

**A STUDY ON BIOCHEMICAL PROPERTIES OF
ALTERNANTHERA SESSILIS IN RESPONSE TO ROADSIDE
POLLUTION**

PROJECT REPORT SUBMITTED TO

**MAHATMA GANDHI UNIVERSITY, KOTTAYAM
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF**

BACHELOR OF SCIENCE

IN

BOTANY



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APRIL 2023

CERTIFICATE

This is to certify that this project work entitled “**A study on biochemical properties of *Alternanthera sessilis* in response to roadside pollution**” is a bona-fide piece of project work done by Ms. MARIYA ROSE PHILIP (Reg.No: 200021023411) in the Department of Botany, Bharata Mata College, Thrikkakara under my guidance and supervision for the award of Degree of Bachelor of Science in Botany during the academic year 2020-2023. This work has not previously formed the basis for the award at any other similar title of any other university or board.

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DECLARATION

I hereby declare that this project entitled “**A study on biochemical properties of *Alternanthera sessilis* in response to roadside pollution.**” is the result of work carried out by me under the guidance of Mrs. Kalyanikrishna, Department of Botany, Bharata Mata College, Thrikkakara. This work has not formed on the basis for the award at any other similar title of any other university of board.

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ACKNOWLEDGEMENT

First, I thank God Almighty for blessing me to make his endeavour a successful one.

I express my sincere gratitude to Mrs. Kalyanikrishna, Assistant professor (on contract) Department of Botany, Bharata Mata College, Thrikkakara for the guidance and support given throughout the project work completion.

I also extend my deep gratitude to Dr. Newby Joseph, Head of the Department, Department of Botany Bharata Mata College, Thrikkakara for all the support and help extended to complete this work

My sincere thanks to Dr. Shahina N K, Dr. Surya Sukumaran and lab assistant Mr. Poly for their support in completing the project.

I thank my batchmates and project group for the and timely help to finish my work.

On my personal note I thank my parents and friends who always support, encourage and bless me in all my endeavours.

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1. INTRODUCTION

Amaranthaceae, the amaranth family, is a predominantly tropical family that consist of 174 genera and around 2,500 species distributed worldwide. Members of this family are normally herbaceous plants or subshrubs, many of which can withstand poor saline soils. They have simple leaves arranged along the stem in alternate/ opposite, the flowers are often small and borne in dense inflorescences (Petruzzello, 2022).

The family is well represented in the tropical regions of the world. The plants are very commonly found in the tropics of New World, Africa and India. Some plants are also found in temperate regions. In India the family is represented with around 17 genera and 50 species in distribution (Akbar et.al, 2020).

Members of Amaranthaceae are used in wide applications including as source of food -both leafy greens and seeds, medicinal herbs and ornamental plants. Many amaranths are deadly agricultural weeds, and several are considered invasive species in areas outside their native ranges. These competitive species generally known as pigweeds, are tolerant to a variety of growing conditions and promptly reseed themselves (Petruzzello, 2022).

One of the most renowned members of *Amaranathaceae* family is *Alternanthera* genus coined by Forsskal in 1775. The genus *Alternanthera* comprises approximately 139 species which are distributed in India, China, Sri Lanka, the United States of America, and Africa (Chandrashekhar, 2019).

1.1. Common *Alternanthera* species found in Kerala and India (Nazreen, 2018).

1) *Alternanthera bettzickiana* (Regel) G, Nicholson :This is a popular ornamental plant that is often grown as border or bedding plants. It has bright green leaves that turn red or bronze in bright sunlight.

2) *Alternanthera sessilis* (L.) R. Br. ex DC: This is a medical plant that is used in ayurvedic medicines. It is also known as sessile joyweed and herbaceous plant with slender stems and the leaves are usually small, lance shaped and green or reddish green in colour.

3) *Alternanthera pungens* Kunth: commonly known as khaki weed, a perennial herbaceous plant very ramified and forming a carpet on the ground.

4) *Alternanthera philoxeroides* (Mart.) Griseb: This is an aquatic plant that is often considered as a weed. It has bright green leaves and can form dense mats on the surface of ponds or other bodies of water.

5) *Alternanthera reineckii* Briq.: This is a popular aquarium plant that has bright red foliage. They are generally slow growers compared to other stem plants, and they can take a certain amount of overcrowding and shading, making them very easy to use in aquascaping.

6) *Alternanthera brasiliana* (L.) Kuntze: They are also known as Brazilian joyweed. It is a fast-growing herbaceous plant with leaves are used in traditional medicine.

7) *Alternanthera tenella* Colla.var.tenella: They are commonly known as slender joyweed. It is a small plant species that is often found in grasslands and open fields.

