NJB, Volume 32(2), December, 2019

EVALUATION OF PIGMENT CONCENTRATIONS OF ROADSIDE TREE SPECIES AS BIOINDICATORS OF AIR POLLUTION IN ABAKALIKI METROPOLIS

[1]Uka, U.N.* and [2]Ajagun, G. A.
[1]Department of Applied Biology, Ebonyi State University, Abakaliki, Nigeria
[2] Nigerian Educational Research and Development Council, Sheda, Abuja, Nigeria

Correspondence: ufereh@gmail.com

Received 17th June, 2019; accepted 15th August, 2019

ABSTRACT
Variations in the concentration of different photosynthetic pigments (Chlorophyll and carotenoids) of leaves of three tree species growing in some polluted sites were evaluated. *Terminalia catappa, Polyalthia longifolia and Mangifera indica* which were in abundance and well distributed in the Residential (Control), Industrial and Traffic sites (Polluted area) were selected for the study. Reduction in chlorophyll 'a', 'b' and carotenoid was recorded in the leaf samples collected from polluted sites when compared with samples from control sites. In the polluted area (Industrial and Traffic sites), the highest decrease in total chlorophyll of the studied plants was in *Mangifera indica* (1.02 mg/g) followed by *Terminalia catappa* (0.89 mg/g) and *Polyalthia longifolia* (0.78 mg/g). There was a decrease in carotenoid content at the polluted area (Industrial and Traffic sites) compared with the control (Residential)**.** The value was highest in *Terminalia catappa* (0.19 mg/g) and lowest in *Mangifera indica* (0.12 mg/g). It is evident from this study that residential tree species have more chlorophyll a/b ratio compared with the tree species exposed to air pollution. The ratio of chlorophyll a+b and carotenoid ratio decreased, which is an indication of stress arising from the industrial and vehicular air pollution. This study clearly showed that the industrial and vehicular-induced air pollution reduced the concentration of photosynthetic pigments in the trees exposed to it. These changes could be used as bioindicator of air pollution of an area.

INTRODUCTION

Chlorophyll activity is a vital tool to evaluate the consequences of air pollutants on plants because it plays a vital role in plant metabolism and reduction in pigment content that enhances plant growth (Wagh *et al*., 2006). Leaf chlorophyll content and carotenoids, therefore, will offer valuable information concerning the status of plants. Air pollutants once absorbed by the leaves can cause a reduction in the concentration of photosynthetic pigments namely, chlorophyll and carotenoids, and directly affect plant productivity. Chlorophyll pigment is the principal photoreceptor in photosynthesis, the light-driven method during which greenhouse gas is "fixed" to yield carbohydrates and oxygen. Carotenoids act as photoprotective agents for the chloroplasts (Joshi and Swami, 2009). Once plants are exposed to the environmental pollution above the normal physiologically acceptable range, photosynthesis gets inactivated (Mizalski and Mydlarz, 1990). As reported by Dwivedi and Tripathi (2007), the distribution of plant diversity is highly dependent on the presence of air pollutants within the surrounding air and sensitivity of the plants.

Studies on the effects of air pollutants on morphology, physiology and biochemistry of plants have been carried out by a number of workers (Raina and Agarwal, 2004; Tripathi and Gautam, 2007) in different parts of the world. Chlorophyll is one of the essential parts of energy production in green plants and its amount is significantly affected by environmental condition. Sulfur dioxide, nitrogen dioxide and CO_2 as well as suspended particulate matter are some of the air pollutants that are absorbed by plant leaves, causing reduction in the levels of photosynthetic pigments, viz., chlorophyll and carotenoids, thereby directly affecting the plant productivity (Joshi and Swami, 2009; Honour *et al.*, 2009). The gradual disappearance of chlorophyll and concomitant yellowing of leaves is one of the most common effects of the pollutants which may be associated with the continuously decreasing photosynthetic

