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Anatomical, Histochemical, and Physiological Analysis of Abutilon Indicum (L.) Sweet, Cassia Auriculata L. and Morinda Tinctoria Roxb. Collected from Polluted and Non-Polluted Habitat

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Abstract

Air pollution has become a major environmental problem facing the world today due rapid increase in industrialization & anthropogenic activity. Vast plant species are facing threats due to specific single pollutants or mixtures of pollutants. The present study analyzed the anatomical, histochemical and physiological parameters in polluted and non-polluted environmental plants such as Abutilon indica, Cassia auriculata, and Morinda tinctoria. The results of anatomical research in polluted plants revealed an increase in the layers of epidermis, hypodermis, cortex, and endodermis compared to non-polluted plants. The total chlorophyll content (sample 1-6) of the leaf in polluted plants was found to be lower (0.414±0.0) when compared to the non-polluted plant. The relative water content was high (0.823±0.0) in non-polluted plants. The highest PH value was recorded in Cassia auriculata (7.2± 0.20) growing in non-polluted habitat, and the lowest PH values were observed in polluted area plants in the range of 5-7.

Key words

Air pollution, Anatomy, Abutilon indica, Cassia auriculata, Morinda tinctoria

Introduction

Environmental pollution is the addition of any substance (solid, liquid, or gas) or any form of energy such as heat, sound, or radioactivity pollution, light to the environment at a rate faster than it can be dispersed, diluted, decomposed, recycle or stored in some harmless form. The pollution may be due to human activities or the natural ecosystem (Arnon, 1949). Modern society is also concerned about specific types of pollutants, such as noise, light, and plastic pollution. Environmental pollution affects the quality of the pedosphere, hydrosphere, atmosphere, lithosphere, and biosphere. Pollution reaches its most severe proportions in the densely settled urban-industrial centers of the more developed countries. The primary pollutants in the air are lead, ground-level ozone, heavy metals, sulphur dioxide, benzene, carbon monoxide, and nitrogen dioxide (Khan *et al.,* 2009).

***Cassia auriculata* L.,** a legume plant commonly known by its local name, matura tea tree, unaware or avaram, belongs to the family Leguminosae, sub-family Caesalpinioideae. The plant is a bushy shrub or small tree; leaves are the oblong-elliptic, mucronate apex, and flowers are corymbose racemes, yellow; fruit is pods.

***Abutilon indicum* (L.)Sweet.** is commonly known as Indian abutilon, Indian mallow, or Thuthi. It is a small shrub belonging to the Malvaceae, native to tropical and subtropical regions and sometimes cultivated as ornaments. It is a small shrub with alternatively arranged leaves and a long petiole with orange-yellow flowers. Fruit schizocarps.

***Morinda tinctoria* Roxb.,** commonly known as Indian mulberry, is a flowering plant in the family Rubiaceae, native to southern Asia. It is an evergreen shrub or small tree growing to 5–10 m tall. The leaves are 15–25 cm long, oblong to lanceolate. The flowers are tubular, white, and scented, about 2 cm long. The fruit is a green syncarp, 2-2.5 cm in diameter. The present study was conducted to analyze the impact of air pollution on the *Cassia auriculata*, *Abutilon indicum*, and *Morinda tinctoria* by observing and recording the difference in anatomical structures and physiological characters and localizing the phytochemicals.

MATERIALS AND METHODS

Study area

Coimbatore is the second largest city in Tamil Nadu, situated in the state's western corner. The city has a pollution index of 66.2, according to the latest reports. The plants from the air-polluted and non-polluted environments were collected from Keeranatham and Sitra, Coimbatore, Tamil Nadu, and identified by the Department of Botany, PSG College of Arts & Science, Coimbatore and a voucher specimen was submitted to the same. The samples collected from the non-polluted area were named sample1, 3 & 5, and samples from a polluted area were called 2, 4 & 6.

