EFFECT OF ROADSIDE POLLUTION ON THE LEAF MORPHOLOGY OF *ALTERNANTHERA SESSILIS*

PROJECT REPORT SUBMITTED TO

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

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IN

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BY

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KERALA, INDIA.

APRIL 2023

CERTIFICATE

This is to certify that this project work entitled **"Effect of Roadside Pollution on the leaf morphology of *Alternanthera sessilis*."** is a bona-fide piece of project work done by Ms. ANJALA ASHRAF (Reg. no: 200021023420) 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.

Place: Thrikkakara

Date : 28-04-2023

Mrs. Kalyanikrishna
Supervising teacher
Department of Botany
Bharata Mata College

DECLARATION

I hereby declare that this project entitled **"Effect of Roadside Pollution on the Leaf Morphology of *Alternanthera Sessilis*."** 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.

ANJALA ASHRAF

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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.) Grisb: 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 sissoo 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, 13C NMR, MS and chemical transformations.

Alternanthera sessilis titanium dioxide nanoparticles (AS@TiO2NP'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.(Hundiwale et al,2012). The plant has been traditionally used in the treatment of jaundice along with other ailments. (Walter et. al, 2014, 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. (Walter et. al, 2014).13. the plant extract is demonstrated to have anti-cancerous properties as it mixes with the blood and removes the toxins from the body.(Zhou & Huang. 2010).

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 (Gupta, 2014). 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 leaf properties of roadside vegetation

Clean air is very precious to life and have to be conserved at any cost. Currently the condition is worsening with respect to air quality as most of the cities in India are gradually turning into metro cities and this adversely affect the health and livelihood of people and other organisms. Burning of fossil fuels, industrial emission, wild fires, microbial decaying process and agricultural activities worsen the air quality.

Air pollution has an adverse effect on plants in all levels, physiological, anatomical and morphological aspects. Leaf is the most sensitive part to be affected by air pollutants instead of all other plant parts such as stem and roots. The sensitivity is based on the fact that the major physiological processes in plants are concerned with leaf. Therefore, the leaf at its various stages of development, serves as a good indicator to air pollutants. Pollutants came from the auto emission can directly affect the plant by entering in to the leaf, destroying individual cells, and reducing the plant ability to produce food (Leghari & Zeidi, 2013).

Recently methyl isocyanate gas has also destroyed the vegetation at Bhopal in 2-3 km vicinity from the factory. Responses of Phaseous aureus to SO2 and HF(hydrogen fluoride) pollutants were carried by Sharma and Rao(1985). It is evident that SO_2 and HF hamper plant growth and suppress the yield in various ways. The seeds collected from the pollutants exposed plants were inferior to control ones. The effects of pollutants on leaves included chlorosis and necrosis, which caused reduction in photosynthetic leaf area. It is known that SO2 induced foliar injury; later developing into necrotic lesions hampers growth and decreases the net assimilation rate of plants (Katz, 1949, Thomas, 1951; Weinstein and Mc Cune, 1970).

In fumigation studies of NO2 on populations of 10 annual and perennial weeds of United States were tested to serve as indicators of plant injury caused by air pollutants. Two types of leaf markings developed: (1) a discoloration associated with cell collapsed, necrosis and (ii) a general overall waxy appearance of the leaf (Benedict and Breen, 1955).

Ammonia is present in the atmosphere of metropolitan areas generally escaped from refrigerators, precooling systems of cold storage rooms and anhydrous ammonia used as fertilizer or escaped during its manufacture (Treshow, 1970). Though ammonia is a minor air pollutant with respect to plant damage (Leone. 1979), yet it has caused necrotic and chlorotic interveinal streaking at considerable distances from an accidental release (Taylor, 1973).

In a field study, Dubey et al. (1984) noted reduction in leaf area of plants growing in polluted atmosphere. The maximum reduction was in Azadiracta indica followed by Mangifera indica while Clerodendrum indicum showed little change. Such reduction in leaf area and growth under stressed environmental conditions have been reported earlier (Mc Cune et al., 1967, Pawar et al.,1978).

Leaf surface temperature and leaf absorption in visible and infra – red regions were observed in Medicago sativa, Triticum aestivam and Zea mays which were exposed to different air pollutants. It has been noticed that fly ash exposed plants had high leaf surface temperatures and high light absorption, as compared to plants exposed in field and artificially to SO2 (Varshney and Garg, 1980).

The effects of vehicular pollution density on leaf anatomy of Cassia siamea, a road side plant, showed that plants growing around polluted sites showed leaf injury symptoms, reduction in thickness of leaf lamina, reduction in thickness of xylem cells and phloem cells and number of palisade and spongy cells per unit area (Pretti Bala, 2001). The effects of pollutants on leaves included chlorosis and necrosis, which caused reduction in photosynthetic leaf area. It is known that SO2 induced foliar injury; later developing into necrotic lesions hampers growth and decreases the net assimilation rate of plants (Katz, 1949, Thomas, 1951; Weinstein and Mc Cune, 1970).

Affected plants show some common effects such as decrease in chlorophyll content, inhabitation in photosynthesis and decreasing plant growth. Air pollutants cause both direct and indirect effects on the metabolism of roadside plants even before visible symptoms appear.

2. REVIEW OF LITERATURE

In 2019, KJ Mitu, MA Islam, P Biswas, S Marzia, MA Ali conducted a study to investigate anatomical changes in leaves and stems of roadsides plants namely Mango (*Mangifera indica*), SilKoroi (*Albizia procera*) and Mahagony (*Sweatenia mahogany*). For anatomical study, fresh leaves and stems collected from controlled and polluted sites. The result of this study revealed that leaf samples were collected from polluted site had reduced cell size however, leaves collected from controlled site had normal anatomy.