1.2 *Alternanthera sessilis*

Alternanthera sessilis is a member of family Amaranthaceae. It is known as sessile joyweed or dwarf copperleaf, and is native to Asia, Africa, and South America. *Alternanthera sessilis* is a flowering plant known by several common names, including sissou spinach, Brazilian spinach, sessile joyweed, dwarf copperleaf, and mukunuwenna in Sri Lanka. They grow as invasive weeds on the tropical areas as well as cultivated as leafy vegetable in some regions (Hwong, 2022). This is a perennial herb with prostrate stems, rarely ascending, often rooting at the nodes. Based on the colour of the aerial part, *A. sessilis* can be identified as a green or red cultivar (Othman et al., 2016). In this study *A. sessilis* green is used and will be the only one hence referred hereafter.

Leaves are obovate to broadly elliptic, occasionally linear-lanceolate, 1–5 cm long, 0.3–3 cm wide, glabrous to sparsely villous, petioles 1–5 mm long. Species plants have elliptic to broad ovate green leaves. However, it is the brightly colored cultivars that have become the popular garden plants, featuring green leaves blotched with yellow, orange, red, brown, copper or purple, sometimes with red veining.

Stem is simple or branched, green – purple, shining at first, terete when old, with longitudinal row of hairs on two opposite sides and Leaves are sessile, opposite, rounded, acute at apex, tapering at base, glabrous or thinly hairy. Inflorescence is spikes. Flowers are small, white in colour. White apetalous flowers appear stalkless or on short stalks in small axillary clusters in late fall to winter, but are insignificant (Shehzad et. al, 2018).

1.3. Application and Uses of *A. sessilis*

Weeds are often considered undesirable as they interfere with the habitat of native plants, and therefore they are underestimated and underutilised. In reality, some edible weeds have beneficial ecological and medicinal values that remain widely unexplored. *A. sessilis* has wide application in the field of medicine, nutrition, pharmacology and ecological regimes.

In *Alternanthera sessilis* the following compounds have been demonstrated to be present: the triterpenes α -spinasterol, β -spinasterol, stigmasterol, β -sitosterol, oleanotic acid and its derivatives and saturated (aliphatic) esters. The leaves contain dietary fibre (about 12 g per 100 g dry matter) and incorporation of about 75 g of this vegetable fibre in the daily diet of diabetics significantly reduced the postprandial blood glucose level.

In tests in India, leaf pastes of *Alternanthera sessilis* exhibited inhibition of mutagenicity in *Salmonella typhimurium* strains. They inhibited the formation of the potent environmental carcinogen nitrosodiethanol amine from its precursors such as triethanolamine.

The aqueous alcohol extract of the entire plant exhibits hypothermic and histaminergic activities and relaxes smooth muscles. An ether extract of *Alternanthera sessilis* yielded an active principle having anti-ulcerative. *A. sessilis* appears to be a cheap, eco-friendly, and alternative approach for curing infectious ulcers on the floor of the stratum corneum.

Alternanthera sessilis (Linn) R. Br. Seed oil contains a moderate source of hydroxy fatty acid (ricinoleic acid, 22.1%), along with the other normal fatty acids such as myristic (3.9%), palmitic (16.9%), stearic (5.9%) oleic (26.0%) and linoleic (25.2%). These fatty acids have been determined and characterized by GLC, TLC, ultraviolet (UV), Fourier transform infrared (FTIR), ^1H NMR, ^{13}C NMR, MS and chemical transformations.

Alternanthera sessilis titanium dioxide nanoparticles ($\text{AS@TiO}_2\text{NP}$'s) were synthesized using tissue-culture grown plant leaves aqueous extracts of *A. sessilis* by the microwave irradiation method. The synthesized nanoparticles showed potential antibacterial activity and dye degradation properties. It has been used for the treatment of dysuria and haemorrhoids. The plant is also believed to be beneficial for the eyes, and is used as an ingredient in the making of medicinal hair oils and kajal. The plant has been traditionally used in the treatment of jaundice along with other ailments. (Chatterjee et. al, 2006).

Alternanthera sessilis has the greatest ability to improve the vision. Persons suffering from night blindness (Nyctalopia) can eat the raw flower of *sessilis* continuously will feel the difference in their eyesight. The plant extract is demonstrated to have anti-cancerous properties as it mixes with the blood and removes the toxins from the body (Hundiwale., 2012).

In many places of the world, the leaves of *Alternanthera sessilis* are eaten as a cooked vegetable or raw as a salad. In tropical Africa its use as a vegetable has been reported from Guinea (where it is used in place of rice as a staple and is said to be satiating), Benin (in sauces and soup), Nigeria (in soup), DR Congo, Tanzania and Zambia (as relish), as well as from Madagascar and Réunion (as a potherb). In Sri Lanka the plant is tied in bundles and sold on markets for use in salads (Chandrika et. al, 2006).

1.4. *Alternanthera sessilis* as an invasive species in Kerala

A. sessilis is a pioneer species typically growing on disturbed areas and in wetland habitats, and regarded as a fast-growing highly invasive weed. It is adapted to grow on a range of soil types ranging from poor sandy or alkaline soils, to loam or black cotton soils. It is also able to grow in seasonally-waterlogged areas as well as in areas with extreme dry conditions (Holm et al., 1997). This species is also a weed in fields with sorghum, millet, *Eleusine* spp., maize, cotton, cassava, cereal crops, pastures, and vegetable farms. Consequently, this species has been listed as invasive in India, South Africa, Namibia, Spain, Hawaii and many other islands in the Pacific Ocean.