*Author for correspondence

capacity of the plant (Joshi *et al.*, 2009). Carotenoids which help in capturing light in the chloroplast are also affected by air pollution. Carotenoids also play an important role in protecting the cells and live organisms as they experience harm from free-radical oxidative cells (Fleschin *et al.*, 2003). Carotenoids are stronger than chlorophyll but much less effective in the capturing of light energy. It helps the important but much delicate chlorophyll, and shields it from photoxidative destruction (Joshi *et al.* 2009). The level of carotenoids decreases with the increase in air pollution load (Tiwari *et al*., 2006; Tripathi and Gautam, 2007; Joshi *et al*., 2009; Gupta *et al*., 2015). The continuous increase in human population in Abakaliki Metropolis has contributed to increased vehicular movement. Motor vehicles account for 60-70% of the pollution found in urban areas (Tripathi and Gautam, 2007; Dwivedi *et al*., 2008). The combustion of fuel in engines of motor generates sulfur dioxide (SO_2), nitrous oxides (NOx) and carbon monoxide (CO), as well as suspended particulate matter. The use of plants as monitors of air pollution has long been established because they are the initial acceptors of air pollution. They act as scavengers for several air-borne particulates within the atmosphere. This study was carried out to assess the changes in the concentration of chlorophyll and carotenoids within the leaf samples of plants growing in automobile-polluted sites.

MATERIALS AND METHODS

Study Area

The study was carried out within Abakaliki township in Ebonyi State, Nigeria. Abakaliki has a land mass of 51 km^2. The area lies on latitude 06^{o}22 ' 26 "N and longitude 08^{o}6' 6"E of the Greenwich meridian. The average atmospheric temperature is 32 - 35 ^{o}C (Epidi *et al*., 2008). The area lies on latitude 06^{o}22'26"N and longitude 08^{o}6' 6"E of the Greenwich meridian. The average atmospheric temperature is 35^{o}C (Epidi *et al.* 2008). Three sites were selected as polluted area as well as for less or non-polluted areas from Abakaliki Township. For polluted area, samples were collected from a rice mill as industrial area and Water works/Ezza roads , an area with high traffic. For non-polluted or less polluted area, a residential area (Democracy estate) of the City was selected. The study sites were located in the city of Abakaliki, Ebonyi State, Nigeria. For the study, three locations were selected: Rice milling site (6°20′54″ N, 8.0900119° E), Water works road (6.3246532° N, 8.0900279°E) and a Residential site (6.3219° N, 8.0845°E). The Rice milling site is an industrial area that has over 2,500 rice milling machines (Cohen *et al*., 1998. Moreover, there is usually smoke emitted from the rice mill continually into the surrounding communities (Njoku *et al.*, 2011).The Democracy estate is a residential area and is considered to be a potentially non-polluted site. The Water works/Ezza roads have intensive road traffic.

Sample Collection

At each study site, 50 physiologically active leaves (third from the tip) were manually harvested from the side of the tree facing the road for chlorophyll determination. The harvested leaves were placed in black self-sealing polyethylene bags, labelled appropriately and transported to the laboratory for analysis.

Estimation of chlorophyll and carotenoid concentrations

Chlorophyll and carotenoid concentrations were determined using the spectrophotometric method described by Arnon (1949). Leaf samples (3 g) were weighed and homogenized in 10 ml of 80% acetone solution using mortar and pestle for 15 minutes. The homogenate was transferred to another test tube and centrifuged at 2,500 rpm for 3 minutes. The supernatant was transferred with a pipette to a cuvette and the absorbance readings were taken against a blank using CECIL 8000 UV-visible spectrophotometer at wavelengths 645 nm, 663 nm and 480 nm. Total chlorophyll was calculated using the formula of Arnon's equation (1949). The chlorophyll and carotenoid concentrations were determined as follows:

Chlorophyll a = $12.7 (A._{663}) - 2.69 (A._{645}) \times V/1000 \times W$ mg/g

Chlorophyll b = $22.9 (A._{645}) - 4.68 (A.663) \times V/1000 \times W$ mg/g

Total Cholorophyll = Chlorophyll a + Chlorophyll b

Carotenoids = $A480 + 11.4 (A.663) - 6.38 (A.645nm) \times V/1000 \text{ x } W$

Where, A = Absorbance of the extract, V= total volume of the chlorophyll solution (ml), and W = weight of the tissue extract (g).

DATA AND STATISTICAL ANALYSIS

The data obtained were subjected to one-way Analysis of Variance (ANOVA) test using SPSS version 23 and mean separation was done according to Turkey b at $p<0.05$.