Anatomical study

Thin sections of stem and leaf samples 1-6 were taken and observed under a light microscope to measure the thickness of different areas such as the epidermis, hypodermis, cortex, endodermis, pericycle, vascular bundle, etc. The number of cells in each part of the samples was counted.

Histochemical study

The thin section of stem samples 1-6 were subjected to histochemical analysis. The unit was stained with various chemicals according to the standard procedure (Bashan et al., 2004) for localizing the multiple phytochemicals in different regions, and the section was viewed under a light microscope.

Table 1: Histochemical analysis of stem in Sample 1 and Sample 2

S.no	Ergastic content	Reaction		Localization	
		Sample 1	Sample 2	Sample 1	Sample 2
1	Alkaloid	+	+	Cortex, vascular bundle, pith	Cortex, vascular bundle, pith
2	Starch	+	+	Hypodermis, pith	Hypodermis, Cortex, vascular bundle, pith
3	Sugar	+	+	Cortex, pith	Cortex, pith

'+' - indicates the positive reaction

Table 2: Histochemical analysis of stem in Sample 3 and Sample 4

S.no	Ergastic content	Reaction		Localization	
		Sample 3	Sample 4	Sample 3	Sample 4
1	Alkaloid	+	+	Hypodermis, cortex, vascular bundle, pith	Hypodermis, cortex, vascular bundle, pith
2	Starch	+	+	The epidermis, vascular bundle, pith	The epidermis, vascular bundle, pith
3	Sugar	+	+	Hypodermis, cortex, pith	Hypodermis, cortex, pith

'+' - indicates the positive reaction

Table 3: Histochemical test of the stem in sample 5 and sample 6

S.no	Ergastic content	Reaction		Localization	
		Sample 5	Sample 6	Sample 5	Sample 6
1	Alkaloid	+	+	Hypodermis, cortex, vascular bundle, pith	Hypodermis, cortex, vascular bundle, pith
2	Starch	+	+	The epidermis, vascular bundle, pith	The epidermis, vascular bundle, pith
3	Sugar	+	+	Hypodermis, cortex, pith	Hypodermis, cortex, pith

'+' - indicates the positive reaction

PHYSIOLOGICAL ANALYSIS

Leaf extraction of pH - Leaf extract pH was determined by the method of Béthoux et al. (2002).

Chlorophyll determination - The Arnon method determined total chlorophyll content Arnon (1949).

Protein fractionation – Protein fractions were estimated by Lowry's method.

Total soluble sugar estimation - The total soluble sugar content of fresh leaf tissue was estimated by the Anthrone method (Tripathi & Gautam, 2007).

Amino acid estimation - The fresh leaves of samples (0.5 g) were blended using 80% ethanol, 0.1 ml of the samples were taken individually in test tubes, and 1 ml of ninhydrin was added. The tubes were heated in a boiling water bath for 20 minutes. The intensity of the purple color against a reagent blank (0.1 ml of 80% ethanol) in a result was compared with the control spectrophotometer at 570 nm.

Ascorbic acid estimation - Ascorbic acid content of the leaf sample was estimated by the method described by Gill & Tuteja (2010).

Carotenoid estimation - Carotenoid content was estimated by the method described by Arnon (1949).

Estimation of relative water content - Leaf relative water content (RWC) was determined by the method by Henson *et al.* (1981).

Identification of air pollution tolerance index (APTI)

APTI of the plants was calculated by incorporating leaf extract pH, total chlorophyll, ascorbic acid content, and relative water content into the following mathematical expression as described by Singh *et al.* (1991).