In Kerala *A. sessilis* is widely found in different environmental habitats including well-watered fertile soil to dry and drought affected areas. It is also one of the prominent members of roadside vegetation in Kerala (Ray & George, 2009, Sasidharan 2004).

Figure 1.1. *Alternanthera sessilis* (L.) R.Br. ex DC. (Source: <https://creativecommons.org/licenses/by-sa/4.0/legalcode>)



1.5. Influence of vehicular pollution on biochemical properties of roadside plants

Vehicular pollution is one of the major contributors to air pollution in today fast developing world and road side vegetation is the primary barrier of the adverse effect from vehicular pollution. The ever-increasing vehicle counts have resulted in significant increase in air pollution affecting human and natural ecosystem (Tripathi & Gautam 2007). Better understanding of roadside vegetation and their properties in response to pollution will lead to an improved management and planning of urban cities that could lower the impacts of harmful pollutants

The leaves absorb airborne pollutants (SO₂, NO_x, PM_{2.5}, etc) and digest heavy metals(Cd, Cr, Cu, Ni, Pb, etc) in dust through different morphological, physiological and biochemical changes such as leaf thickness, leaf size, trichomes, ascorbic acid, pH, etc. (Banerjee et al., 2022). It is a reliable tool for atmospheric restoration.

Carbohydrate is the primary food material that helps plants to counter the stress condition. The concentration of carbohydrate reflects the carbon assimilative capacity of plants (Tripathi & Gautam 2007). Significant reduction of the carbohydrate content of plants in the polluted environment could have possibly been due to less photosynthetic activity consequent to the significant depletion of chlorophyll content of leaves.

Davison and Barnes (1986) mentioned that pollutants like SO₂, NO₂ and H₂S 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.

Protein is the most important bio-molecule responsible for the growth and development of plants. Previous studies report varied data on the protein levels of polluted samples. Some shows a reduction in protein content in plant (Priyadarshini & Sahu, 2019) while in others more free amino acids at severe air pollution site were observed probably may be due to more nitrate reductase activity or may also be due to more protein denaturation at polluted site (Tripathi & Gautam, 2007).

It has been revealed that pollution led to elevation in total phenolics and total flavonoids, when compared with samples of plants from non-polluted sites. This suggests that elevations in total phenolics and total flavonoids can be considered as stress defence mechanisms in plants against pollution

Alternanthera sessilis showed high amount of energy rich nutritional factors, such as carbohydrates, proteins and lipids. High content of amino acids and flavonoids have been observed in *A. sessilis* and *A. philoxeroides*. *Alternanthera versicolor* recorded higher amount of vitamin A while *A. tenella* showed the least. Vitamin C content was predominantly high in all the species investigated carbohydrate and starch was found to be 3.60 g/100g and 3.24 g/100g in *Alternanthera sessilis* leaves.

2. REVIEW OF LITERATURE

Ability of each plant species to absorb pollutants can be known by examine the biochemical parameters of leaves (Taylor et al, 1973). Biochemical parameters that act as a key indicator in plants are used to evaluate the changes in the tolerance level of plants to air pollution.

Several studies have been conducted to analyse the nutritional and biochemical profile of *A. sessilis*. It has been found that the plant leaves have high carbohydrate and protein content (Nuñez-Estevez et al, 2021). Chemical profiling and evaluation of antioxidants potential of leaves and stem of *A. sessilis* showed total phenolic content ,total flavonoid concentration and antioxidant activity of various solvent extract obtained from the plant *Alternanthera sessilis* (Khan et al, 2018, Shreshtha et al, 2018).

Urbanization and industrialization processes contribute significant amount of various air pollutants such as SO₂, NO₂, CO, particulate matter, etc. These pollutants affect plant health and emit various forms of SO₂, NO_x, and O₃ which may act in combination of a variety of ways: additive, synergistic, and antagonistic. These pollutants can have a deleterious effect on a variety of biochemical and physiological processes and on the structural organization within the cells. Certain plant species are very sensitive to these pollutants resulting in well visible and measurable symptoms while others might adapt physiological and biochemical mechanisms to cope with the pollutants.

Several biochemical parameters can be analysed to understand a plant's response to pollution. The concentration of soluble sugars is indicative of the physiological activity of a plant which determines the sensitivity of plants toward air pollution. Reduction in soluble sugar content at polluted sites can be attributed to the increased rate of respiration and decreased CO₂ fixation because of chlorophyll deterioration (Wilkinson and Barnes 1973). Increased proline content and enzyme activity can be found in polluted site samples which in turn leads to increased total protein content in such plants (Singh et al, 1985, Sanaeirad et al, 2017).