Due to the effect of pollution, the plants grow in roadside shows reduced leaf length and leaf width when compared to non-polluted plants (Madhumonisa A. S, Saradha M 2021). A quantitative analysis of leaf morphology parameters by Leghari & Zeidi, 2013 in 13 different plant species including trees, shrubs and climbers showed that there was significantly ($p<0.05$) slow growth in all the investigated parameters in all the plants species at polluted site with respect to non-polluted site, which might be due to severe air pollution in the city center.

Leaf characters in plants are greatly affected by air pollution. The plants growing in road sides, reduces leaf surface area and length of petiole. The leaf length, leaf width, leaf area and length of petiole were smaller in polluted areas compared to controlled area. (Assadi et al, 2011).

A study was conducted to examine the effect of pollution in the morphological characters of some plant leaves. Seven plant species from Baghdad was collected from polluted and less polluted area. Plant leaf morphological characteristics which included length, width and area were measured. (A. Wood and J. Roper, 2006) (S.K Pandey and H.A Singh,2011). The study result shows variation in the leaf length, width and area when comparing polluted region with less polluted region. It can be explained as the reduction of leaf length, area and width is as one of the plant adaptation mechanisms to survive under environmental stress and air pollution. (N. Efe and O. Ozbay, 2000)

Reduction of leaf area, length and width may lead to reduced contact between air pollutants and plant leaves and thus protect the plant from pollution.(A. Pourkhabbaz and N. Rastin, 2010). The absence of anatomical changes in roadside plants in control sites is because of the low concentration of pollutants in that area.(Giel wanowaska et al., 2005)

3. OBJECTIVE OF THE STUDY

The objective of the present study is to analyse and compare the leaf properties of *Alternanthera sessilis* plants from control and polluted sites. Quantitative leaf characters including leaf length, leaf width and leaf area will be investigated to understand the effect of vehicular pollution on roadside plants. Leaf morphology is an important parameter to assess the response of a plant to environmental stress and will help to explore the application of these parameters as adaptability indicators.

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.

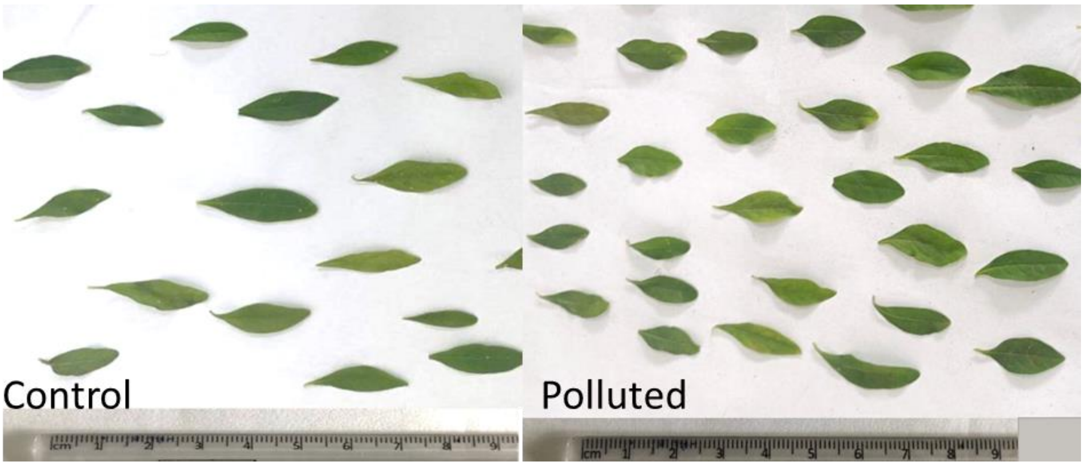
Table 4.1: *Site description*

Control	Kandanthara village, Vazhakulam, Perumbavoor, Ernakulam	GPS Latitude: 10^{0}2’1.308” N Longitude: 76^{0}20’8.666” E
Polluted	Seaport-Airport Road, Thrikkakara, Kalamassery, Ernakulam	GPS Latitude:10^{05}’58.824” N Longitude: 76^{0}28’3.01” 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

