for the purposes of air monitoring regulations and policy formulations by relevant body of the State including the prediction of the weather.

2. MATERIALS AND METHODS

2.1 Study Locations

For this study, a total of six locations and a control site within Uyo Metropolis were intentionally selected on the basis of some features including nature of human activities (commercial, residential and industrial), traffic densities, human population, presence of dual carriage road surrounded by adjoining streets and availability of *C. sinensis* tree. A road which is more of a reserved or fallow area with no human activities and not affected by any extrinsic emissions from automobile was used as a Control. Names of the locations, codes used in this study including their coordinates are presented in Table 1.

2.2 Determination of Gaseous and Particulate Pollutants in Air Samples

Air sampling for sulphur (iv) oxide (SO₂), nitrogen (iv) oxide (NO₂), carbon monoxide (CO), volatile organic compounds (VOCs) was carried out twice daily between the hours of 8:00 am – 11:00 am (morning) and 3:00 pm – 6:00 pm (evening) for 7 days (each day for one location). Sampling of air was done in two peak traffic periods in a day - morning (when workers and students will

be leaving for work and schools), and evening (closing period for workers and students) to ensure comparative good results. Absorption train techniques involving the adsorption of pollutant gases from air samples in a particular location into appropriate reagent solutions was adopted for this study [15]. Solutions used for the absorption of gases were sodium tetrachloromercurate for SO₂, acidified potassium dichloro blend with mixture of N - (1- naphthyl) ethylene diamine dichloride and sulphamic acid in the presence of glacial acetic acid for NO₂, and silica gel impregnated with ammonium molybdate for CO. Each air contaminant samples was collected using this procedure and the resulting solutions after absorption with suitable reagents was used to estimate the concentrations of SO₂, NO₂ and CO by calorimetric techniques using standard methods described in details elsewhere [16,17]. Quantification of particulate matters (PM₁₀ and PM_{2.5}) in the air samples was done following the method of Hakim [18], while concentration of volatile organic compounds (VOCs) was carried out using the equation adopted by Chattopadhyay et al. [19] given below:

$$QVOCs = 100 \times V/V_s \tag{1}$$

Where $QVOCs$ = quantity rating ; V = observed value ;
 V_s = permissible threshold value

2.2.1 Traffic density

Traffic density of each location was determined using the method of Ogunbe *et al.*, [20]. At each location, direct observation and counting of traffic was done at two peak traffic periods 8. 00 am – 11.00 am (morning) and 3.00 pm – 6.00 pm (evening). Traffic density of each location was determined using electronic counting machine.

Table 1. Sampling stations and coordinates

Station code	Name of location	Coordinate
APR	Airport Rd	Lat 50° 07! N – 50° 08! N and Longitude 70° 46! E – 70° 47! E
CIR	Calabar – Itu Rd	Lat 50° 04! N – 50° 05! N and Longitude 70° 55! E – 70° 56! E
IDR	Idoro Rd	Lat 50° 02! N – 50° 03! N and Longitude 70° 50! E – 70° 51! E
ENR	Ekpri Nsukara Rd	Lat 50° 01! N – 50° 02! N and Longitude 70° 52! E – 70° 53! E
UAR	Uyo – Abak Rd	Lat 50° 06! N – 50° 07! N and Longitude 70° 48! E – 70° 49! E
NWR	Nwaniba Rd	Lat 50° 05! N – 50° 06! N and Longitude 70° 54! E – 70° 55! E
UVR (Ctrl)	Uyo village Rd	Lat 50° 03! N – 50° 04! N and Longitude 70° 56! E – 70° 58! E

Ctrl = control

2.3 Determination of Biochemical Markers in *C. sinensis* Leaves

2.3.1 Plant material, sample preparation and extraction

Leaves of *C. sinensis* were collected from *C. sinensis* tree in six major roads and a Control site within Uyo Metropolis, Akwa Ibom State. Fifty (50) grams leaves were taken of the same age at each location. Care was taken to avoid selecting *C. sinensis* leaves with insect infestation, honey-dew and bird droppings. Samples were preserved in clean envelop papers before being taken to a botanist in the Department of Botany and Ecological Studies, University of Uyo, Nigeria for identification, authentication and specimen referencing and a voucher specimen deposited at the Herbarium of the Department of Botany and Ecological Studies, University of Uyo. The leave samples were taken to Chemistry Laboratory, University of Uyo for air drying at room temperature for forty-eight hours and then being crushed to a fine powder using a pestle and mortar. The dried powdery substance was then packed into an envelope and stored in an air tight container. Fresh samples of the leaves was also preserved in the refrigerator to avoid denaturation prior to use. For solvent extraction, about 10 g ground *C. sinensis* leaves was uniformly packed into thimble and extracted with 125 cm³ methanol. The extraction continued for 24 h till it becomes colorless. Afterwards, the extract was then put into a beaker and heated at 30 – 40 °C on a hot plate till all the solvent is evaporated to yield the crude extract. Some of the plants' biochemical markers were determined using the methanolic extract, dried powdered sample, and others using the fresh *C. sinensis* leaves.