Phenolics are diverse secondary metabolites (flavonoids, tannins, hydroxycinnamate esters and lignin) abundant in plant tissues (Polidoros and Scandalios 1999). antioxidative properties of polyphenols arise from their high reactivity as hydrogen or electron donors, from the ability of the polyphenol-derived radical to stabilize and delocalize the unpaired electron (chain-breaking function), and from their ability to chelate transition metal ions (termination of the Fenton reaction) (Ferreira et al. 2002).

3. OBJECTIVE OF THE STUDY

The objective of the current study is to examine the biochemical parameters of roadside *Alternanthera sessilis* plants in response to vehicular pollution. Studying the biochemical responses could provide a deep insight regarding the applicability of biochemical attributes that can be used as bioindicator to assess the level of air pollution. In this study three biochemical attributes are analysed:

- A. Total Protein**
- B. Total Carbohydrate**
- C. Total Phenol**

4. MATERIALS AND METHODS

4.1. Study Area & Plant Material

For the present study, we selected the Seaport-Airport Road (SPAP Road) which is a four-lane, 30 km (19 mi) highway from Cochin seaport to the Cochin International Airport developed to improve the transport infrastructure in the city of Kochi, India (under development). Our college is located in this highway and we selected the polluted site around 4km from the campus towards Kalamassery. This is a heavy traffic saturated highway that contribute to heavy vehicular pollution.

The control site is located in the Kandanthara village of Vazhakkulam Block, Perumbavoor in Ernakulam District of Kerala. It is a rural area with very less traffic and the site of collection of at least 50m away from any main roads.

Table 4.1: Site description

Control	Kandanthara village, Vazhakulam, Perumbavoor, Ernakulam	GPS Latitude: 10 ⁰² '1.308" N Longitude: 76 ⁰²⁰ '8.666" E
Polluted	Seaport-Airport Road, Thrikkakara, Kalamassery, Ernakulam	GPS Latitude: 10 ⁰⁵ '58.824" N Longitude: 76 ⁰²⁸ '3.01" E

Alternanthera sessilis plants were collected from both control and polluted site for the below experiments three times. The plants were collected with main shoot. Soil samples were also collected from the control and polluted site. Fresh leaves were used for all the experiments.



4.2. Sample Preparation

Plant leaves collected from control and polluted sites were shade dried thoroughly and ground into a fine powder. The samples were stored in air tight bottles and kept at room temperature. For each biochemical analysis 100mg of this powder is used in triplicates.

4.3. Estimation of total Protein by Lowry's method

Total protein in control and polluted site samples were estimated using Lowry's method (Sadashivam & Balasubramaniam, 1987). Briefly 100mg of each sample were homogenised in 100ml of distilled water (1mg/ml). the samples were centrifuged @1000rpm for 5min. From this 0.2ml was taken and made up to 1ml for the assay. To this 4.5 ml of Reagent I (48ml of 2% Na₂CO₃ in 0.1N NaOH + 2ml of 0.5% CuSO₄.5 H₂O) was added and incubated for 10 minutes. After incubation 0.5 ml of reagent II (1-part Folin-Phenol : 1 part water) was added and incubated for 30 minutes in dark. Measured the absorbance at 660 nm and plotted in standard graph to obtain the concentration. Standard graph was prepared using BSA as standard with a stock concentration of 1mg/ml.

4.4. Determination of total Carbohydrates by Anthrone reagent

Total carbohydrate in control and polluted site samples were estimated using Lowry's method (Sadashivam & Balasubramaniam, 1987). In short, carbohydrates in the samples were hydrolysed in 5ml of 2.5N HCl. After hydrolysis, the samples were neutralized with solid sodium carbonate until the effervescence ceases. Make the volume into 100 ml with distilled water and centrifuged @1000rpm for 5 min. from this 0.5ml was used for the assay, made up to 1ml, to 4ml of Anthrone reagent is added each test tube. After boiling for 8 minutes the assay tubes were cooled rapidly. After cooling, the absorbance was measured at 630 nm in a visible spectrophotometer (Systronics). Standard graph values for glucose were adapted from Bhardwaj & Dubey, 2019.