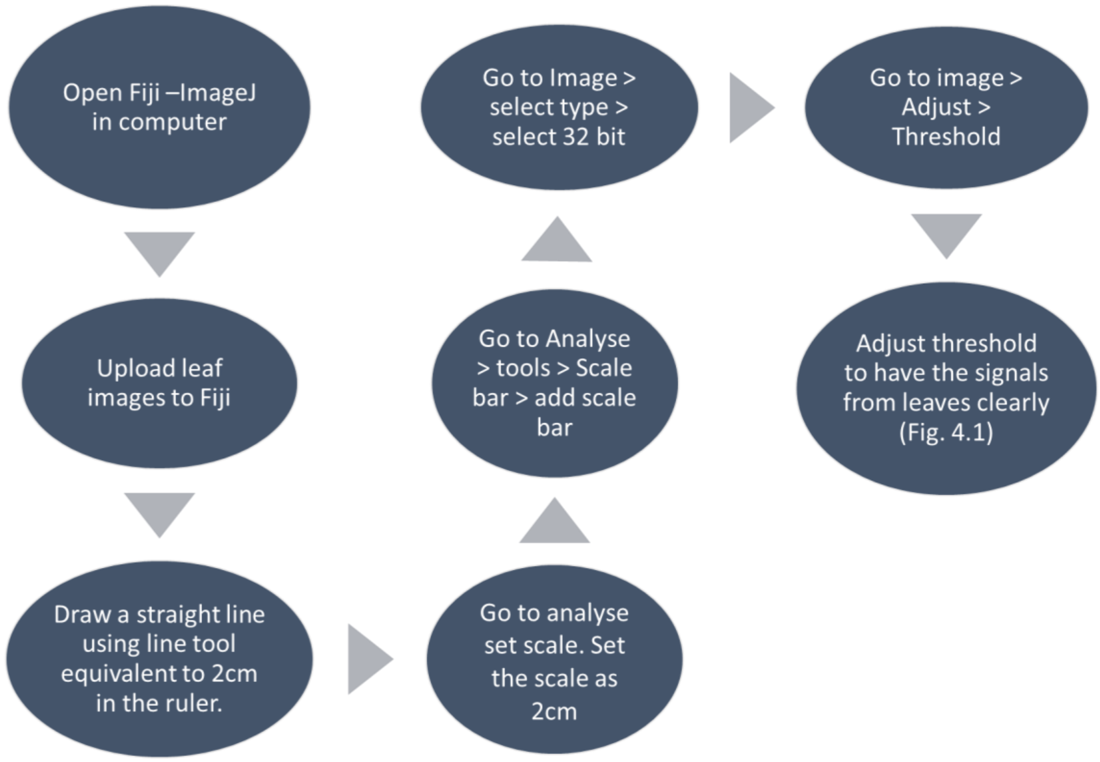
Mature leaves of similar age were taken from branched having almost same thickness from both control and polluted site samples of *A. sessilis*. Three replicates were performed , from each replicate 15 leaves were used for measurement. Highly damaged and necrotic leaves were excluded. Before taking photographs of the leaves, they were placed uniformly on a white paper and pressed with the help of heavy books to make sure all leaves had proper lamina and boundaries in the picture. A ruler was also placed along with leaves to adjust the scale while taking measurements.



4.3. Leaf morphology measurements using IMAGE-J software

For measuring the leaf morphology parameters, Fiji extension of Image-J software is used (Schindelin et.al, 2012). Fiji is a distribution of the popular open-source software ImageJ focused on biological-image analysis. In this study images of leaves taken were uploaded into Fiji-ImageJ and leaf morphology parameters like leaf length, leaf width and leaf area were measured as described below in the workflow:

Phase 1: Image thresholding



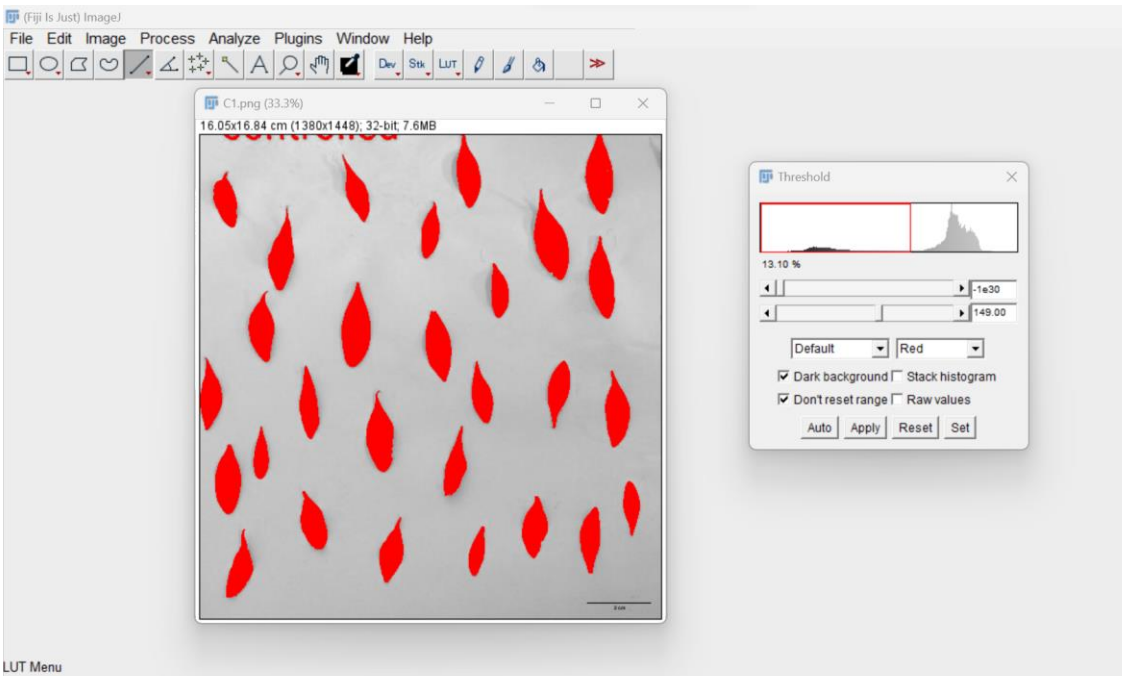
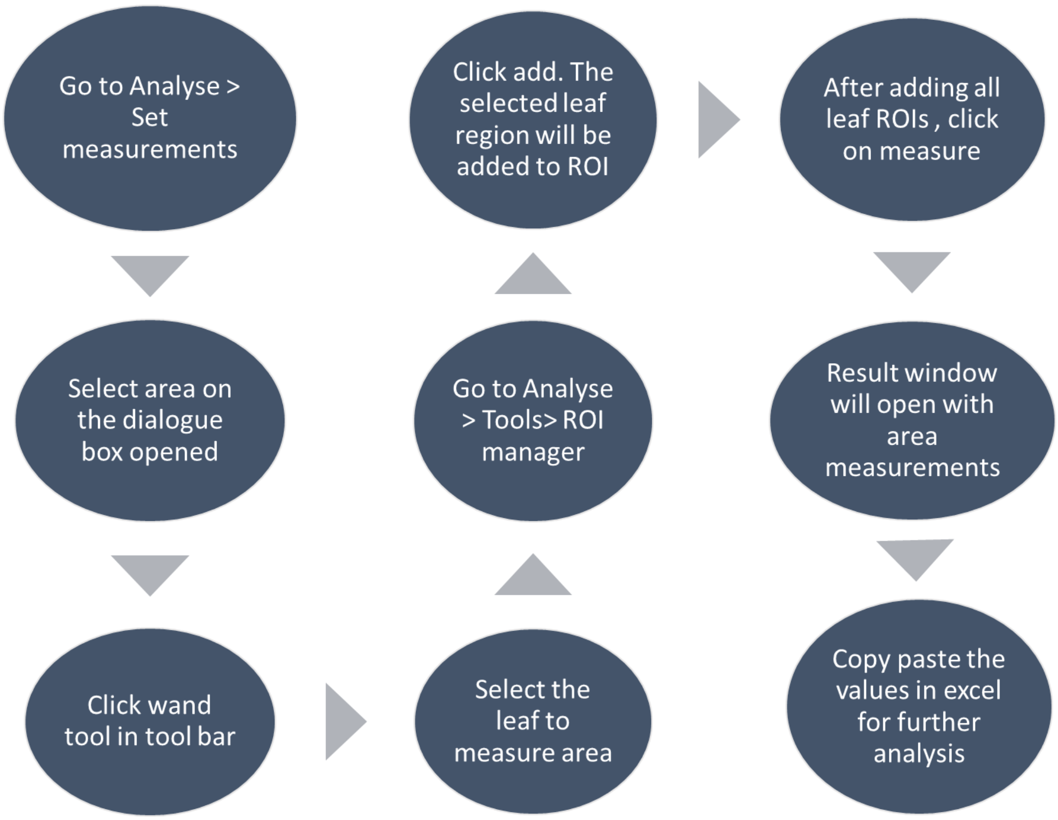


