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Bio monitoring of air pollution around urban and industrial sites

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ABSTRACT:

Plants are the only living organisms which have to suffer a lot from automobiles exhaust pollution and industrial pollution because they remain static at their habitat. Experiments on air and bio monitoring were conducted to evaluate pollution impact on the vegetation along the road in Dindigul town TamilNadu. The plantation along the roads mainly include *Azadiracta indica, Delonix elata, Morinda tinctoria, Calotrophis, Thyme rosemary* and *Cyandan dactylon.* For bio monitoring, total chlorophyll, carotenoid, ascorbic acid, protein and total sugar were analyzed to study the impact of air pollutants. It was observed that vegetation at the road side with heavy traffic and industries was much affected by air pollutants. Significant reduction of total chlorophyll, carotenoid, protein and total sugar was observed. These variations can be used as an indicator of air pollution for early diagnosis of stress or as a marker for physiological damage to trees prior to the on set of visible injury symptoms. It is concluded that plants can be used as indicators for urban air pollution, and it is need to protect the road side plants from air pollution.

Keywords:
Bio monitoring, chlorophyll, carotenoid, plant protein, visible injury, air pollution, bio indicators, field plants.

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INTRODUCTION

Due to sharp increase in the roadway diesel in the last few decades the emission of gases now constitutes an important source of environmental pollution all over the world. In developing countries like India, the problem is much more aggravated due to factors such as narrow and congested road and old and poorly maintained engines. Exhausted emissions from the diesel powered vehicles have low carbon monoxide (CO) and unburnt hydrocarbons, while nitrogen oxide (NO) is present in high concentration than petrol powered vehicles. Beside, these emissions contain smoke particles, Sulfurdioxide (SO_2), aldehydes and odour producing compounds (Agarwal, 1991).

Air pollution is influenced by four major factors, namely industrialized expansion of the cities, increase in traffic, rapid economic development, and higher level of energy consumption. The growth of both an industrial and residential area is unplanned in many developing cities of India. Thus, contributing to the air pollution problems. Automobile produces volatile organic compounds (VOC), Suspended particulate matter (SPM) oxides of sulfur (SO_x), oxides of nitrogen (NO_x) and carbon monoxide (CO) which have adverse effects on surrounding ecosystem.

Regional impact of air pollution on different local plant species is one of the major ecological issues. The climatic conditions, the physico - chemical properties of air pollutants and their residence time in the atmosphere have impact on surrounding plants and animals.

Monitoring of air pollutant is a prerequisite to air quality control. Their impact on the chemical composition of plants is often used as an indicator and a tool for monitoring environmental pollution. (Rao,1977; Posthumus, 1984, 1985; Agrawal and Agrawal, 1989; Kulump etal., 1994; Dmuchowski and bytnerowicz, 1995, Sarala etal 2010). Monitoring with the help of biological indicators is a simple, cheap and convenient method to ensure the state of local environment. The effect of environmental factors on plants increases with exposure time. Plant response to air pollution can be used to assess the quality of air that may provide early warning signals of air pollution trends.

The dying rates change with respect to apparent injury, chlorophyll reduction, cell size reduction and reduction in leaf area are used as parameters for monitoring air pollution impacts on plant metabolism (Leblanc and Rao, 1975).

Industrial effluents are constantly adding up toxic substances into the ground water reservoir at a very high rate, especially in industrial zones. Many regions all over the globe are heavily depending on ground water for various purposes (Babiker *et al*., 2004).

The main focus of this work is to provide an assessment of the use of biochemical parameters of plants as indicator of air pollution so that these biochemical indicators can be used for air quality monitoring in urban areas of Dindigul the capital of Tamil Nadu.

MATERIALS AND METHODS

Study site

The study area is located in the southern part of India, close to Kodaganar river basis, mainly in hard rock terrain. The area is known for its leather industries. It lies between 10° 13'44" – 10°26'47" N latitude and 77 °55'08" – 78 °01'24" E longitude and falls in survey of India Top sheet No.58 F/15 & J/3, in the state of Tamil nadu, India. The selected area is located in the central part of Dindigul town and along Madurai, Batlagundu and Ponmandurai roads.