2.3.2 Estimation of total phenolic content

Total phenolic content in leaves of *C. sinensis* was determined by Folin's phenol reagent described in details elsewhere [21]. An aliquot of the extract was mixed with 0.5 ml Folin's reagent and 1.5 ml sodium carbonate (20%) was added to small aliquot. The sample tube was placed in hot water, cooled and absorbance measures at 650 nm using UV/visible spectrophotometer (JENWAY 7305). Quantification of total phenolic content was done using a calibration curve prepared with varying concentrations of 1, 2 dihydroxy benzene.

2.3.3 Estimation of proline content

About 0.5 g plant leaves were homogenized in 5 ml sulphosalicylic acid using prewashed mortar and pestle. The homogenate were filter using whatmann paper. Two milligrams of the extract were decanted into a test tube and 2 ml of glacial acetic acid and 2 ml of ninhydrin were added. The reaction tube was heated in hot water bath for 1 h, placed in ice bath, before adding toluene with constant stirring to separate the toluene layer. The absorbance of the filtrate was measured at 520 nm by UV/visible spectrophotometer (JENWAY 7305) before estimating the quantity of proline using calibration curve prepared with different concentration of pure proline [22].

2.3.4 Estimation of malonaldehyde content

The sample extract was prepared by dissolving the crushed powdery sample in water before collecting the supernatant. Twenty percent of trichloroacetic acid (TCA) and 0.5% tribarbituric acid (TBA) were added to the supernatant and mixed thoroughly. The resulting mixture was boiled, cooled in ice bath before centrifuging. The supernatant was collected and absorbance measured at 532 nm. The concentration of malonaldehyde content in leaf of *C. sinensis* was found by graphical plotting against a standard calibration curve using different concentration of malonaldehyde [23].

2.3.5 Estimation of chlorophyll content

Chlorophyll content of *C. sinensis* leaves was estimated using the method of Mukherjee et al., 2019 described in details elsewhere [24]. About 1.0 g fresh leaves was blended with a little quantity of distilled water and extracted with 10 ml acetone (80% v/v) acetone and left for 15 mins. The liquid portion was decanted into another test tube and centrifuge for 3 mins. The supernatant was collected and 1 ml of the solution was used to determine the amount of chlorophyll. The absorbance of the extract was measured at 663 nm and 645 nm with UV/visible spectrophotometer using cell with 1 cm path against 80% (v/v) acetone blank. The levels of chlorophyll A and that of B, and total chlorophyll (A+ B) in the leaves extracts was calculated using equations (2), (3) and (4)

$$\text{Chlorophyll A } \left(\frac{\text{mg}}{\text{g}} \right) = \left(\frac{\text{Absorbance @ 663 nm} - \text{Absorbance @ 645 nm}}{1000W} \right) \times V \quad (2)$$

$$\text{Chlorophyll B } \left(\frac{\text{mg}}{\text{g}}\right) = \left(\frac{\text{Absorbance @ 645 nm} - \text{Absorbance @ 663 nm}}{1000W}\right) \times V \quad (3)$$

$$\begin{aligned} \text{Total chlorophyll in the leaves extracts} \\ = \text{Chlorophyll A} \\ + \text{Chlorophyll B} \end{aligned} \quad (4)$$

Where W

= fresh weights of leaf sample taken in grams; and V
= volume of leaf extract

2.3.6 Estimation of cellulose content

The sample extract was treated with acetic / nitric acid reagent, boiled in water bath for 30 mins, cooled, and the residue washed with distilled water, 67% sulphuric acid was added, diluted with distilled water before adding anthrone reagent. The tubes were boiled in water bath and cooled before measuring the absorbance at 630 nm. The concentration of cellulose was extrapolated using graphical plotting against a standard calibration curve using varied concentration of cellulose [22].

2.3.7 Estimation of ascorbic acid content

Concentration of ascorbic acid in *C. sinensis* leaves was estimated using spectroscopic method. One gram fresh leaf of *C. sinensis* was placed in a test tube, 4 ml oxalic acid-EDTA extracting solution was added. About 2 ml ammonium molybdate was added to the resulting solution followed by 3 ml distilled water. The resulting mixture was allowed to stand for 15 mins before measuring its absorbance at 760 nm UV/visible spectrophotometer using cell with 1 cm path length. The concentration of ascorbic acid in the leaves was then extrapolated using calibration curve prepared using different concentration of pure ascorbic acid [25]. For all estimations, at least three different absorbance readings were taken and the determination done for each absorbance for all the biochemical parameters before taking the mean concentrations by simple average.

3. RESULTS AND DISCUSSION

3.1 Concentrations of Gaseous and Particulate Pollutants in Air Samples

Results of air gaseous pollutants in air samples from six major roads and a control site are presented in Table 2.