4.5. Estimation of total Phenol

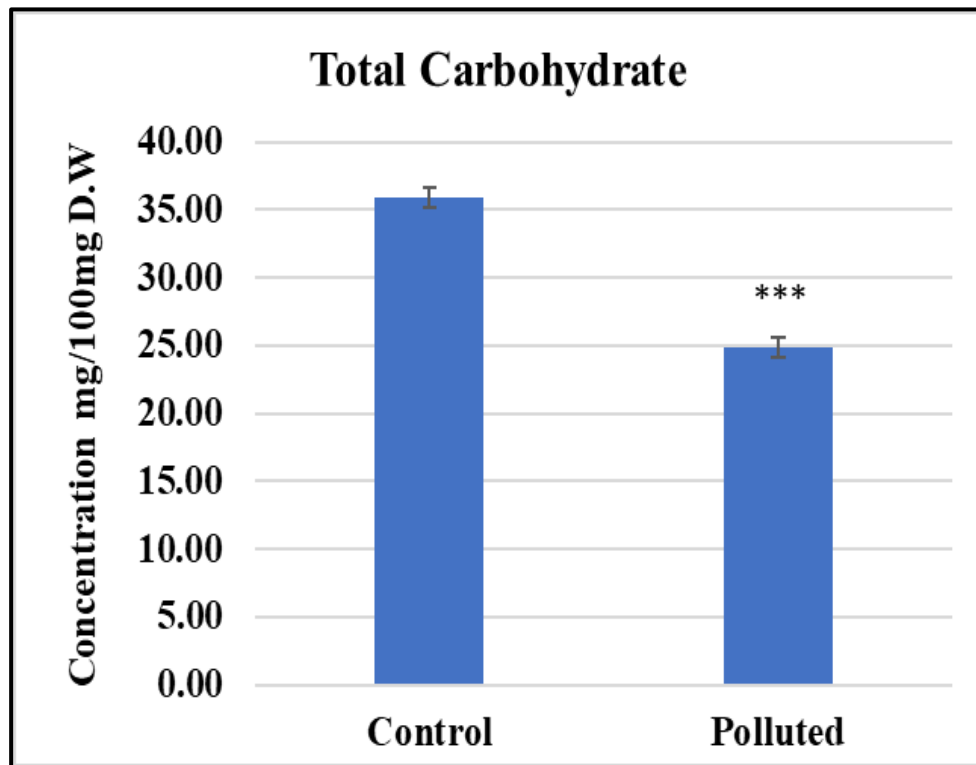
Total phenol in control and polluted site samples were estimated using Lowry's method (Sadashivam & Balasubramaniam, 1987). 100mg of sample was homogenised in 5ml 80% acetone and centrifuged. The supernatant was saved and reextracted with another 5ml of 80% acetone. The supernatant was evaporated to dryness in air and dissolved the sample residue in 5ml of distilled water. From this 0.2ml of sample solution was made up to 3ml and 0.5ml of phenol reagent is added. After 3 minutes 2ml of 20% sodium carbonate were added to each tube after thoroughly mixing the tubes were placed in a boiling water bath for 1 minutes and then cooled. The absorbance was measured at 650nm against reagent blank. Standard graph was prepared using catechol with a stock concentration of 1mg/ml.

4.6. Statistical Analysis

All experiments were performed in triplicates. The values were entered into MS-Excel for statistical analysis. Mean, standard deviation and standard error were calculated. Test of significance was performed using Student's t-test and p values were determined.

5. RESULTS

5.1. Total Carbohydrate



*Figure 5.1: Total carbohydrate quantified from control and polluted samples of *A. sessilis* leaves. Concentrations were calculated from standard graph and expressed in mg/100mg of D.W. Error bars represent standard error. Student's *t*-test was performed, *** - *p* value < 0.001.*

The total carbohydrate content in control and polluted leaf samples of *A. sessilis* was quantified as described in section 4.4. It has been observed that there is a significant reduction in the total carbohydrate of samples collected from polluted site in comparison with that of control site (Figure 5.1). The mean value of carbohydrate from polluted samples were 24.87 mg/100mg D.W while that of control plants were 35.95 mg/100mg D.W (Table 5.1)