Figure 4.1: *Thresholding the leaf images using Fiji-ImageJ software.*

Phase 2: To Measure Leaf Area



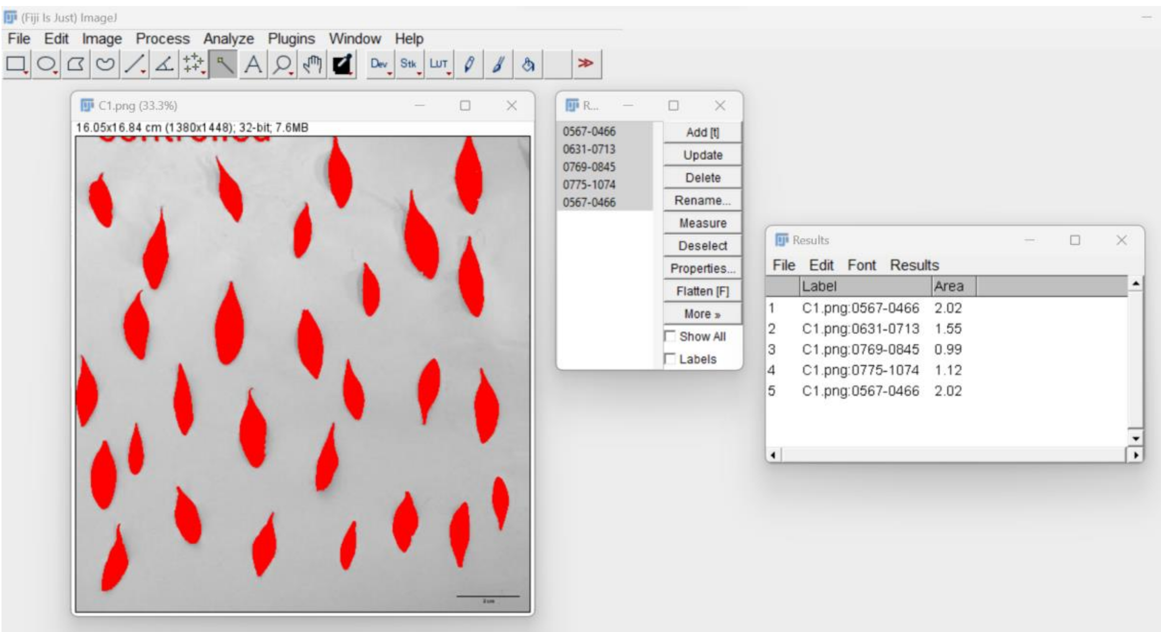
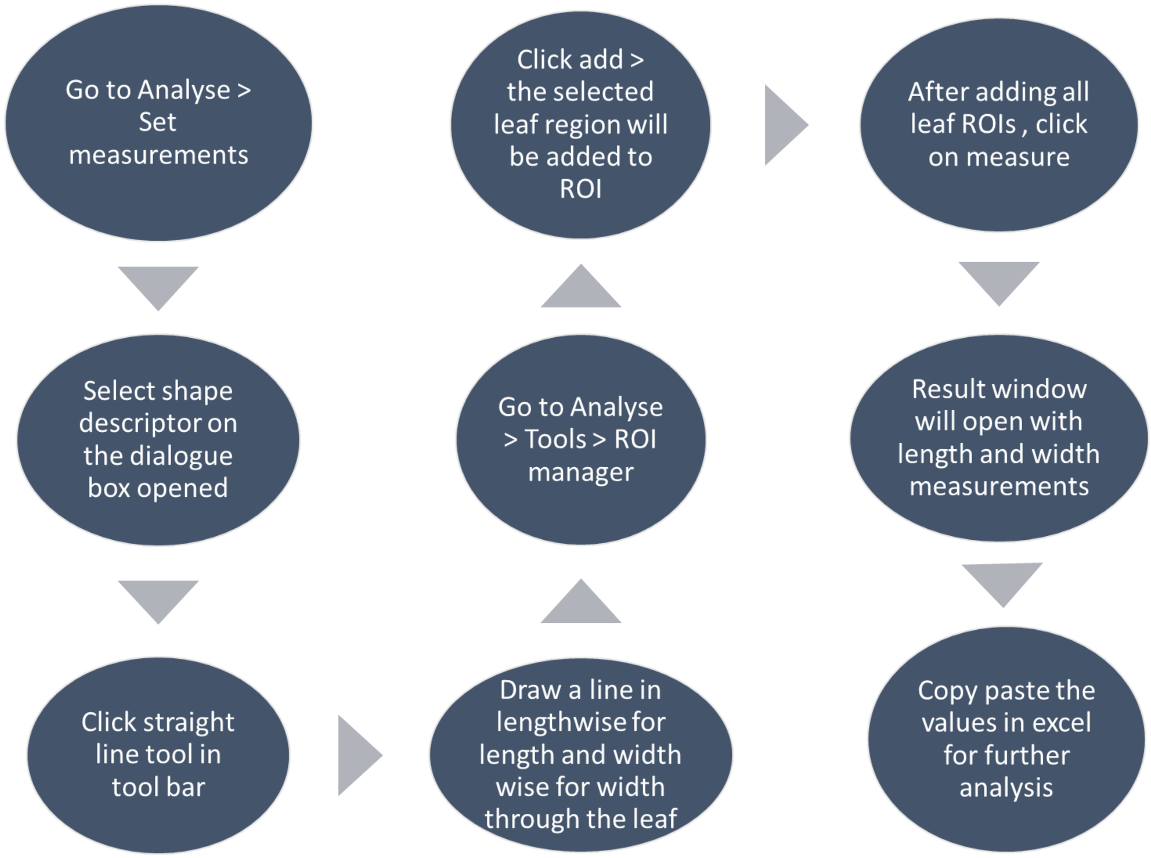