Site selection / bio indicator Station selection

Totally three bioindicator stations including urban and suburban sites close to streets with heavy and light pollution load were identified and Lakshmanapuram was treated as control site. Details of sites are

Bio indicator Station1 – Residential area (Lakshmanapuram)
Bio indicator Station2 – Dindigul Bus stand (Traffic area)
Bio indicator Station3 – Tannery area (Thomaiyar puram)

Air Quality Analysis (SO_2, NO_x and SPM):

During the exposure period ambient air quality in terms of common air pollutants that is SO_2, NO_x and SPM were analyzed at all three bioindicator stations.

Sampling was done for 24hr and twice in a week. Average was taken for final calculation. For the collection of samples for SPM from ambient air, GF / A filter paper was used in high volume sampler (HVS) at the flow rate of 0.1 to 1.5 m^3 / min. SPM was computed as per standard method. Filter paper was weighted before and after sampling. West and Gaeke method (1956) and modified Jacob and Hochheiser method (1958) were used for the analysis of SO_2 and NO_x respectively.

Air Pollution Index (API)

The average of the sum of the ratios of three major pollutant concentrations to their respective air quality standards were obtained. The average was then multiplied by 100 to get the index (Rao and Rao, 1989).

$$API = 1/3\ [(SPM)/(S_{spm}) + (SO_2)/(S_{So2}) + (NO_x)/(S_{NOX})]*100$$

Where $S_{spm,}$ S_{So2} and S_{NOX} represent the ambient air quality standards for SPM, SO_2 and NO_x.

Air pollution index of bioindicator stations were developed on the basis of ambient air quality analyzed at specified bioindicator stations through instrumental monitoring of SPM, SO_2 and NO_x and correlated with the variation in biochemical indicator. On the basis of air pollution index, bio indicator station 1 was categorized as light air pollution site (air pollution index for summer and winter are (28.66 and 42), station 2 as moderate air pollution site (air pollution index for summer and winter are 61.66 and 60) and station 3 as moderate air pollution site (air pollution index for summer and winter are 47 and 50.56) (**Table1 and Table 2**).

Biochemical parameters

After three months of the exposure plants were brought back to the institute and leaf samples were analyzed for different biochemical parameters. Total chlorophyll and carotenoid were analyzed following the method of Arnon (1949),ascorbic acid by Sadasivam and Bala Subramanian (1987), protein by Lowry etal (1951), total soluble sugars by phenol sulphuric acid method of Dubois *et al*. (1951).

Estimation of Chlorophyll and Carotenoid

500mg of fresh leaf material was taken and ground with help of pestle and mortar with 10ml of 80% acetone. The homogenate was centrifuged at 800 rpm for 15 minutes. The supernatant was saved. The residues were re-extracted with 80% acetone. The supernatant was saved and utilized for chlorophyll estimation. Absorbance was read at 645, 663 and 480 nm in the UV – Spectrophotometer.

Chlorophyll 'a' ($mg.g^{-1}$ FW) = (0.0127)*(0D663)-(0.00269)*(0D645)

Chlorophyll 'b' ($mg.g^{-1}$ FW) = (0.229)*(0D645)-(0.00488)*(0D663)

Total chlorophyll ($mg.g^{-1}$ FW) = (0.0202)*(0D645) + (0.00802)*(0D663)

Carotenoid = 80% acetone (1000 A470 – 3.27 [chla]-1.04 [chl b] / 227 (X + c = (1000 A470 – 2.27 chl a – 81.4 chl b) /230.

Estimation of protein

Protein content was determined by the method of lowry etal(1951). 0.5g of plant sample (shoot) was homogenized in 10 ml of 20% Trichloro Acetic acid (TCA). The homogenate was centrifuged in 10 minutes. The supernatant was discharged and the pellet was re extracted with 5 ml of 0.1N NaOH. One ml of the extract was taken in a test tube and 5 ml of reagent 'c' (protein reagent) was added. This solution was mixed well and kept in dark for 10 minutes. Later 0.5 ml of folin – ciocal teau reagent was added and the mixture was kept in dark for 30 minutes. The sample was read at 660 nm in the uv spectrophotometer.