RESULTS

The effect of air pollution on chlorophyll a concentrations on the leaf samples of selected tree species at the various study sites

The effect of air pollutants on chlorophyll a concentrations on the leaf samples of selected tree species at the various study sites are presented in Table 1. The chlorophyll a concentration of leaf samples of all the three tree species at the Industrial and Traffic sites were lower and significantly differed from those of the Residential sites (p= 0.05). The mean concentration of chlorophyll a ranged from 0.22 mg/g in *Terminalia catappa* at the Industrial area to 0.73mg/g in *Mangifera indica* at the Traffic site , while at the Residential area, chlorophyll a concentration ranged from 0.82 mg/g in *Terminalia catappa* to 1.21 mg/g in *Mangifera indica*. The concentration of chlorophyll a in *Terminalia catappa* was significantly different among the sites (p = 0.000). When compared with the control, the highest percentage decrease in *Terminalia catappa* chlorophyll a concentration was 73.17% at Industrial area and the lowest decrease was 32.93% at Traffic area (Table 1). The chlorophyll a concentration of *Mangifera indica* had significant differences between the Industrial and Traffic sites. When compared with the Residential area, the highest percentage reduction in *Mangifera indica* chlorophyll a concentration was 58.68% at the Industrial area and lowest at the Residential area with a percentage decrease of 39.67 % (Table 1).

The concentration of chlorophyll a in *Polyalthia longifolia* was significantly different (p= 0.000) compared with the Industrial and Traffic sites. When compared with the Residential area (control), the highest percentage decrease of 70.23% occurred in *P. longifolia* at the Industrial site while the lowest percentage decrease of 45.74% was observed at the Traffic site (Table 1).

Table 1. The effect of air pollution on concentration of chlorophyll a (mg/g) of selected tree species across the study sites

Sampling site	**Tree Species**					
	Terminalia catappa	**% R**	*Mangifera indica*	**% R**	*Polyalthia longifolia*	% R
Residential	0.82±0.02^{c}		1.21±0.01^{c}		0.94±0.01^{c}	
Industrial	0.22±0.02^{a}	**73.17**	0.50±0.01^{a}	**58.68**	0.28±0.01^{a}	**70.23**
Traffic	0.55±0.07^{b}	**32.93**	0.73±0.02^{b}	**39.67**	0.51±0.02^{b}	**45.74**

Mean + SE in the same column with different letters in superscript differs significantly ($p < 0.05$)
% R = Percentage reduction

Chlorophyll b

The effect of air pollutants on chlorophyll b concentrations of the leaf samples of selected tree species at the various study sites is presented in Table 2 . The chlorophyll b concentration of leaf samples of all three tree species located at the Industrial and Residential sites was lower and significantly different from those of the Residential site (p=0.000). The mean concentration of chlorophyll b ranged from 0.34 mg/g in *Polyalthia longifolia* at the Industrial and Traffic sites to 0.54 mg/g in *Terminalia catappa*, while at the Residential site, chlorophyll b concentration ranged from 0.59 mg/g in *Polyalthia longifolia* to 0.68 mg/g in *Terminalia catappa*. The chlorophyll b concentration of *Terminalia catappa* in the Industrial and Traffic sites was significantly different from each other (p<0.05). When compared with the Residential area, the highest percentage decrease in *Terminalia catappa* chlorophyll b concentration was 29.41% at the Industrial area while the lowest percentage decrease was 20.59% at the Traffic area (Table 2). The chlorophyll b concentration of *Mangifera indica* was significantly different between the Industrial and Traffic areas (p= 0.000). When compared with the Residential area, the highest percentage decrease in *Mangifera indica* chlorophyll b concentration was 46.97% at the Industrial area and the lowest percentage decrease was 30.30% at the Traffic area (Table 2).

Table 2. The effect of air pollution on concentration of chlorophyll b (mg/g) concentration in selected tree species across the study sites

Sampling site	Tree Species					
	Terminalia catappa	**% R**	*Mangifera indica*	**% R**	*Polyalthia longifolia*	% R
Residential	0.68±0.01^{a}		0.66±0.01^{c}		0.59±0.01^{c}	
Industrial	0.48±0.00^{a}	**29.41**	0.35±0.01^{a}	**46.97**	0.42±0.01^{b}	**28.81**

Mean + SE in the same column with different letters in superscript differs significantly (P < 0.05), % R = Percentage reduction

Total chlorophyll

The effect of air pollution on total chlorophyll concentration of selected tree species at the various study sites is presented in Table 3. The total chlorophyll concentration of all the tree species at the Industrial area was lower and significantly different from that of the Residential site (p= 0.000). The mean concentration of total chlorophyll ranged from 0.62 mg/g in *Polyalthia longifolia* at the Industrial area to 1.19 mg/g in *Mangifera indica* at the Traffic site, while at the Residential site, total chlorophyll concentration ranged from 1.50 mg/g in *Terminalia catappa* to 1.86 mg/g in *Mangifera indica.*

The total chlorophyll concentration of *Terminalia catappa* was significantly higher at the Industrial than at the Traffic site (p= 0.000). When compared with the Residential site, the highest percentage decrease in *Terminalia catappa* total chlorophyll concentration was 53.33% at the Industrial site and lowest at the Traffic site with a percentage decrease of 27.33% (Table 3).