Figure 4.2: *Leaf area measurement steps.*

Phase 3: To Measure Leaf Length and Width



4.4. Statistical Analysis

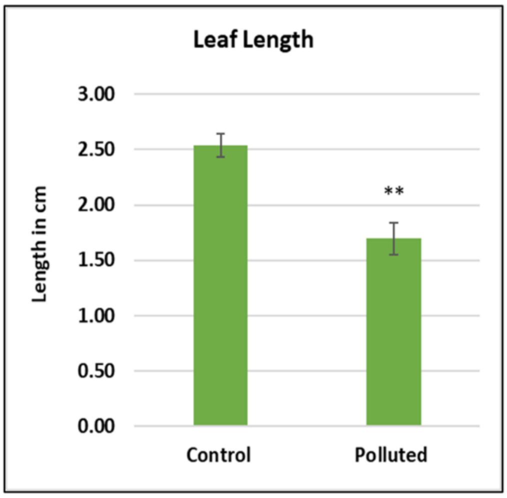
After taking all measurements in Fiji-ImageJ, the values were copied to MS-Excel. From each replicate 15 leaves were measured for both control and polluted samples. Mean, standard deviation and standard error were calculated. To test the significance, student's t-test is performed and p-values were determined.

5. RESULTS

The effect of roadside pollution on the morphology of the *Alternanthera* plants growing in polluted site is compared with the plants growing at the control site. Preliminary examination of the leaves from control and polluted site showed increased chlorosis and mechanical damage in leaves collected from polluted site. Nevertheless, in both sites *A. sessilis* showed extensive growth as an invasive weed.

5.1. LEAF LENGTH

A



B

Parameters	Control	Polluted
Replicate 1	1.84	1.32
Replicate 2	1.72	1.22
Replicate 3	1.83	1.19
Mean	1.80	1.24
Standard Deviation	0.06	0.07
Standard Error	0.08	0.09
f-test		0.93
Student's t test		0.000501

Figure 5.1: A- *Leaf length of A. sessilis collected from control and polluted sites. Error bars represent standard error. Student's t-test is performed, ** - p value <0.01.* ***B -*** *Statistical analysis using MS-Excel.*

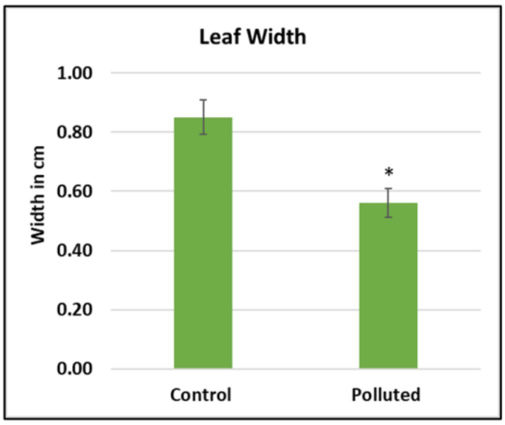
Leaf length of the leaves were measured in triplicates using ImageJ software (in 3 biological replicates, 15 leaves from each replicate, table 5.1). Control site plants have an average leaf length of 1.8cm while that of polluted site was 1.24 cm. Polluted site plants showed significant reduction in the leaf length compared to that of leaves collected from control site.