Table 1. Ambient air quality and air pollution index for different bio indicators stations

Bio indicators stations	Pollutants (µg / m³)				Air pollution index	Remarks
		SPM	SO_2	NO_x		
Station no:1(Residential)	winter	111.2	11.4	32.0	42	light air pollution
	Summer	99.4	9.5	3.3	28.66	light air pollution
Station no:2(Traffic)	winter	158.4	15.0	25.6	60	Moderate airpollution
	summer	147.6	18.7	29.7	61.66	Moderate airpollution
Station no:3(Tannery)	winter	98.0	14.2	20.0	50.56	Moderate airpollution
	summer	65.5	9.9	14.7	47	Moderate airpollution

Ambient air quality standards taken for calculation of air pollution index 140 µg / m³ for SPM, 60 µg / m³ for SO_2 and 60 µg / m³ for NO_X.

Table 2 . Rating Scale for indices (reference) [Both winter and summer]

Index value	Remarks
0 - 25	Clear air
20 - 50	light air pollution
51 - 75	Moderate air pollution
76 - 100	Heavy air pollution
> 100	Severe air pollution

Estimation of Sugars

Five hundred mg of plant materials were weighed and macerated in a pestle and mortar with 10ml of 80% ethanol. The homogenate was centrifuged for 10 minutes at 800 rpm. The Supernatant was saved. Then the ethanol is evaporated in water both at 50°c. The net content was made up to 20ml with distilled water and the extract was used for the estimation of reducing sugar. One ml of extract was taken in a 25ml marked test tube. One ml of reagent 'c' was added. Then the mixture was heated for 20 minutes at 100° c in a boiling water bath, cooled and 1 ml of arsenomolybdate reagent was added. The solution was thoroughly mixed and diluted to 25 ml with distilled water. The sample was read in the uv spectrophotometer at 520 nm. The sugar contents were expressed were in mg/g fresh weight basis.

Estimation of Ascorbic acid:

Ascorbic acid is a reducing agent it is determined by reacting with a selective agent namely 2, 6 dichlorophenol indophenoldye 10 gm of the leaf samples were transferred to a glass pestle mortar and macerated well with 4% oxalic acid. The contents were transferred to a 100 ml volumetric flask by filtering through a muslin cloth. Repeated the extractions with 4% oxalic acid 3 – 4 times so that the extraction was perfectly completed. This volume of solution was made up with 4% oxalic acid. Titrated against 0.02% dye solution taken in the burette, a permanent pale pink colour is obtained.

RESULT AND DISCUSSION

Leaf samples of the plant species were analyzed for chlorophyll, carotenoid, ascorbic acid, protein and soluble sugars. All the biochemical indicators exhibited significant variations from species to species and sampling site to site. These were listed in the **Table- 3.**

Azadiracta indica

The total chlorophyll content of *Azadiracta indica* at the control site is 0.85 mg/g . It exhibited

Table 3: Biochemical Indicators of different species at different bioindicator stations