The results indicated that there was a significant variations in the spatial distribution of gaseous (CO, NO₂, VOC, SO₂) and particulate pollutants (PM₁₀, PM_{2.5}) in air samples from the roads. For all the sampling locations including the Control, mean nitrogen (iv) oxide concentration in µg/m³ ranged from 12.40 obtained at Uyo village road (UVR) to 47.50 obtained at Calabar- Itu road (CIR). Uyo village road (UVR) sampling location recorded the least NO₂ concentration when compared to other locations. This could be explained by the fact that there are no traces of any commercial activity, industry / residential buildings and the location is not affected by extraneous local automobile emissions with only an average of 23 automobiles per hour as shown in Table 2. Highest concentration of NO₂ obtained in air samples from Calabar- Itu Road is significantly related to the heavy traffic density including the presence of many construction company's yard, heavy trucks, and existence of a mega abattoir. Generally, the trend in terms of NO₂ abundance in air samples for all the locations and the Control was CIR > IDR > ENR > APR > UAR > NWR > UVR (ctrl). The concentration of NO₂ obtained in this study for all the sampling locations and the Control site were within values of 20.0 µg/m³, 15.0 – 36.7 µg/m³, 31.0 µg/m³ and 20.0 µg/m³ respectively reported elsewhere by other researchers [10,12,17,26]. However, the concentration of NO₂ were far below and inconsistent with 313.0 µg/m³ reported by Ogunbe and co-workers [20], 70.1 µg/m³ by Ameh and co-workers [27], and 63.0 µg/m³ reported by Adakayi and others [28]. Results presented in Table 2, also indicated that traffic density of sampling locations were directly related to NO₂ concentration of the sampling locations, with locations with higher traffic density recording higher NO₂ concentrations. The result shows that mean NO₂ concentrations for all the sampling locations were within the range of 75.0 – 200.0 µg/m³ daily average of hourly value recommended by World Health Organization.

Results for the spatial distribution of sulphur (iv) oxide in the six sampling locations and the Control is presented in Table 2. The result reveals that SO₂ concentrations varied from one location to another within Uyo Metropolis. As also obtained in the case of NO₂, concentrations of SO₂ were observed to be directly related with traffic densities of the studied areas. Areas with high traffic density recorded SO₂ concentrations higher than areas with less traffic density. Mean range of SO₂ concentrations recorded for all the sampling locations and the Control was 3.46

$\mu\text{g}/\text{m}^3$ obtained in air from the Control to $34.10 \mu\text{g}/\text{m}^3$ obtained for Calabar-Itu road samples. Higher SO_2 concentration obtained for CIR is as a result of high population of people, increase traffic density, commercial and industrial activities observed in the area. It is already documented that anthropogenic sulphur emission is principally originated from fossil fuel combustion. The area (CIR) hosts several construction companies' yards handling major road constructions works in the Metropolis and also has industry like aluminum, quarry site and motor parks. About 99% of SO_2 concentrations in the atmosphere comes from human sources such as industrial activities that processes materials that contain sulphur such as the generation of electricity from coal, oil and gas [29]. Low sulphur (iv) oxide concentrations obtained for Uyo village road presented in Table 2 may be as result of absence of industrial and commercial activities in the area in addition to fewer numbers of automobile plying the road. Concentration of SO_2 obtained in this study for all sampling locations and the Control indicated that the results are consistent with $20 \mu\text{g}/\text{m}^3$ reported by Uka and co-workers [14], $30 \mu\text{g}/\text{m}^3$ by Ipeiyeda and co-workers [17], and $34 \mu\text{g}/\text{m}^3$ reported by Ekoh [30] with slight variations. Also, the result is higher than $0.31 - 2.33 \mu\text{g}/\text{m}^3$, $2.0 \mu\text{g}/\text{m}^3$ and $0.6 \mu\text{g}/\text{m}^3$ respectively reported previously [12,31,32]. However, comparing the result of SO_2 concentrations obtained in this study with allowable concentrations in air by World Health Organization [33], the result indicated that all the locations recorded SO_2 concentrations that were within the stipulated value of $26 \mu\text{g}/\text{m}^3$ daily average of hourly value except air samples from Calabar – Itu and Idoro road locations.

Table 2 shows the mean concentrations of carbon monoxide in part per million recorded for all the sampling locations studied. The result showed a direct relationship with traffic density

history of the studied major roads. From the result, mean range was 0.06 ppm obtained at Uyo village road to 6.21 ppm obtained at Calabar – Itu road. CO concentration obtained at CIR indicated the highest value and can be explained based on the increase volume of automobiles plying the road. Anthropogenic contributions to CO level in the atmosphere usually comes from burning or combustion of carbon containing fuels such as gasoline, natural gas, oil, coal and wood. Ekoh [30] opined that the greatest sources of CO to outdoor air pollution comes from cars, trucks, and machinery that uses fuel for their operations. Heavy machines, trucks and equipment used by different construction companies, operating their yards at CIR including higher traffic density (the road serves as the only exit out of the state to other state) and activities of abattoirs (one of the biggest in Uyo metropolis) may constitute possible causes of higher CO observed in this road. In general, and for the entire sampling locations including the Control, CO concentrations in air samples followed the trend; CIR > IDR > ENR > APR > UAR > NWR > UVR (ctrl). Range of CO concentration obtained in this study agrees with some previous reports by other researchers for air around other major roads in Nigeria and other parts of the world. However, the obtained mean range is lower than mean value of 19 ppm reported by Njoku and co-workers [34], 8.01 ppm by Francis and co-workers [32], but higher than 0.03 ppm earlier reported by Ideriah and co-worker [35] in their respective studies. Differences in the reported range of CO obtained in this study and previously reported ones in literature, may be occasioned by differences in traffic flow, vehicular intersection, activities carried out in the locations, and meteorology between studied locations. The result of CO concentrations obtained for all the studied locations within Uyo metropolis were all below the stipulated bench mark of 10 – 20 ppm daily average of hourly value (8 – hourly average) by the World Health Organization [33].