Statistical analyses (p<0.05) revealed that significant differences were observed in the total chlorophyll concentration of *Mangifera indica* between the industrial and traffic sites (p= 0.05). When compared with the Residential site, the highest percentage decrease in *Mangifera indica* total chlorophyll concentration was 54.30% at the Industrial site and lowest at the Traffic site with percentage decrease of 36.02% (Table 3).

The total chlorophyll concentration of *Polyalthia longifolia* was significantly different from the study site (p= 0.000). When compared with the control, the highest percentage decrease in *Polyalthia longifolia* total chlorophyll concentration was 59.48% at the Industrial site and lowest at the industrial site with a percentage decrease of 39.22% (Table 3).

Table 3. The effect of air pollution on total concentration of chlorophyll (mg/g) in selected tree species across the study sites

Sampling site	**Tree Species**					
	Terminalia catappa	**% R**	*Mangifera indica*	**% R**	*Polyalthia longifolia*	% R
Residential	1.50±0.02[c]		1.86±0.01[c]		1.53±0.01[c]	
Industrial	1.09±0.05[b]	**27.33**	1.19±0.03[b]	**36.02**	0.93±0.01[b]	**39.22**
Traffic	0.70±0.02[a]	**53.33**	0.85±0.01[a]	**54.30**	0.62±0.01[a]	**59.48**

Mean + SE in the same column with different letters in superscript differs significantly ($p < 0.05$)
% R = Percentage reduction

The carotenoid concentration of *Polyalthia longifolia*

The effect of air pollution on carotenoid concentration of tree species at the sampling sites is presented in Table 4. The carotenoid concentration in leaf samples of all the three tree species at the industrial and Traffic sites were lower than and varied significantly from those at the Residential sites except for *Polyalthia longifolia* ($p<0.05$). The mean concentration of carotenoid ranged from 0.11 mg/g in *Polyalthia longifolia* at the Industrial site to 0.26 mg/g in *Terminalia catappa* while at the Residential sites, it ranged from 0.20 mg/g in *Polyalthia longifolia* to 0.39 mg/g in *Terminalia catappa.*

The carotenoid concentration of *Terminalia catappa* at the Industrial sites was significantly different from that of the Traffic and Residential sites ($p=0.000$). When compared with the residential sites, the highest percentage decrease in concentration of carotenoid in *Terminalia catappa* was 69.23% at the Industrial and lowest at the Traffic sites with a percentage decrease of 33.33% (Table 4).

The carotenoid concentration of *Mangifera indica* was significantly different within the study sites ($p < 0.05$). When compared with the Residential sites, the highest percentage decrease in carotenoid concentration of *Mangifera indica* was 50. 00 % at the Industrial sites and lowest at the Traffic sites with a percentage decrease of 43.33 (Table 4). ($p> 0.05$). When compared with the Residential sites, the highest percentage decrease in *Polyalthia longifolia* carotenoid concentration was 45.00 % at the Industrial sites and lowest at the residential sites with a percentage decrease of 35.00% (Table 4).

Table 4. The effect of air pollution on carotenoid (mg/g) concentrations of selected tree species in the Abakaliki City

Sampling site	**Tree Species**					
	Terminalia catappa	**% R**	*Mangifera indica*	**% R**	*Polyalthia longifolia*	% R
Residential	0.39 ± 0.01^{c}		0.30 ± 0.01^{c}		0.20 ± 0.01^{b}	
Industrial	0.26 ± 0.01^{b}	**33.33**	0.17 ± 0.01^{b}	**43.33**	0.13 ± 0.01^{a}	**35.00**
Traffic	0.12 ± 0.01^{a}	**69.23**	0.15 ± 0.01^{a}	**50.00**	0.11 ± 0.01^{a}	**45.00**

Mean + SE in the same column with different letters in superscript differs significantly ($P < 0.05$)
% R = Percentage reduction

Variation in the assimilating pigments of selected tree species at different sampling sites

The variation in the assimilating pigments of selected tree species at the different sampling sites is shown in Table 5. *Mangifera indica* had the highest chlorophyll a/b (1.43, 1.59 and 1.83) while *Terminalia catappa* had the lowest (0.46, 1.02 and 1.21) at the Industrial, Traffic and Residential sites, respectively. Chlorophyll a/b ratio was less at the Industrial and Traffic sites than at the Residential sites.