Name of the Species	Sampling Station	Site No	Total Chlorophyll	% of Variation	Carotenoid	%of Variation	Ascorbic Acid	%of Variation	Protein	%of Variation	Total Sugar	%of Variation
Azadiracta Indica	Residential	S1	0.85		4.4		2.08		5.52		49.60	
	Traffic	S2	0.478	43.76	2.65	39.77	4.05	-94.71	1.58	71.37	25.3	48.99
	Tannery	S3	0.69	18.82	12.2	-177.2	2.62	-25.96	3.28	40.57	50.94	-2.70
Delonix elata	Residential	S1	0.531		6.8		2.65		2.56		40.58	
	Traffic	S2	0.384	27.68	5.5	19.11	3.58	-35.09	1.15	55.078	32.6	19.66
	Tannery	S3	0.76	-43.12	0.471	93.07	3.15	-18.86	3.8	-48.43	0.291	99.28
Morinda tinctoria	Residential	S1	0.948		8.5		1.65		25.6		46.35	
	Traffic	S2	0.636	32.91	5.5	35.29	3.55	-115.15	15.5	39.45	36.5	21.25
	Tannery	S3	0.48	49.36	10.6	-24.70	3.68	-123.03	12.34	51.79	31.88	31.21
Calotrophis	Residential	S1	0.674		6.6		2.56		6.68		35.15	
	Traffic	S2	0.47	30.26	3.5	46.96	3.65	-42.57	3.58	46.40	38.60	-9.81
	Tannery	S3	0.43	36.20	7.6	-15.15	1.89	26.17	2.38	64.37	45.47	-29.35
Thyme Rosemary	Residential	S1	0.80		10.3		1.96		4.28		48.96	
	Traffic	S2	0.458	42.75	8.5	17.47	2.35	-19.89	3.12	27.10	25.36	48.20
	Tannery	S3	0.56	30	5.4	47.57	1.08	44.89	2.72	36.44	56.66	-15.60
Cyanadon dactylon	Residential	S1	0.66		3.58		3.88		2.85		31.88	
	Traffic	S2	0.42	36.36	2.58	27.93	4.56	-17.5	3.56	-24.91	28.5	10.60
	Tannery	S3	0.37	43.93	9.6	-168.15	2.34	39.69	5.68	-99.29	45.28	-42.03

Results are significant at 0.1% (r<0.001)

43.76% reduction at the sampling site 2 and 18.82% reduction at the sampling site 3. The carotenoid content at the control site (s_1) is 4.4 mg/g significant reduction (39.77%) in carotenoid content was absorbed at sampling site S_2 whereas at sampling site S_3 again of >100% was evident. The ascorbic acid at the control site S_1 traffic area (s_2) and at the industrial site (s_3) were found to be 2.08 mg/g 4.05 mg/g and 2.62mg/g respectively. Protein content at the control site S_1 was found to be 5.52mg/g but maximum reduction (71.37%) at site S_2 followed by 40.57% at site S_3 was found out. Total sugar showed decreased over control site S_1 48.99% reduction in total sugar was observed at sampling site s_2 followed by 2.70% increase at sampling site S_3. It is evident from data that total chlorophyll, carotenoid protein and total sugar showed significant reduction at sampling site S_2 and S_3. But the ascorbic acid showed increase at all the two sampling sites (S_2 or S_3) compared to control site S_1.

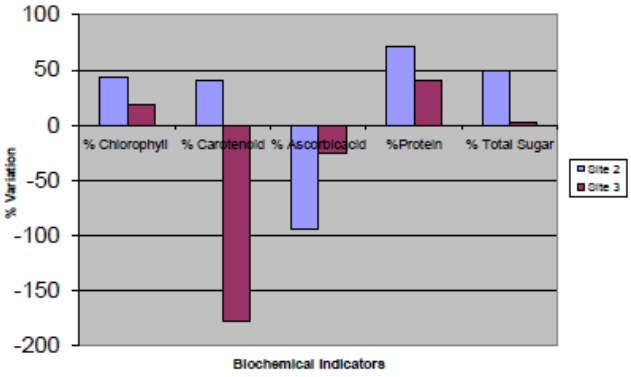

Fig: 1. Variation in bio-chemical indicators *Azadirachta indica* at different bio indicator stations

Delonix elata

Bio chemical indicators of *Delonix elata* at all the sampling sites are varied significantly. The total chlorophyll content, carotenoid, ascorbic acid, protien and total sugar are 0.531 mg/g , 6.8 mg/g , 2.65mg/g , 2.56mg/g and 40.58 mg/g respectively. Maximum reduction (27.68%) in chlorophyll content was observed at sampling site s_2 and gain of (43.12%) was observed at sampling site s_3. Maximum reduction (93.07%) of carotenoid was exhibited at sampling site s_3 followed by 19.11% reduction at sampling site s_2. Ascorbic acid was found to be increased in all the sampling sites as compared to control. Maximum increasing of 35.09% was evident at sampling site s_2 followed by 18.86% at sampling site s_3. Maximum reduction (55.08%) in protein was exhibited at sampling site s_2 but it showed an increasing trend (48.43%) at sampling site s_3. The significant reduction (99.28%) in total sugar was exhibited at sampling site s_3 followed by (9.66%) at sampling site s_2 respectively.