Table 2. Concentrations of gaseous pollutants in air around major roads within Uyo Metropolis

Locations	Traffic density	NO_2 ($\mu\text{g}/\text{m}^3$)	SO_2 ($\mu\text{g}/\text{m}^3$)	CO (ppm)	VOC ($\mu\text{g}/\text{m}^3$)
APR	201	26.70 ± 1.03	17.52 ± 1.08	27.54 ± 0.43	4.82 ± 0.03
CIR	825	47.50 ± 1.32	34.10 ± 1.10	63.40 ± 0.12	16.10 ± 0.01
IDR	643	38.40 ± 1.28	31.43 ± 2.11	51.51 ± 1.01	10.44 ± 0.05
ENR	321	28.60 ± 1.00	24.21 ± 0.73	30.63 ± 0.31	6.53 ± 0.03
UAR	121	20.80 ± 2.01	12.23 ± 0.32	20.03 ± 0.02	3.04 ± 0.01
NWR	89	18.70 ± 1.21	9.45 ± 0.11	13.40 ± 0.00	1.07 ± 0.00
UVR (ctrl)	23	12.40 ± 0.74	3.46 ± 0.00	4.12 ± 0.03	0.04 ± 0.00

Each value represents mean \pm SEM of three determinations; ctrl – control

Mean concentration of volatile organic compounds (VOCs) in $\mu\text{g}/\text{m}^3$ obtained in air samples from different sampling locations and the Control is presented in Table 2. VOCs represent other carbon containing gases and vapor such as gasoline fumes, solvents other than carbon dioxide, carbon monoxide, methane and chlorofluorocarbons. The results presented in Table 2 shows that air samples around the different sampling locations within Uyo Metropolis accumulated different amounts of VOCs. Similar to other gases analyzed, VOCs abundances was also related directly to the traffic density of the locations. Mean VOCs concentrations obtained at all the sampling locations and the Control ranged from $0.54 \mu\text{g}/\text{m}^3$ obtained at Uyo village road (ctrl) to $16.10 \mu\text{g}/\text{m}^3$ obtained at CIR sampling location. Comparing results of individual sampling locations in terms of the amount of VOCs in air, CIR recorded mean amount that was 1.5 times higher than IDR, 2.5 times than ENR, 3 times than APR and about 3 times higher than UVR which was the control. The highest concentration of VOCs obtained in air sample obtained at CIR, could be traceable to the impact of traffic and other anthropogenic activities. Again, fuel is used to drive automobiles and heavy equipment and with its complex composition (containing mostly hydrocarbons) in addition to its ability to activate other pollutants suspending in the air, could lead to an increase in level of VOCs in the location. The mean range of VOCs obtained in this study agrees with $12 \mu\text{g}/\text{m}^3$ previously reported by Uka and co-workers [14] with slight variations. However, the results disagrees with $24.82 \mu\text{g}/\text{m}^3$ and $31.05 \mu\text{g}/\text{m}^3$ mean VOCs concentration reported by others researchers [27,12]; however, the levels did not exceed the recommended limit of $113 \mu\text{g}/\text{m}^3$ hourly amount by the World Health Organization [33].

Fig. 1, shows result of mean content of atmospheric particulate matters (PM_{10} , $\text{PM}_{2.5}$) of air samples collected around major roads within Uyo Metropolis. PM_{10} and $\text{PM}_{2.5}$ as used in this work are particulate matters in air with an aerodynamic diameters of $10 \mu\text{m}$ or less, and $2.5 \mu\text{m}$ or less respectively. PM_{10} content for all the sampling locations indicated that air samples collected at the control site contained the lowest ($24.10 \mu\text{g}/\text{m}^3$), while air samples collected from CIR had the highest ($124.48 \mu\text{g}/\text{m}^3$) particulate matter. Similarly, for $\text{PM}_{2.5}$, same trend as in the case of PM_{10} was observed, with from the