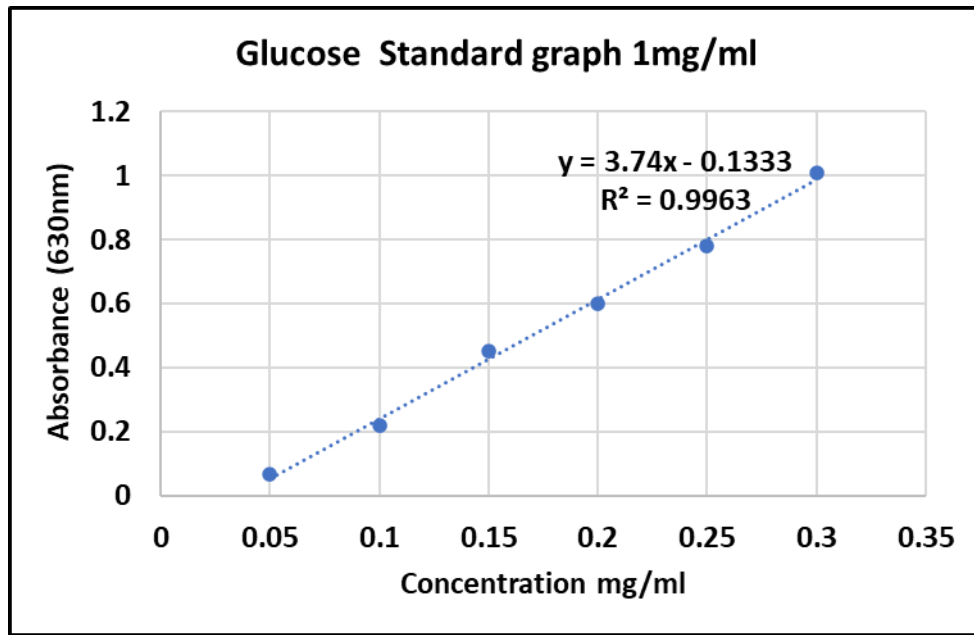
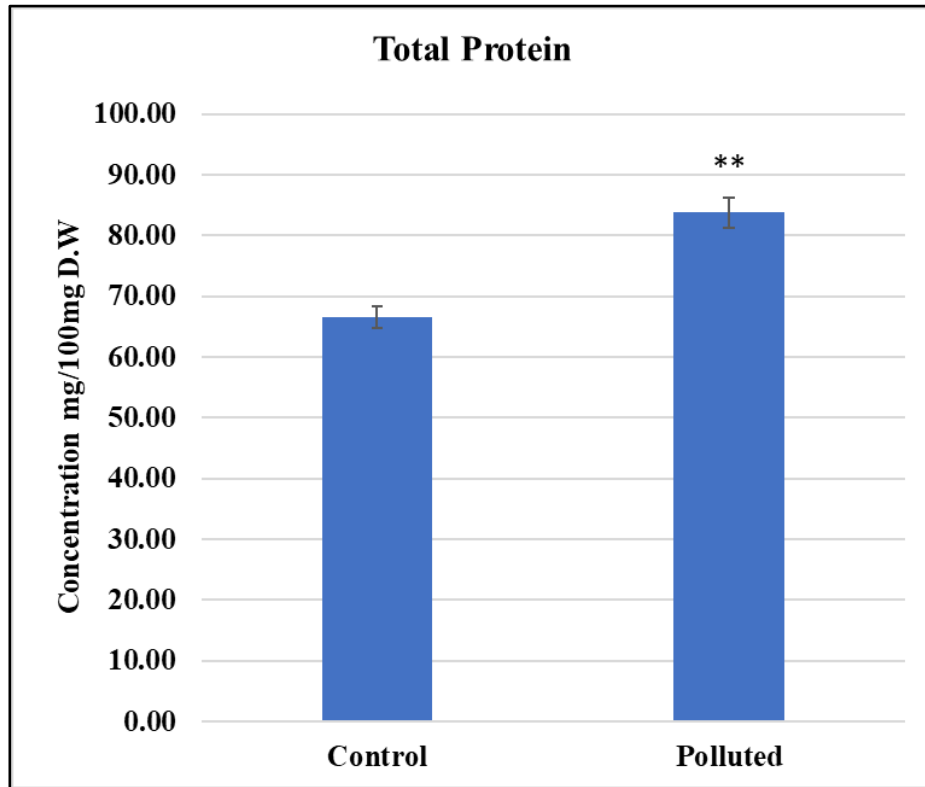


Figure 5.2: Standard graph of glucose for carbohydrate estimation

Parameters	Control	Polluted
Replicate 1	36.79	26.42
Replicate 2	34.49	24.33
Replicate 3	36.58	23.85
Mean	35.95	24.87
Standard Deviation	1.27	1.36
Standard Error	0.73	0.79
f-test		0.93
Student's t test		0.000501

Table 5.1: Statistical analysis of total carbohydrate content

5.2. Total Protein



*Figure 5.3: Total protein quantified from control and polluted samples of *A. sessilis* leaves. Concentrations were calculated from standard graph and expressed in mg/100mg of D.W. Error bars represent standard error. Student's t-test was performed, ** - p value < 0.01.*

Total protein content in polluted samples of *A. sessilis* leaves demonstrates a 20% increase when compared to that of control plants. The average protein content in control site leaves was observed to be 66.5 mg/100mg D.W and polluted site was 83.7 mg/100mg D.W. the increase in protein content could be due to the stress response in terms of increased enzyme activity or proline production in plants.