Table 4: Leaf extracts pH of the sample

S.no	Leaf Sample	pH
1	Sample 1	7.2± 0.20
2	Sample 2	5.6 ± 0.15
3	Sample 3	6.0 ± 0.11
4	Sample 4	4.7 ± 0.05
5	Sample 5	7.2 ± 0.11
6	Sample 6	6.5 ± 0.10

Values are expressed as mean ± standard deviation of the observation

Table 5: Total chlorophyll content

S.no	Sample	Chl a	Chl b	Total chlorophyll
1	Sample 1	0.1±0.0	0.3±0.0	0.414±0.0
2	Sample 2	0.3±0.0	0.6±0.0	0.712±0.0
3	Sample 3	0.4±0.0	0.2±0.0	0.607±0.0
4	Sample 4	0.3±0.0	0.5±0.0	0.823±0.0
5	Sample 5	0.2±0.0	0.3±0.0	0.425±0.1
6	Sample 6	0.3±0.0	0.2±0.0	0.510±0.0

Values are expressed as mean ± standard deviation of observation.

Table 6: Protein Estimation of plants

S.no	Protein content	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
1	Prolamine	0.4 ± 0.1	0.5±0.3	0.2±0.0	0.3±0.1	0.4 ± 0.1	0.5 ± 0.2
2	Glutaline	0.4 ± 0.2	0.6±0.2	0.5±0.2	0.4±0.1	0.5 ± 0.0	0.5 ± 0.1
3	Albumin	0.7± 0.0	0.5±0.1	0.3±0.2	0.3±0.0	0.6 ± 0.2	0.5 ± 0.1
4	Globulin	0.5 ±0.1	0.2±0.1	0.3±0.1	0.2±0.0	0.5± 0.2	0.4 ± 0.1

Values are expressed as mean ± standard deviation of the observation

Table 7: Total sugar estimation of the sample

S.no	Sample	Total sugar content
1	Sample 1	3.1± 0.0
2	Sample 2	2.0±0.0
3	Sample 3	3.8±0.0
4	Sample 4	4.5±0.0
5	Sample 5	6.0±0.0
6	Sample 6	5.2±0.0

Values are expressed as mean ± standard deviation of the observation

Table 8: Total amino acid content

S.no	Samples	The total amino acid content
1	Sample 1	2.27±0.01
2	Sample 2	3.04±0.02
3	Sample 3	2.42±0.02
4	Sample 4	4.01±0.01
5	Sample 5	2.45±0.01
6	Sample 6	3.25±0.02

Values are expressed as mean ± standard deviation of the observation

Table 9: Ascorbic acid content in samples 1-6.

S. no	Sample	Ascorbic acid content
1	Sample 1	2.02±0.02
2	Sample 2	1.78±0.17
3	Sample 3	3.01±0.04
4	Sample 4	2.06±0.09
5	Sample 5	3.78±0.12
6	Sample 6	2.57±0.06

Values are expressed as mean ± standard deviation of the observation

Table 10: Carotenoid estimation of the sample

S. no	Habit	Total carotenoid content
1	Sample 1	0.4 ± 0.0
2	Sample 2	0.1 ± 0.0
3	Sample 3	0.9 ± 0.0
4	Sample 4	0.2 ± 0.0
5	Sample 5	0.6 ± 0.0
6	Sample 6	0.19 ± 0.0

Values are expressed as mean ± standard deviation of the observation

Table 11: Relative water content of the sample

S.no	Habit	Relative water content
1	Sample 1	82.5±0.05
2	Sample 2	76.4±0.08
3	Sample 3	86.2±0.02
4	Sample 4	80.3±0.13
5	Sample 5	78.9±0.03
6	Sample 6	72.8±0.05

Values are expressed as mean ± standard deviation of the observation

Table 12: Air pollution tolerance index (APTI) of the sample

S.no	Habit	APTI content
1	Sample 1	17.28± 0.0
2	Sample 2	13.50±0.1
3	Sample 3	16.44±0.0
4	Sample 4	14.41±0.0
5	Sample 5	18.69±0.2
6	Sample 6	15.09±0.0

Values are expressed as mean ± standard deviation of observation.