Control recording the lowest ($10.46 \mu\text{g}/\text{m}^3$) amount, while air samples obtained from CIR had the highest ($91.40 \mu\text{g}/\text{m}^3$). From the result, PM_{10} and $\text{PM}_{2.5}$ contents were also seen to relate with the number of automobiles (traffic density) of the sampling locations. For all the locations, both PM_{10} and $\text{PM}_{2.5}$ contents increased with traffic density though not in all cases. Variations in the contents of PM_{10} and $\text{PM}_{2.5}$ among sampling locations as observed in the results presented in Fig. 1, may be as a result of a range of factors including varying temperatures of the locations, relative humidity of the area, extent of fuel/wood combustions, and even the mineral (sulphate, nitrate, organic carbon etc) contents of the locations. Aside from the influence of heavy traffic density, continuous burning of fuel by automobiles and heavy equipment by construction companies operating at CIR, there are a lot of ongoing construction works including excavation of lands / rocks that can trigger higher contents of particulate matters in this area. Also, the higher content of PM_{10} , and $\text{PM}_{2.5}$ in air samples obtained at CIR could be due to elevated level of VOCs obtained in the location (Table 2), since VOCs are known precursors to secondary formation of fine particulate matters [4]. Sources of particulate matters to the atmosphere could be both primary and secondary, since some particulate matters are formed in the atmosphere due to atmospheric reactions of gaseous vegetable emission, motor vehicle emission and wood smoke emissions. Mean results of PM_{10} and $\text{PM}_{2.5}$ obtained for all sampling locations in this study are consistent with those previously reported [30, 34] for some major roads in some Nigerian Cities. However, the result is far greater than result reported [36,37] for roads within some major Cities in some part of Europe. Higher content of particulate matters in major roads within Uyo metropolis, Akwa Ibom State, Southern Nigeria, than other Cities in developed Countries may be largely due to lack of development. Most developed Countries have strict vehicular emission control mechanism and good road networks, whereas in Uyo, Southern Nigeria, there is no such vehicular control mechanism in addition to presence of heavy traffic and unpaved curbsides [36]. However, mean content of particulate matter in studied location obtained were below the recommended level of $250 - 600 \mu\text{g}/\text{m}^3$ daily average value allowed for air samples considered to be of good qualities by WHO [33].

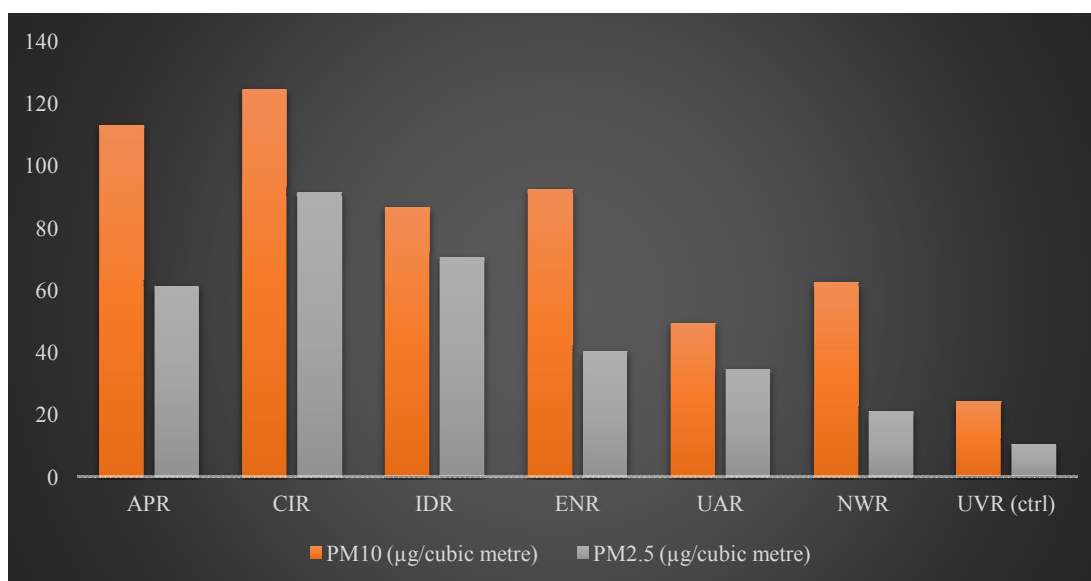


Fig. 1. Mean concentrations of particulate pollutants in air around major roads within Uyo Metropolis

From the findings and results obtained for all the sampling locations in terms of gaseous and particulate pollutants distribution, studied areas were laddered with different levels (low to moderate) of gaseous pollutants as indicated by the different amounts of these substances in the air samples studied. Although all the gaseous and particulate pollutants measured in this study were below recommended concentrations, their presence in the air of the studied locations were above the background level, and is mainly due to anthropogenic induced activities. This implies that these locations were likely to have poor air quality since air quality index of any given place is directly associated with the presence and amounts of these pollutants in air (Clean Air Act 1970). Consequently, and based on the results presented in Table 3 and Fig. 1, it could be inferred that Calabar-Itu road among the six studied locations excluding the Control (UVR), is likely the most highly air pollution stress location (as it contain higher concentrations of gaseous and particulate pollutants). In general and in lieu of the aforementioned, air pollution stress pattern (occasioned by the presence of gaseous and particulate pollutants in air) for the entire sampling locations increases in the order NWR < UAR < ENR < APR < IDR < CIR.

To ascertain the observations of increase gaseous and particulate pollutant concentrations in studied air samples obtained and the fact that the studied locations have been stressed by

gaseous and particulate pollutants at levels above background values, changes in some important plant biochemical markers of *C. sinensis* under this condition of pollution were also studied and the results are presented in Table 3.