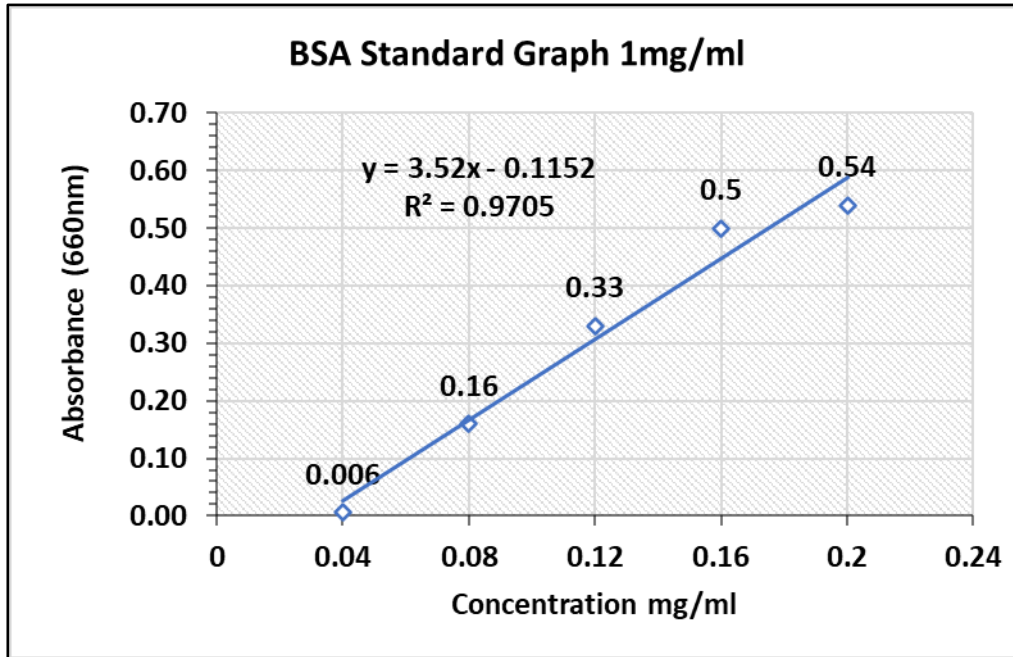
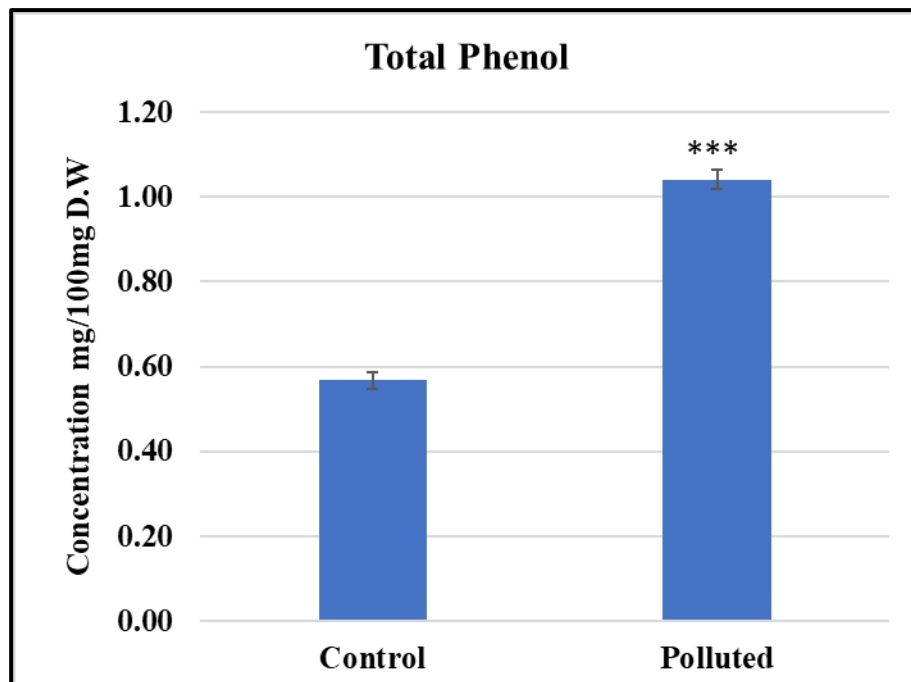


Figure 1.4: Standard graph of BSA for protein estimation

Parameters	Control	Polluted
Replicate 1	70	88.66
Replicate 2	64.66	81.28
Replicate 3	64.94	81.42
Mean	66.53	83.79
Standard Deviation	3.00	4.22
Standard Error	1.73	2.44
f-test		0.67
Student's t test		0.004493

Table 5.2: Statistical analysis of total protein content

5.3. Total Phenol



*Figure 5.5: Total phenol quantified from control and polluted samples of *A. sessilis* leaves. Concentrations were calculated from standard graph and expressed in mg/100mg of D.W. Error bars represent standard error. Student's *t*-test was performed, *** - *p* value < 0.001.*

Phenols are crucial secondary metabolites in plants that play important role in its physiology and defence mechanisms. The total phenol content significantly increased in polluted site leaf samples of *A. sessilis* compared to control site. The average phenol content in control sample was 0.57mg/100mg D.W while that of polluted sample was nearly doubled (1.04 mg/100mg D.W).