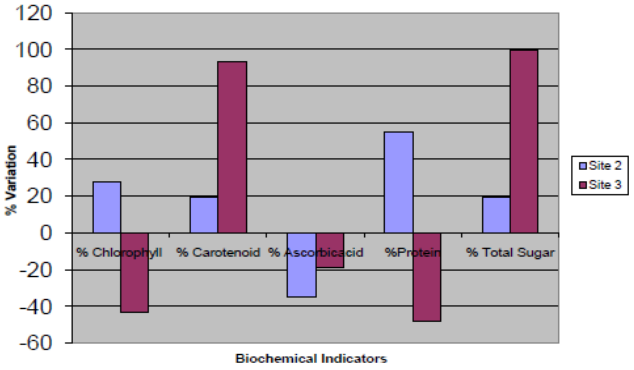

Fig : 2. Variation in bio-chemical of *Delonix elata* at different bio indicator stations

Morinda tinctoria

The total chlorophyll, carotenoid, ascorbic acid, protein and total sugar of M*orinda tinctoria* at the control site was found to be 0.94 mg/g , 8.5 mg/ g, 1.65 mg/g, 25.6 mg/g and 46.3 mg/g respectively. Chlorophyll content at sampling site s_3 showed 49.36% reduction followed by 32.91% reduction at sampling site s_2. Caroteniod was found to be significantly reduced (35.29%) at sampling site s_2 but a gain (24.70%) was observed at sampling site s_3. Ascorbic acid was found to be more at all the sampling sites as compared to control site. Maximum enhancement (>100%) in ascorbic acid was exhibited in sample site s_3 followed by sampling site s_2. Protein content at sampling site s_3 showed 51.79% reduction followed by 39.54% reduction at sampling site s_2. Soluble sugar was significantly reduced and the maximum reduction (31.21%) was revealed at sampling site s_3 followed by sampling site s_2 (21.25%).

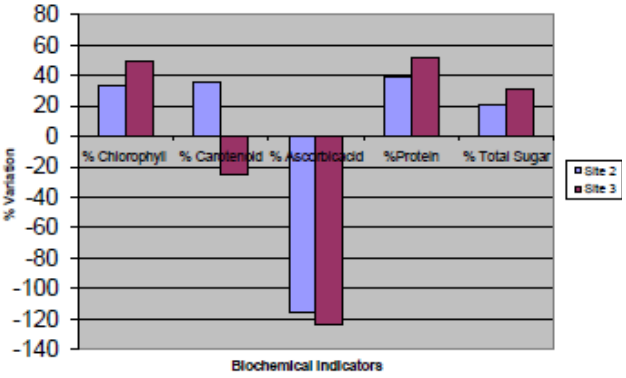

Fig : 3. Fig: 3. Variation in bio-chemical *Morinda tinctoria* at different bio indicator station

Calotrophis

The chlorophyll content of *calotrophis* at the control site was found to be 0.674 mg/g. It exhibited 36.20% reduction at the sampling site s_3 followed by 30.26% reduction at the sampling site s_2. The caroteniod at the control site was found to be 6.6 mg/g at which it showed 46.96% reduction at the sampling site s_2 but maximum increase of 15.15% was found at sampling site s_3. *calotrophis* exhibited 26.17% reduction in ascorbic acid at sampling site s_3 while increase of 42.57% was observed at sampling site s_2. All the two sampling sites showed a decreasing trend of protein over control site s_1. Maximum reduction (64.37%) at sampling site s_3 followed by 46.40% at sampling site s_2 was observed. Total sugar showed increase over control at all the sampling sites. Maximum increase (29.35%) was observed at sampling site s_3 followed by sampling site s_2 (9.81%).