Chlorophyll (a+b)/carotenoid ratio was less at the Residential sites for *Terminalia catappa (*3.85) when compared with the other sampling sites. It was evidently lower in *Mangifera indica* (5.67) and *Polyalthia longifolia* (5.64) at the Industrial sites. The ratio of Chl a+ b and carotenoid was lower at the Industrial and Traffic sites in comparison to the Residential sites. *Mangifera indica* had the highest value at the Industrial (1.00) and Traffic sites (1.36) while *Polyalthia longifolia* had the lower values of 0.73 and 1.06 at the Industrial and Traffic sites, respectively.

NJB, Volume 32(2), December, 2019 Uka, U.N. and Ajagun, G. A.

Table 5. The weight ratio of Pigments of Leaves Sampled from Selected Tree Species across the study sites

Tree Species	**Chlorophyll a/b ratio**			**Chlorophyll (a+b)/ Carotenoid**			**Chlorophyll (a+b) + Carotenoid**		
	Industrial	**Traffic**	**Residential**	**Industrial**	**Traffic**	**Residential**	**Industrial**	**Traffic**	**Residential**
Terminalia catappa	0.46	1.02	1.21	5.86	4.19	3.85	0.82	1.35	1.89
Mangifera indica	1.43	1.59	1.83	5.67	7.00	6.25	1.00	1.36	2.73
Polyalthia longifolia	0.82	1.21	1.59	5.64	7.15	7.65	0.73	1.06	1.73

DISCUSSION

The decrease in the concentration of chlorophyll at polluted sites (Industrial and Traffic sites) might be as a result of shading effects due to deposition of suspended particulate matter on the leaf surface. This can clog the stomata and interfere with the gaseous exchange, leading to increase in leaf temperature and retarding chlorophyll synthesis (Seyyednejad *et al.*, 2013). A similar reduction has been recorded in the leaves of *Azadirachta indica*, *Nerium oleander*, *Mangifera indica* and *Dalbergia sissoo (Giri et al., 2013)*. Damage in chlorophyll content in *Azadirachta indica* L., *Conocarpus erectus* L., *Guiacum officinale* L. and *Eucalyptus* sp. growing along the roads of a city has also been recorded by *Iqbal et al*. (2015). Dusted or encrusted leaf surface is responsible for reduced photosynthesis, thereby causing reduction in chlorophyll content (Joshi and Swami, 2009)). A similar impact of air pollutants on the concentration of chlorophyll contents has been reported by a number of other workers (Saxena, 1991; Swami *et al*., 2004; Tripathi and Gautam, 2007).

At the polluted sites (Industrial and Traffic sites), the pattern of decrease in total chlorophyll of the studied plants was in *Mangifera indica* (1.02 mg/g) followed by *Terminalia catappa* (0.89 mg/g) and *Polyalthia longifolia* (0.78 mg/g). The degradation of chlorophyll pigments could be attributed to the action of SO_2 and NO_2 on the metabolism of chlorophyll (Lauenorth and Dodd, 1981). These gases are the constituents of vehicular emissions. The reduction in the concentration of chlorophyll might be due to the increase in chlorophyllase enzyme activities, which in turn affects the chlorophyll concentration in plants (Mandal and Mukherji, 2000). The results of this study are in agreement with the work of Wali *et al.* (2004) who reported that the chlorophyll was significantly lower in the plants fumigated with different levels of SO_2. Rao and Le Blane (1966) had reported that Chlorophyll a was degraded to phaeophytin through replacement of Mg^{+2} ions in chlorophyll molecules, while chlorophyll b formed chlorophyllide b through the removal of the phytol group of the molecule. The decrease in carotenoid content at polluted sites (Industrial and Traffic sites) compared to control (Residential) was highest in *Terminalia catappa* (0.19 mg/g) and lowest in *Mangifera indica* (0.12 mg/g). The decrease in carotenoid contents in the leaves of the studied tree species at the Industrial and Traffic sites agrees with Joshi and Swami (2009) who reported that vehicular emission or vehicle- induced air pollution reduced photosynthetic pigments in trees exposed to roadside pollution. Carotenoid acts as photoprotective agents for the chloroplasts (Joshi and Swami, 2009). Under stressed conditions, the routine protective process may become burdened, resulting in a cellular degradation including pigment destruction. Chauhan (2010) reported that carotenoids are sensitive to SO_2. Since SO_2 is a byproduct of vehicular air pollution, it is suggested that this pollutant could have caused the reduction of carotenoid content of the leaves of the studied species at the road sites. Several researchers have reported reduced carotenoid content under air pollution (Verma and Singh, 2006; Tripathi and Mukesh, 2007; Sharma and Tripathi, 2009). Swami *et al*. (2004) have also reported significant reduction in carotenoid content in *Shorea robusta* and *Mallotus phillipinensis*, due to road side automobile emission. Aquil *et al.* (2003) reported a reduction in carotenoid content in the plants of *Albizzia lebbek* Benth exposed to coal smoke.