Leaf Length	Control			Polluted		
Sl. No:	**I**	**II**	**III**	**I**	**II**	**III**
1	3.09	2.08	2.31	2.35	1.44	1.43
2	3.05	2.46	2.27	2.39	2.11	1.54
3	2.61	2.91	2.21	1.94	1.38	1.81
4	2.81	2.25	3.07	1.77	1.45	1.42
5	2.7	2.67	2.44	1.62	1.84	1.46
6	2.74	2.14	2.45	2.28	1.76	1.19
7	2.66	2.92	2.37	1.74	1.61	1.25
8	2.64	2.46	2.7	1.95	2.21	1.26
9	3.02	2.27	2.95	1.85	2.17	1.12
10	2.72	2.56	2.65	1.69	1.46	1.16
11	2.59	2.12	2.29	1.46	1.72	1.35
12	2.81	1.82	2.18	1.89	1.84	1.59
13	2.35	2.3	2.23	1.76	1.88	1.64
14	2.83	2.58	2.16	1.82	2.13	1.79
15	2.55	2.82	2.37	1.71	1.88	1.19
Mean	**2.74**	**2.42**	**2.44**	**1.88**	**1.79**	**1.41**

Figure 5.1: *Leaf length values from three replicates of control and polluted samples.*

5.2. LEAF WIDTH

A



B

Parameters	Control	Polluted
Replicate 1	0.96	0.65
Replicate 2	0.78	0.55
Replicate 3	0.81	0.48
Mean	0.85	0.56
Standard Deviation	0.10	0.09
Standard Error	0.06	0.05
f-test		0.86
Student's t test		0.018487

Figure 5.2: ***A-*** *Leaf width of A. sessilis collected from control and polluted sites. Error bars represent standard error. Student's t-test is performed, * - p value <0.05.* ***B -*** *Statistical analysis using MS-Excel.*

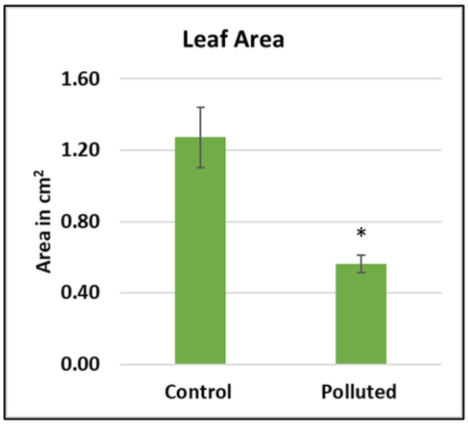
Width of the leaves were measured from the broadest part of the lamina. Leaf width was also measured in triplicates from control and polluted site plants. It was observed that the average leaf width of control site plants was 0.85cm while that of polluted site plants was 0.56cm. there was a significant decrease in the leaf width of the plants collected from polluted site.

Leaf Width (cm)	Control			Polluted		
Sl.No:	**I**	**II**	**III**	**I**	**II**	**III**
1	0.93	0.72	0.62	0.8	0.58	0.42
2	0.98	0.9	0.84	0.86	0.44	0.46
3	0.93	0.9	0.73	0.61	0.6	0.53
4	1.17	0.71	1.08	0.76	0.57	0.39
5	0.94	0.75	0.75	0.68	0.46	0.48
6	0.97	0.84	0.82	0.75	0.41	0.52
7	0.97	0.74	0.64	0.6	0.64	0.49
8	1.04	0.86	0.66	0.6	0.67	0.38
9	0.86	0.66	0.97	0.73	0.54	0.52
10	0.79	0.69	1.02	0.62	0.69	0.57
11	0.89	0.65	0.86	0.57	0.65	0.44
12	1.04	0.82	0.84	0.47	0.51	0.51
13	1.06	0.71	0.81	0.52	0.49	0.45
14	0.97	0.94	0.79	0.5	0.54	0.45
15	0.9	0.74	0.78	0.67	0.52	0.56
Mean	**0.96**	**0.78**	**0.81**	**0.65**	**0.55**	**0.48**

Figure 5.2: *Leaf width values from three replicates of control and polluted samples.*

5.3. LEAF AREA

A



B

Parameters	Control	Polluted
Replicate 1	1.61	0.65
Replicate 2	1.09	0.55
Replicate 3	1.12	0.48
Mean	1.27	0.56
Standard Deviation	0.29	0.09
Standard Error	0.17	0.05
f-test		0.16
Student's t test		0.015447

Figure 5.3:* *A- *Leaf area of A. sessilis collected from control and polluted sites. Error bars represent standard error. Student's t-test is performed, * - p value <0.05.* ***B -*** *Statistical analysis using MS-Excel.*

Leaf area from selected plants were measured using ImageJ software as explained in materials and methods. Leaf area is an important parameter that indicate the proper growth and development of a plant and directly related to its biomass production. In this study, the polluted site plants showed a significant reduction in leaf area when compared to control site plants. The control site plants have a leaf area of 1.27cm^2 while that of polluted site plants reduced to nearly half (0.56 cm^2).