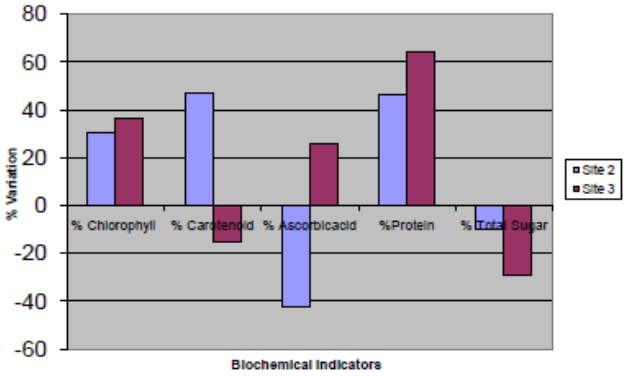

Fig : 4. Fig: 4. Variation in bio-chemical indicators *Calotrophis* at different bio indicator stations

Thyme rosemary

Biochemical indicators such as Total chlorophyll, Caroteniod, Ascorbic acid, Protein and Total sugar were 0.80 mg /g , 10.3 mg /g , 1.96 mg/ g , 4.28 mg/g and 48.96 mg/g respectively. *Thyme rosemary* showed maximum reduction of total chlorophyll (42.75%) at sampling site s_2 followed by 30% at sampling site s_3. The caroteniod of *Thyme rosemary* at the control site was observed as 10.3 mg/ g. It exhibited maximum reduction (47.57%) at sampling site s_3 followed by 17.47% at sampling site s_2. Ascorbic acid showed the reduction (44.89%) at sampling site s_3 while a increase of 19.89% was observed at sampling site s_2. Protein showed the significant reduction at the sampling site s_2 and s_3 respectively. Sampling site s_3 showed 36.44% reduction in protein followed by sampling site s_2 (27.10%). The total sugar at the control site s_1 and other the sampling sites s_2 and s_3 are 48.96 mg/g, 25.36 mg/g and 56.60 mg/g respectively.

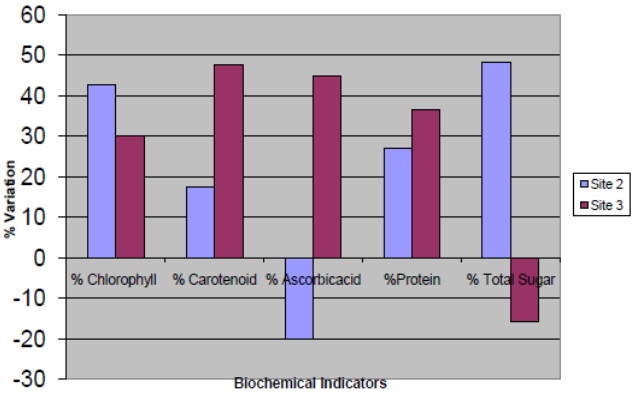

Fig : 5. Variation in bio-chemical indicators *Thyme rosemary* at different bio indicator stations

Cyandan dactylon

Cyandan dactylon showed decrease in trend of total chlorophyll at sampling sites s_2 and s_3 compared to control values site s_1. Sampling site s_3 exhibited maximum decrease (43.93%) followed by sampling site s_2 (36.36%). The carotenoid at the control site was observed as 3.58 mg/g it showed 27.93% reduction at sampling site s_2 but > 100% increase at sampling site s_3 was observed. Ascorbic acid was found to be as increasing trend (17.5%) at the sampling site s_2 but decreasing trend at (39.69%) at sampling site s_3. Protein was found to be increasing at all the sampling sites as compared to control. Maximum increase 99.29% was evident at sampling site s_3 followed by 24.91 at sampling site s_2. Total sugar showed maximum loss (10.60%) at sampling site s_2 and maximum stimulation (42.03%) at sampling site s_3. Although all the species showed significant variations in all the biochemical parameters. The extent up to which plant species were affected varied from species to species and station to station. Almost all the species showed maximum variation in biochemical indicators at sampling site s_3 which is found to severe air pollution site. A considerable loss in total chlorophyll and carotenoid in the leaves of the plants exposed at sampling site s_2 and s_3 (Moderate air pollution sites) supports the argument that the chloroplast is the primary sites of attack by air pollutant such as SPM, SO_2 and NO_x. Air pollutants make their entrance into the tissues through the stomata and cause partial denaturation of the chloroplast and decreases pigment contents in the cells of polluted leaves. Rao and Leblanc (1966) mentioned that high amount of gaseous SO_2 cause destruction of chlorophyll and that might be due to