3.2 Biochemical Responses of *Citrus sinensis* Leaves

Results of common biochemical markers in leaves of *C. sinensis* locally sourced from six major roads and Control within Uyo Metropolis is presented in Table 3. Biochemical markers determined in the leaves of *C. sinensis* included total phenols, proline, malonaldehyde, chlorophyll, cellulose and ascorbic acids. Results of total phenolic content measured in milligram of gallic acid equivalent (mg GAE/g) indicate that the *C. sinensis* leaves from different locations responded differently in terms of the accumulation of phenolic compounds. From the result, mean range was 8.48 mg GAE/g obtained in *C. sinensis* leaves collected from UVR (control) to 63.5 mg GAE/g obtained for leaves from CIR. The result indicated that phenolic content in the leaves increase with increase in pollution stress of the sampling locations. Trend of mean phenolic content was CIR > IDR > APR > ENR > UAR > NWR > UVR, with the highest pollution stress area (CIR) showing about 7.5 fold higher than the Control (UVR). The reason for this observation may be attributed to the varied amount of gaseous and particulate pollutants in

air around the different studied areas which can cause the production and accumulation of various hydroxyl containing compounds. Range of 14.33 – 63.50 mg GAE/g obtained for phenolic content in leaves of *C. sinensis* obtained in this study is lower than ranges reported previously by other researchers [12,10], but higher than what was reported by Johnson and co-workers [38] for different tree leaves in their respective studies. Increased phenolic contents of plant leaves after exposure of the plant to several toxic gaseous pollutants have previously been reported [39, 12]. The use of changes in the phenolic content of leaves of plants with respect to exposure to different array of toxic pollutants from air serving as a biomarker has previously been reported by Mira and co-worker [40].

Proline contents ($\mu\text{mol/g DW}$) of *C. sinensis* leaves sourced from major roads within Uyo Metropolis is presented in Table 3. Like the case of total phenolic, proline contents of the leaves varied directly with pollution loads of the locations. Mean proline content in $\mu\text{mol/g DW}$ for each locations were 2.84, 1.67, 1.42, 1.82, 1.15, 0.93, and 0.52 for CIR, IDR, APR, ENR, UAR, NWR, and UVR respectively. Mean range obtained was 0.52 $\mu\text{mol/g DW}$ obtained for *C. sinensis* leaves from UVR to 2.48 $\mu\text{mol/g DW}$ for those collected from CIR. The result shows that proline content in the leaves increased with increase in pollution stress, with highest pollution stress location (CIR) showing higher proline content than others. Comparing individual results of proline content with others for all locations, CIR showed about 1.7-fold higher proline content higher than those from IDR, and 5.46-fold higher than the Control. Range (0.93 – 2.84 $\mu\text{mol/g DW}$) obtained for proline in leaves of *C. sinensis* obtained in this study is lower than values reported previously [13,41], but higher than values reported by Ogagaoghene [9] and Kavi-Kiskor and co-workers [42] for different plant leaves in their respective researches. Elevated contents of proline in plant leaves after exposure to different environmental stresses have previously been reported [12]. Nayer and Reza [43] opined that, proline itself can be of principal importance in air pollution monitoring and can serve as a selective marker in the study of air pollution of any location.

Leaves of *C. sinensis* collected from six major roads within Uyo Metropolis were analyzed to determine the malonaldehyde (MDA) content which gives information about the membrane lipid peroxidation in leaves of plants.

Environmental stress such as exposure of plant leaves to gaseous and particulate pollutants usually leads to increase production of reactive oxygen species (ROS) which leads to chlorophyll degeneration and increase lipid peroxidation, which then enhances increase in MDA in plant leaves [44]. Mean result of MDA content in *C. sinensis* leaves obtained in this study is presented in Table 3. Trend observed in terms of variation in MDA contents for the leaves based on extent of pollution stress determined, followed the same order with that of total phenolic and proline contents. There was a clear and significant difference observed in the MDA contents of leaves obtained from different major roads within Uyo Metropolis. Leaves from UVR (control) recorded lowest (0.16 $\mu\text{mol/g DW}$) MDA content, while leaves from the most pollution stress location (CIR) recorded highest (1.25 $\mu\text{mol/g DW}$) MDA content. Leaves from CIR showed about 1.2-fold higher MDA content than leaves from IDR (next highly pollution stress location after CIR), and about 7.8-fold than leaf from UVR. Results of MDA obtained in the present study is lower than values earlier reported [4], but higher than values reported by Tripathi and co-workers [45]. Various reports abound on the effect of some abiotic stresses such as high salinity, excessive light, water deprivation, toxic metals, and gaseous pollutants on plants leading to elevated MDA contents in plant leaves [46 – 49]. Their researches indicated that lipid peroxidation was a common phenomenon in plants under different kinds of environmental stress and MDA could be used as an important pointer of physiological status during plant growth and a marker of oxidative lipid injury caused by environmental stress [48].