Leaf Area (cm²)	Control			Polluted		
Sl.No:	**I**	**II**	**III**	**I**	**II**	**III**
1	1.83	0.81	0.63	1.03	0.5	0.36
2	1.41	1.54	1.18	1.22	0.86	0.43
3	1.56	1.16	1.4	0.82	0.46	0.46
4	2.23	1.12	1.48	0.8	0.57	0.49
5	1.62	0.88	1	0.65	0.59	0.4
6	1.55	1.35	1.15	1.06	0.78	0.4
7	1.38	0.92	0.98	0.67	0.55	0.55
8	1.93	1.19	1.87	0.72	0.92	0.6
9	1.38	0.88	0.92	0.88	0.75	0.56
10	1.19	0.76	1.17	0.63	1	0.49
11	1.43	0.63	1.31	0.57	0.51	0.5
12	1.98	1.18	0.69	0.61	0.68	0.35
13	1.37	1.4	0.68	0.53	0.67	0.41
14	1.72	1.48	0.93	0.58	0.95	0.5
15	1.54	1	1.42	0.48	0.58	0.45
Mean	**1.61**	**1.09**	**1.12**	**0.75**	**0.69**	**0.46**

Figure 5.3: *Leaf area values from three replicates of control and polluted samples.*

6. DISCUSSION

Plants are exposed to many external stress factors. The intensity of the stress may vary according to the environment in which they live. Leaves are the first part of the plant body that exhibit the symptoms of these stresses including air pollution (S. Lata and M.Poonam, 2010)

Pollution can affect the morphology and structure of plants. It can also affect the plant growth and development through a variety of mechanisms, including the deposition of toxic substances on the plant's leaves. In the case of *Alternanthera sessilis*, pollution can lead to changes in leaf morphology, including alterations in leaf shape, size, area, and colour.

In 2019,Abdul -Hameed M.J, Israa and Abdul -Rahman of University of Technology Baghdad, Iraq conducted a study in which the result showed reduction in the plant leaf characteristics like leaf area, width and length in polluted samples. They observed that it may be a survival mechanism of plant under severe stress and pollution.

Studies have shown that exposure to vehicular pollution can reduce leaf lengths in plants. When pollutants are absorbed by leaves, they can block sunlight and reduce the amount of energy available for photosynthesis, which can lead to reduced leaf growth (Lobo A.P, Tandon, Rao and Mishra, 2015).

In the present study, we observed that the leaf length of plants in control region have an average of leaf length 1.8 cm while that of polluted region was 1.24 (Figure 5.1). This result shows that the leaf length of the plant sample taken from polluted site shows reduction in growth.

Previous studies have shown that vehicular pollution can also lead to reduction of leaf width. For example, a study was conducted in Delhi, India, and it is found that leaves of plants growing near busy roads were significantly smaller and narrower than those of plants growing in controlled areas. (Kumar, P & Mor, 2015)

The current study also demonstrates a significant decrease in the leaf width of the plants collected from polluted site. Here, the leaf width of control site plants was 0.85cm while that of polluted site plants was 0.56 cm. (Figure3)

In the case of leaf area, previous studies stated that pollution which includes particulate matter, nitrogen dioxide, sulphur dioxide and other harmful pollutants can lead to reduction in leaf area of the *Alternanthera* plants. This is because these pollutants can block the stomata, and also interfere with photosynthesis. This results in a decrease in the plants growth rate.(Sarkar, S & Patra, 2018)

Leaf area is an important parameter that shows the proper growth and development of a plant. According to this study, the mean value of leaf area of the investigated sites was 0.56 cm^2 (polluted) and 1.27 cm^2 (control). This shows a significant reduction in the leaf area of *A. sessilis* plant from polluted sites.

The pollutants and dust particles stick on the young and matured leaves. Due to the sticking of dust particles in the young leaves will affect the further growth of leaves. The overall result indicates that the morphological parameters like length, area and width of leaves are comparatively diminished in polluted area. This could be due to the adaptation of plant by using the reduction as a survival mechanism. By reducing the leaf size, plants can reduce the quantity of polluted particles absorbed by the leaves and it can grow by adapting to it. Even though the environment is polluted, the leaf overcome this stress by reducing the leaf length. In control site plants the leaf size is usually large and it can grow normally due to reduced exposure to pollutants and also due to good atmosphere and temperature.

7. CONCLUSION & APPLICATIONS

This study demonstrated that the plants grown in polluted area are exposed to many external stress factors. Pollution affects the morphology and structure of *Alternanthera sessilis* plants in the roadside vegetation. The effect of pollution is displayed as reduced leaf size , shape, area and colour of the plant. Similar to obtained results, previous studies have shown that plants exposure to vehicular pollution have reduced leaf length. The plants grown in the polluted area have smaller leaves than the plants grown in control area. In polluted area, plants might be using reduction as a survive mechanism , by reducing the leaf size , plants can reduce the quantity of polluted particles absorbed by the leaves and it can grow by adapting it . If the environment is polluted the leaf overcome the stress by reducing the overall leaf size.

However, despite of these changes, *A. sessilis* plants were survived well at the polluted environment of Seaport Airport Road. This indicates the importance of morphological data for careful analysis of pollution tolerance limits and to determine the sensitivity of different plant species to the action of air pollutants. Combined with structural analysis these parameters could be applied to analyse the impact of air quality on vegetation in the urban areas.