the replacement of Mg^{++} by two hydrogen and degradation of chlorophyll molecules to phaeophytin. In *Azadiracta indica, Delonixelata, Moringa tinctoria, Calotrophis, Thyme rosemary* and *Cyandan dactylon*, maximum depletion in chlorophyll content and carotenoid s_2 and s_3 may be due to the maximum pollution load at sampling sites.

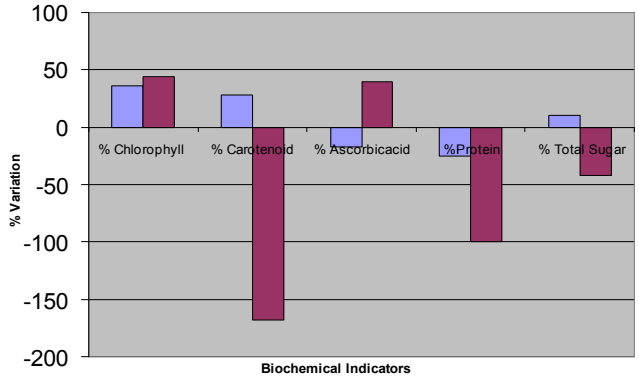

Fig :6. Variation in bio-chemical indicators *Cyanodon dactylon* at different bio indicator station

Reduction in protein content in *Azadiracta indica*, *Delonixelata, Moringa tinctori, calotrophis* and *Thyme rosemary* at sampling sites s_2 and s_3 might be due to the enhanced rate of protein denaturation which is also supported by the findings of Prasad and Inamdar (1990). Constantinidou and Kozlowski (1979) found enhanced protein denaturation and break down of existing protein to amino acid as the main causes of reduction in protein content. Soluble sugar is an important constituent and source of energy for all living organisms. Plants manufacture this organic substance during photosynthesis and break down during respiration. Our study revealed significant loss of soluble sugar in all the species at all the sites. All the species showed maximum loss at severe air pollution site that is s_2 and s_3. The concentration of soluble sugars is an indicative of the physiological activity of a plant and it determines the sensitivity of plants to air pollution. Reduction in soluble sugar content in polluted stations can be attributed to increase respiration and decreased CO_2 fixation because of chlorophyll deterioration. Davison and Barnes (1986) mentioned that pollutants like SO_2, NO_2 and H_2S under hardening conditions can cause more depletion of soluble sugars in the leaves of plants grown in polluted area. The reaction of sulfite with aldehydes and Ketones of carbohydrates can also cause reduction in carbohydrate content Present investigation revealed a great variation in the levels of ascorbic acid in all the sites. Pollution load dependent increase of all the species may be due to the more rate of production of reactive oxygen species (ROS) such as SO_3^-, HSO_3^-, OH^-, O_2^- etc. During photo oxidation of SO_3^- to SO_4^- where sulfites are generated from SO_2 observed. The free radical production under SO_2 exposure would increase the free radical scavengers, such as ascorbic acid (Pierre and Queirz, 1981) based on dosage and physiological status of plant. Increased level of ascorbic acid may be due to the defense mechanism of the plant. Data on ambient pollutant concentrations do not allow direct conclusions to be drawn on potential impacts on plants and the environment. Evidence of effects can only be provided by using plants itself as monitors. These types of plant bio indicators integrate the effects of all environmental factors. Therefore use of plants, as bio indicators is inexpensive and easy technique. Merely by analyzing the present parameters, an early diagnosis of the extent of pollution can be done in the absence of visible injury.

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