Chlorophyll is a photoreceptor during plant photosynthesis and variations in its content usually arises as a result of exposure of plant to different abiotic environmental stresses. Variations in chlorophyll content of plant leaves due to exposure to environmental stress is owing to plant pigment undergoing different photochemical responses such as oxidation and reduction, pheophytinisation, and even reversible bleaching [50]. Results of chlorophyll content expressed in mg/g FW (milligrams per gram fresh wet basis) of *C. sinensis* leaves from major roads within Uyo Metropolis is presented in Table 3. From the result, mean range was 3.52 mg/g FW in leaves from UVR (control) to 1.36 mg/g FW obtained in leaves from CIR. Trend in terms of abundance of chlorophyll in the leaves of *C. sinensis* was UVR > NWR > UAR > ENR > APR >

IDR > CIR. The result also indicates a contrast in the variation of chlorophyll content with respect to locations based on the extent of air pollution stress from those of total phenolic, proline and MDA contents. Locations with less pollution stress (lower amounts of gaseous and particulate pollutants as in Table 1 and Fig. 1) recorded higher chlorophyll contents than those with higher pollution stresses. Leaves of *C. sinensis* from UVR showed about 2.6-fold higher than leaves from CIR, and about 1.9-fold higher than leaves from IDR which represented locations with highest and next higher pollution stress locations according to this study. Differences in the chlorophyll contents of *C. sinensis* leaves from major roads within Uyo Metropolis obtained in this study is an indication of varied concentrations of gaseous and particulate pollutants at the different locations where the test leaves were obtained for analysis. Indu and co-workers [51], reported similar results in chlorophyll contents of *C. papaya* and *F. religiosa* leaves from major roads in Asaba, Delta State, Sothern Nigeria, as a result of influence of automobile exhaust and industrial pollutants at different concentrations. In their studies, the authors reported a decrease in chlorophyll content of *C. papaya* and *F. religiosa* leaves in roadsides leaves with increased level of automobile pollutants. Range of 1.36 to 3.03 mg/g FW obtained for chlorophyll in leaves of *C. sinensis* obtained in this study is lower than values previously reported [10], but higher than values reported by Banerjee and co-workers [52] for plant leaves in their respective studies. Other authors have also worked and reported a decrease in chlorophyll contents of many different plant leaves with respect to varied environmental pollution stresses [52-53].

Table 3 shows the mean cellulose content in g/100g DW of *C. sinensis* leaves from six major roads and a control location within Uyo Metropolis. From the result, cellulose content of the leaves varied directly with locations based on extent of pollution stresses. Mean range of cellulose was 1.82 g/100g DW in leaves obtained from UVR to 10.84 g/100g DW in leaves obtained from CIR, with order of abundance UVR < NWR < UAR < ENR < APR < IDR < CIR. The results reveal that *C. sinensis* leaves from the most pollution stress location (CIR) showed about 5.9-fold high cellulose content than leaves from the Control, 4.2-fold higher than leaves from NWR, and 2.7-fold higher than UVR. The observed differences in cellulose content between different sampling locations indicate

variance in environmental stresses in which the test plant has been exposed to. Mean range of cellulose content obtained in this study is lower than ranges earlier reported [7,14], but higher than ranges reported [12] and Thawale and co-workers [10] in their independent studies. When plants are exposed to environmental stresses, there is an increase deposition of cellulosic materials inside the cell wall which makes the cell wall thicker, resulting in a higher capacity of the plant to withstand varied environmental stresses [54]. Studies on variations of cellulose content in different plant leaves under different abiotic stresses such as varied salt concentrations, flood, trace metals and others have been previously reported [54–55]. The findings of reduced cellulose content of *C. sinensis* leaves from locations with less pollution stress and vice versa obtained in this study agrees with the report of Mukherjee and co-workers [12]. The authors reported a similar observation in *M. indica* leaves with respect to increased environmental stress conditions. However, the result is inconsistent with the report of Wang and co-workers [7] who reported a decrease in amounts of cellulose content in *D. regia* leaves with increase concentrations of arsenic salt.

Influence of gaseous and particulate air pollutants on ascorbic acid content of *C. sinensis* leaves collected from major roads within Uyo Metropolis is presented in Table 3. The result expressed in mg/g DW reveals that *C. sinensis* leaves from major roads within Uyo Metropolis contained varied concentrations of ascorbic acid. The result unlike chlorophyll content, showed a direct relationship between ascorbic acid concentrations with sampling locations based on extent of pollution stress. Mean range of ascorbic acid content of *C. sinensis* leaves from all the locations was 5.48 mg/g DW obtained in leaves from UVR to 51.16 mg/g DW obtained in leaves from CIR. Location with lowest pollution stress (UVR) recorded least ascorbic acid content, while location with highest pollution stress recorded the highest ascorbic acid content. *Citrius sinensis* leaves obtained from most pollution stress location (CIR) showed about 1.2-fold higher than ascorbic acid content in leaves from IDR, and about 9.3 – fold higher than leaves from the Control location. Variations in ascorbic acid content of the tested leaves from different locations obtained in this study reveals the effect of environmental pollutions occasioned by the presence of gaseous pollutants in the troposphere. Mean range of ascorbic acid