8. REFERENCES

- A.H.M.J. Al-Obaidy, I.M. Jasim, & A.R.A. Al-kubaisi. (2019). Air Pollution Effects in Some Plant Leave Morphological and Anatomical Characteristics within Baghdad City, Iraq. Engineering and Technology Journal, 37(C)(1), 84-89.
- Aasawari A. Tak, Umesh B. Kakde. (2017)Assessment of air pollution tolerance index of plants:a comparative study. Department of Botany, The Institute of Science, 15, Madame Cama Road, Fort, Mumbai 400032, Maharashtra.
- Akbar, Syed & Senavarayan, Narthanaa. (2020). Genetic diversity of Amaranthaceae family of Ashokapuram panchayat, Coimbatore, Tamilnadu.
- Assadi, A., Ghasemi, A., Pirbalouti, A.G., Malekpoor, F., Teimori, N., & Assadi, L. (2011). Impact of air pollution on physiological and morphological characteristics of Eucalyptus camaldulensis Den. Journal of Food, Agriculture & Environment: 9(2), 676-679.
- Benedict, H.M., & Breen, W.H. (1955). The use of weeds as a means of evaluating vegetation damage caused by air pollution. Proc. Third Natl. Air Pollut. Symp. 177-190, U.S.A.
- Chandrashekhar, K. (2019). Ethnobotanical and Phyto-Pharmacological Overview of Matsyakshi (Alternanthera Sessilis R. Br. Ex DC.). J. Ayu. Her. Med. 5 (4), 152–155.
- Chandrika UG, Svanberg U, Jansz ER. (2006). In vitro accessibility of β -carotene from cooked Srilankan green leafy vegetables and their estimated contribution to vitamin A requirement. J Sci Food Agric; 86(1): 54-61
- Chatterjee I, Chakravarty AK, Gomesa A. (2006). Current and future status of herbal medicines. Br J Ethnopharmacol.;106(1):38–43. Doi: 10.1016/j.jep.2005.11.031.
- Efe, N., & Ozbay, O. (2000). Effect of air pollutant sulphurdioxide on the morphology and anatomy of some plants (Maize and soybeans). Doga Turk Botanik Dergisi, 17(4), 207-214.
- Hundiwale Jogendra C. et al. . (2012) A current update on phytopharmacology of the genus Alternanthera. J. Pharm. Res
- Hwong, C. S., Leong, K. H., Abdul Aziz, A., Mat Junit, S., Mohd Noor, S., & Kong, K. W. (2022). *Alternanthera sessilis*: Uncovering the nutritional and medicinal values of an edible weed. *Journal of Ethnopharmacology*
- Katz, M. (1949). SO2 in the atmosphere and its relation to plant life. Ind. Eng. Chem., 42, 2450-2465.

- Kumar, P., & Mor, S. (2015). Impact of vehicular pollution on leaf morphological traits of tree species planted along highways in Delhi. Pollution Research, 34(4), 679-684.
- Leone, I.A. (1976). Indian J. Air poll. cont., 2, 1-5.
- Lobo, A.P., Tandon, H.L., Rao, D.N., & Mishra, S. (2015). Effect of air pollution on leaf characteristics of urban trees in Delhi. Environmental monitoring and assessment, 187(4), 1-9.
- Nazreen, Salma & S, AnilKumar & V, Viji & S, AnilkumarV. (2018). Foliar features of Alternanthera (Forssk.) species of southern western ghats of Kerala. International Journal of Advanced Research. 6. 144-151. 10.21474/IJAR01/7346.
- Othman, Azizah et al. "Comparative Evaluation of Nutritional Compositions, Antioxidant Capacities, and Phenolic Compounds of Red and Green Sessile Joyweed (Alternanthera Sessilis)." Journal of Functional Foods 21 (2016): 263–271
- Petruzzello, Melissa. "List of plants in the family Amaranthaceae". *Encyclopedia Britannica*, 20 Jan. 2022, https://www.britannica.com/topic/list-of-plants-in-the-family-Amaranthaceae-2042049.
- S.K. Pandey, & H.A. Singh. (2011). Simple, cost-effective method for leaf area estimation. J. Bot., 1(2), 1-6.
- Sarkar, S., & Patra, A.K. (2018). Impact of vehicular pollution on leaf epidermal characteristics of Alternanthera philoxeroides (Mart.) Griseb. International Journal of Environmental Sciences & Natural Resources, 8(5), 1-6.
- Sasidharan N (2004). Biodiversity documentation for Kerala Part 6: Flowering plants, Kerala Forest Research Institute Handbook No. 17. KFRI, Peechi, Trichur, Kerala, India.
- Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., … Cardona, A. (2012). Fiji: an open-source platform for biological-image analysis. Nature Methods, 9(7), 676–682.
- Shehzad, Aamir & Qayyum, Abdul & Rehman, Rafia & Nadeem, Farwa & Shehzad, Muhammad. (2018). A Review of Bioactivity Guided Medicinal Uses and Therapeutic Potentials of Noxious Weed (Alternanthera sessilis). 95-103.

- Taylor, O.C. (1973). In J.A. Naegele (Ed.) Air pollution damage to the vegetation, Advances in chemistry series. 122. Washington, D.C., American Chemical Society, pp. 9-20.
- Treshow, M. (1970). Environment and Plant Response. McGraw Hill, New York.
- Wood, A., & Roper, J. (2000). A simple and nondestructive technique for measuring plant growth and development. Amer. Bio. Teach., 62(3), 15-17.