The ratio of Chl a and Chl b (Chl a/b ratio) is an indicator of the functional pigment equipment and light adaptation of photosynthetic apparatus (Pierre and Queiroz, 1981). It is evident from the data that residential tree species have more chlorophyll a/b ratio compared with the tree species exposed to air pollution. Chlorophyll a+b/ carotenoid are an indicator of greens of plant. The lower values of the ratio at the polluted sites indicate the stress and the damage to the plants. Since the plants are exposed to the air pollutant, the ratio of chlorophyll a+b and carotenoid ratio decreased, which is an indication of stress and damage due to air pollutant. In conclusion, this study revealed a reduction of photosynthetic pigments (chlorophyll a, b, total chlorophyll and carotenoid) resulting in industrial and vehicular-induced air pollution in the leaves of *Terminalia catappa, Polyalthia longifolia and Mangifera indica* tree species. It is, therefore, suggested that in the absence of visible injury, analysis of photosynthetic pigments of plants could result in the early diagnosis of the pollution level of an area.

REFERENCES

Aquil, S., Syed Hilal Ahmad, A., Reshi, Z. M. and Muhammad, I. (2003). Physiological and bio-chemical response of *Albizzia lebbek* Benth to coal smoke pollution. Pollut. Res., 22: 489-493.

Arnon, D. I. (1949). Copper enzyme in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol.*, 24: 1-15.

Chauhan, A. (2010). Photosynthetic pigment changes in some selected trees induced by automobile exhaust in Dehradun. *J. New York Sci*., 3(2): 45-51.

Cohen, S. B. (1998). *Abakaliki: The Columbian Gazetteer of the World*: Columbian University Press. New York, NY.

Dwivedi, A. K. and Tripathi, B. D. (2007). Pollution tolerance and distribution pattern of plants in surrounding sites of coal-fired industries. *Journal of Environmental Biology*, 28: 257-263.

Dwivedi, A. K., Tripathi, B. D. and Shashi, T. (2008). Effect of ambient air sulphur dioxide on sulphate accumulation in plants. *Journal of Environmental Biology*, 29, 377-379.

Epidi, T.T., Nwani, C. D. and Ugorji, N. P. (2008). Prevalence of malaria in blood donors in Abakaliki Metropolis, Nigeria. *Scientific Research and Essay,* 3 (4): 162-164.

Fleschin, S., Fleschin, M., Nhta, S., Pavel, E. and Mageara, V. (2003). Free radicals mediate protein oxidation in biochemistry. *Rom Biotechnol Lett.,* 5:479–495.

Giri, S., Shrivastava, D., Deshmukh, K. and Dubey, P. (2013). Effect of air pollution on chlorophyll content of leaves. *Current Agriculture Research Journal*, 1(2): 93-98.

Gupta, G. P., Singh, S., Kumar, B. and Kulshrestha, U. C. (2005). Industrial dust sulphate and its effect on biochemical and morphological characteristics of Morus (*Morus alba*) in NCR Delhi. *Environmental Monitoring and Assessment*, 187 (3): 67.

Honour, S. L., Bell, J. N., Ashenden, T.W., Cape, J. N. and Power, S. A. (2009). Responses of herbaceous plants to urban air pollution: Effects on growth, phenology and leaf. *Environmental Pollution*,157:1279–1286.