Table 3. Concentrations of biochemical markers of *C. sinensis* leaves

Locations	Total phenolic (Mg GAE/g)	Proline (μ mol/gDW)	MDA (μ mol/gDW)	Chlorophyll (mg/gFW)	Cellulose g/100gDW	Ascorbic acid (mg/g)
APR	32.51 \pm 2.10	1.42 \pm 0.02	0.84 \pm 0.04	2.02 \pm 0.03	8.65 \pm 0.21	31.62 \pm 1.10
CIR	63.50 \pm 1.52	2.84 \pm 0.01	1.25 \pm 0.01	1.36 \pm 0.01	10.84 \pm 0.10	51.16 \pm 2.21
IDR	46.00 \pm 1.12	1.67 \pm 0.00	1.02 \pm 0.00	1.81 \pm 0.01	10.13 \pm 0.05	43.24 \pm 1.17
ENR	24.63 \pm 0.32	1.83 \pm 0.01	0.55 \pm 0.00	2.31 \pm 0.00	6.32 \pm 0.02	20.85 \pm 0.98
UAR	20.46 \pm 0.12	1.15 \pm 0.02	0.42 \pm 0.02	2.64 \pm 0.00	4.06 \pm 0.01	16.46 \pm 0.54
NWR	14.33 \pm 0.11	0.93 \pm 0.04	0.38 \pm 0.00	3.03 \pm 0.01	2.61 \pm 0.00	10.34 \pm 0.06
UVR(ctrl)	8.48 \pm 0.05	0.52 \pm 0.00	0.16 \pm 0.00	3.52 \pm 0.00	1.82 \pm 0.01	5.48 \pm 0.01

Each value represents mean \pm SEM of three determinations; MDA – malonaldehyde; GAE – gallic acid equivalent; FW – fresh weight; DW – dry weight

Table 4. Correlation coefficients between gaseous and particulate pollutants

Variables	PM ₁₀	PM _{2.5}	SO ₂	NO ₂	CO	VOCs
PM ₁₀	1					
PM _{2.5}	0.962*	1				
SO ₂	0.989*	0.963*	1			
NO ₂	0.982*	0.934	0.971*	1		
CO	0.976*	0.763	0.879	0.767	1	
VOCs	0.827	0.961*	0.856	0.993*	0.783	1

Correlation is significant at the 0.05 level (2 tailed); VOCs – Volatile organic compounds

content (10.34 – 51.16 mg/g DW) obtained in the present study is higher than range reported [9], but lower than range reported by Thawale and co-workers [10]. Results of increase ascorbic acid content with increasing pollution load obtained in this study agrees with the report [56], but disagrees with previously respective reports by other authors [57-59]. Increase ascorbic acid content with increasing pollution loads observed in this study may be attributed to increase rates in the generation of reactive oxygen species through light oxidation of sulphate present in the leaves to sulphite by the plant [4]. The role of ascorbic acid in plants include synthesis of cell wall, defense and in cell division. Being a strong reductant, it usually triggers most physical and defense mechanism in plants.

3.3 Correlation Coefficient between Gaseous and Particulate Pollutants

Result presented in Table 4 shows the Pearson's correlation between air gaseous and particulate pollutants in the air environment around six major roads within Uyo metropolis.

The result indicated that there were some perfect (values close to +1), and high degree (values between 0.5 to +1) relationship existing between the gaseous and particulate pollutants. From the result, there was a strong positive and significant associations between PM₁₀ and PM_{2.5} ($r = 0.962$ $p < 0.05$), SO₂ and PM₁₀ ($r = 0.989$ $p < 0.05$), SO₂ and PM_{2.5} ($r = 0.963$ $p < 0.05$). Also, a strong association existed between NO₂ and PM₁₀ ($r = 0.982$ $p < 0.05$), as well as PM₁₀ and CO ($r = 0.976$ $p < 0.05$). Other parameters in the study that showed a strong positive relationship which were very significant included PM_{2.5} and VOCs ($r = 0.961$ $p < 0.05$), NO₂ and SO₂ ($r = 0.971$ $p < 0.05$), VOCs and NO₂ ($r = 0.961$ $p < 0.05$). This means that when increasing the concentration of PM₁₀, there will also be an increase in the concentrations of PM_{2.5}, SO₂, NO₂, and CO. In terms of PM_{2.5}, its increase in air around the major roads within the metropolis

will also lead to increase in the concentrations of SO₂ and NO₂, whereas increase in SO₂ will result in the increase in concentration of NO₂ and so on. There were also some parameters that showed positive but not significant association as in the case PM₁₀ and VOCs and SO₂ with CO and VOCs. It can also be deduced from the result of Pearson's correlation analysis presented in Table 3, that the parameters that showed a strong and significant relationship (perfect and high degree correlation) have similar emission sources and could be influenced by similar factors which can be anthropogenic or other environmental factors [60].

4. CONCLUSION

Increase in human pollution with related increase in number of automobiles, various commercial and industrial activities, as a way of making movement easy and existence possible are common features of most cities in Nigeria. This development usually leads to many negative impacts such as pollution of the environment including air. The present study reveals that increase in the number of automobiles (heavy traffic density), presence of construction sites and yards, fossil fuel combustion and many other anthropogenic activities directly increases the spatial distribution pattern and concentrations of some gaseous and particulate pollutants (PM₁₀ and PM_{2.5}) from their background concentrations. Also, biochemical markers in *C. sinensis* leaves from major roads within Uyo Metropolis showed different responses in terms of accumulation of these biochemical markers, and were observed to increase as concentrations of the gaseous and particulate pollutants increases throughout the studied locations. This is as a result of various alteration in the rate of production of bioactive molecules that could withstand and oppose the aftereffect and resultant strain on the plant. It can be concluded that based on the result of this work, changes in biochemical markers of *C. sinensis* leaf can serve as a biomarker to air

qualities of major roads within Uyo metropolis, Akwa Ibom State, Southern Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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