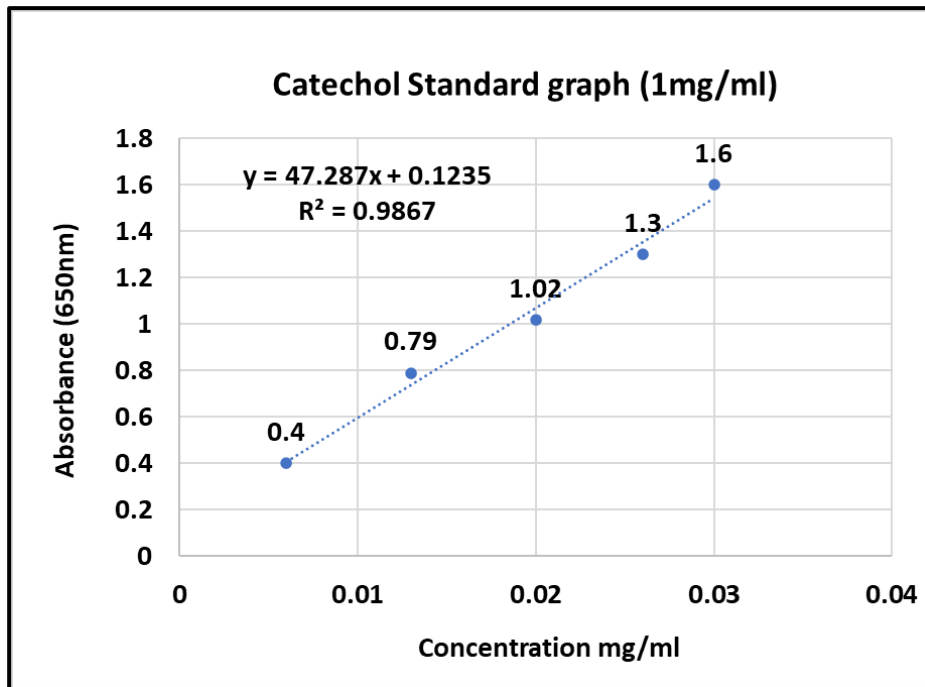


Figure 5.6: Standard graph of catechol for phenol estimation

Parameters	Control	Polluted
Replicate 1	0.55	1.06
Replicate 2	0.61	1.07
Replicate 3	0.54	1.00
Mean	0.57	1.04
Standard Deviation	0.03	0.04
Standard Error	0.02	0.02
f-test	-	0.86
Student's t test	-	8.99496E-05

Table 5.3: Statistical analysis to total phenol content.

6. DISCUSSION

Air pollution is one of the severe problems world is facing today. It deteriorates ecological condition and can be defined as the fluctuation in any atmospheric constituent from the value that would have existed without human activity (Tripathi and Gautam, 2007). In recent past, air pollutants, responsible for vegetation injury and crop yield losses, are causing increased concern (Joshi and Swami, 2007). Urban air pollution is a serious problem in both developing and developed countries (Li, 2003). The increasing number of industries and automobile vehicles are continuously adding toxic gases and other substances to the environment (Jahan and Iqbal, 1992). All combustion release gases and particles into the air. These can include sulphur and nitrogen oxides, carbon monoxide and soot particles, as well as smaller quantities of toxic metals, organic molecules and radioactive isotope (Agbaire and Esiefarienrhe, 2009).

Vegetation is an effective indicator of the overall impact of air pollution and the effect observed is a time-averaged result that is more reliable than the one obtained from direct determination of the pollutant in air over a short period. The species which adapts maximum fitness under adverse environmental conditions show maximum capacity to ameliorate air pollution. Plants adapt to such changes in their environment by balancing their morphological and biochemical variables such as leaf size, stomatal frequency, trichomes, ascorbic acid, protein, carbohydrates and flavonoids (Banerjee et al, 2022)

In the present study the aim was to investigate the effect of vehicular pollution in roadside *A. sessilis* plants. Similar to previous studies we observed several significant changes in the parameters studied. In case to total carbohydrates, the polluted site plants showed a significant reduction which in accordance with the previous results of similar studies. Reduction in carbohydrates could be attributed to the adverse effect of pollutants leading to less efficient light consumption by plants and deterioration of plant photosynthetic pigments (Woo et al, 2007., Tzvetkova & Kolarov 1996).

Increased protein content in polluted site samples were observed for *A. sessilis* when compared to control sites. When plants experience any environmental stress, they switch on different pathways in response to the stress and to mitigate its negative effects. This could involve an array of enzyme activities, especially the ones involved in antioxidant pathway.

Previous studies show contradictory results in case of protein content, some plants respond by decreasing the protein content while others didn't show any significant changes. In this study we observed a significant increase in protein content which could be a defensive adaptation by *A. sessilis* in response to vehicular pollution.

Phenols are an important class of secondary metabolites that are essential for plant growth and development, as well as for their defence against various environmental stresses. In polluted site *A. sessilis* leaves high phenol increase was observed compared to the control plants. This result is in accordance with previous studies on pollution effects and high phenol content is a plant's response to stress.

7. CONCLUSION & APPLICATIONS

The current study investigated the effect of vehicular pollution on the biochemical parameters of *A. sessilis* plants in roadside vegetation. The biochemical parameters analysed were total protein, total carbohydrate and total phenol in dried leaf samples. It has been previously shown that the biochemical parameters of plants are good indicators of pollution and provide a tool to understand the load of pollution at a particular region at a particular time (Tripathi & Goutam, 2007). From the present study we observed similar changes in the biochemical characters of *A. sessilis* from polluted site. The total carbohydrate content showed reduction on the other hand total protein was increased in the polluted sample. Total phenol was significantly increased in *A. sessilis* in response to pollution stress. These changes can be explained by the adverse impact of pollution on plant forcing the plant to adapt to changes by modulating its biochemical, physiological and morphological responses.

The main application of this study is to understand the use of roadside vegetation as indicators of environmental stress and changes in biodiversity in particular area over the course of time. Further studies are required to analyse the pathways behind this preliminary response we observed for instance which class of phenols get activated in response to vehicular pollution and how plants monitor this.

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