Iqbal, M., Shafik, M. and Athar, M. (2015). Effect of automobile Pollution on chlorophyll content of road side urban trees. *Global Journal of Environmental Science and Management*, 1: 283-296.

Joshi, N., Chauhan, A. and Joshi, P.C. (2009). Impact of industrial air pollutants on some biochemical parameters and yield in wheat and mustard plants. *Environmentalist*, 29:398–404.

Joshi, P.C. and Swami, A. (2009). Air pollution-induced changes in the photosynthetic pigments of selected plant species. *Journal of Environmental Biology*, 35:295–298.

Lauenorth, W.K. and Dodd, J. L. (1981). Chorophyll reduction in western wheat grass (*Agropyron smithii* Rydb.) exposed to sulphur dioxide. *Water Air Soil Pollut*ion, 15, 309-315.

Mandal, M. and Mukherji, S. (2000). Changes in chlorophyll contents, chlorophyllase activity, Hill reaction, photosynthetic CO_2 uptake, sugar and starch content in five dicotyledonous plants exposed to automobile exhaust pollution. *Journal of Environmental Biology,* 21, 37-41.

Miszalski, Z. and Mydlarz, J. (1990). SO_2 influence on photosynthesis of tomato plants at different CO_2 concentrations. *Photosynthetica*, 24: 2-8.

Njoku, C., Mbah, C. N. and Okonkwo, C. I. (2011). Effect of Rice Mill Wastes Application on Selected Soil Physical Properties and Maize Yield (*Zea mays* L.) on an Ultisol in Abakaliki, Southeastern Nigeria. *Journal of Soil Science and Environmental Management*, 2(11):375-383.

Pierre, M. and Queiroz, Q. (1981). Enzymic and metabolic changes in bean leaves during continuous pollution by necrotic levels of SO_2. *Environ Pollution*, 25, 41 – 51.

Raina, A.K. and Aggarwal, B. (2004). Effect of vehicular exhaust on some trees in Jammu-II. *J. of Industrial Pollution Control*, 20.(2) : 229-232.

Rao, D. N. and Le Blanc, F. (1966). Effect of SO_2 on the lichen algae with special reference to chlorophyll. Bryologist, 69, 69-75.

Saxena, R. M. (1991). Effect of motorway pollution on seed health of some vegetable crops. *Ind. J. Environ. Hlth*., 33, 385- 387.

Seyyednejad, S. M., Koochak, H. and Vaezi, J. (2003). Changes in oxidative enzyme activities, protein content and ascorbic acid level in *Prosopsis juliflora* exposed to industrial air pollution. *Journal of Biology and today's World*, 10: 482-492.

Sharma, A. P. and Tripathi, B. D. (2009). Biochemical response in tree foliage exposed to coal-fired power plant emission in seasonally dry tropical environment. *Environmental Monitoring Assessment*, 158: 197–212.

Swami, A., Bhatt, D. and Joshi, P.C. (2004). Effects of automobile pollution on sal (*Shorea robusta*) and rohini (*Mallotus phillipinensis*) at Asarori, Dehradun. *Himalayan Journal of Environment and Zoology*, 18 (1): 57- 61.

Tiwari, S., Agarwal, M. and Marshall, F. M. (2006). Evaluation of ambient air pollution impact on carrot plants at a suburban site using open top chambers. *Environmental Monitoring and Assessment,* 119:15–30.

Tripathi, A. K. and Mukesh, G. (2007). Biochemical parameters of plants as indicators of air pollution. *Journal of Environmental Biology*, 28(1): 127-132.

Tripathi, A.K. and Gautam, M. (2007). Biochemical parameters of plants as indicators of Pollution. *Journal of Environmental Biology*, 28: 127-132.

Verma, A. and Singh, S. N. (2006). Biochemical and ultrastructural changes in plant foliage exposed to auto-pollution. *Environmental Monitoring and Assessment*, 120: 585–602.

Wagh, N.D., Shukla Poonam, V., Tambe Sarika, B. and Ingle, S.T. (2006). Biological monitoring of roadside plants exposed to vehicular pollution in Jalgaon city. *Journal of Environmental Biology*, 27(2): 419-421.

Wali, B. M. and Iqbal, M. (2004). Plant growth, stomatal response, pigments and photosynthesis of Althea officinalis as affected by SO_2 stress. *Ind. J. Plant Physiol*., 3: 224-233.