RESULTS AND DISCUSSION

Anatomical study

The anatomical studies in the investigated samples (1-6) showed significant changes in the number and shape of the cells. For example, there was a compression of parenchymatous cells in the pith region. Hence, the differences mentioned above in the present study could impact photosynthesis, which may alter the functions of the plants. Leaf anatomy of *Thevetia*

peruviana also showed a reduction in the mesophyll, palisade parenchyma, and upper and lower epidermis in the polluted area as compared to leaves collected from the non-polluted site. Changes in the shape and structure of thin-walled mesophyll cells have been widely reported. In addition, the palisade parenchyma cells become flattened due to continuous exposure to pollutants. The unifying characteristic of all parenchymatous cells is that they are capable of cell division and play an important role in photosynthesis, storage, transport, regeneration, and wound healing Singh & Verma (2007).

Histochemical study

Histochemical studies in the present study revealed a positive response for alkaloids in the stem of samples 1-6. Localization of alkaloid was found in the cortical and pith region in sample 1; its absence was noted in the cortex of sample 2. Alkaloids are significant for the protection and survival of plants because they ensure their survival against micro-organisms, insects, and herbivores and also against other plants using allopathically active chemicals (Joshi & Swami, 2009) (Table 1, 2 & 3).

Physiological analysis

Leaves extract pH: The highest leaf extract pH value was observed in Sample 1 (**7.2± 0.20**), followed by Sample 5 (**7.2 ± 0.11**). Sample 4 showed (**4.7 ± 0.05**) and sample 2 (5.6 ± 0.15). However, most plants showed pH in the 5-7 (Table 4). Plants from the polluted area showed acidic pH, and non-polluted plants exhibited neutral pH. In the presence of the pollutants, cell sap pH shifted towards acid, decreasing the conversion of hexose sugars to ascorbic acid. Due to this, the leaf pH was lowered and the decline was observed in sensitive species. The reducing activity of ascorbic acid at higher pH gives tolerance to plants against pollution (Villanueva *et al.*, 1997).

Total chlorophyll content: The three different plant species total chlorophyll content (mg/g of fresh weight) was recorded. Sample 4 have high chlorophyll content **(0.823±0.0)** and sample1 has lowest chlorophyll content (**0.414±0.0**). The Highest total chlorophyll content was recorded in sample 4 and the lowest in Sample 1 (Table 5). Higher chlorophyll content in plants might favor tolerance to pollutants. Chlorophyll is the main triggering molecule of green plants and its significance is unavoidable while assessing the resistance of a plant against stress (Roy et al., 2012). The non-polluted plants are found to contain a higher amount of chlorophyll. The chlorophyll content was high in sample 4. One of the common impacts of air pollution is the gradual disappearance of chlorophyll and senescence of leaves which results in the consequent decrease in photosynthesis activity. The chlorophyll content is an index of productivity, certain pollutants increase the total chlorophyll content, and others decrease it (Joshi & Swami, 2009).

Total sugar estimation: The maximum amount of total sugar was found in sample 5 (**6.0±0.0**), followed by sample 6(5.2±0.0), sample 4(4.5±0.0), sample 3 (3.8±0.0), sample 1(3.1± 0.0). The minimum amount of total sugar was recorded in sample 2 (**2.0±0.0**) (Table 7). Soluble sugars are indicative parameters of plants' physiological activity to air pollution. Reduction of sugar concentration in polluted plants attributed to increased respiration and decreased CO_2 fixation because of chlorophyll deterioration. Pollutants like SO_2, NO_2, and H_2S cause more depletion of soluble sugars in the leaves of polluted areas Singh & Verma (2007).

Ascorbic acid estimation: The maximum content of ascorbic acid (mg/g of fresh weight) was found in sample 5 (3.78±0.12) followed by sample 3 (3.01±0.04), sample 6 (2.57±0.06), sample 4(2.06±0.09) and sample 1(2.02±0.02). The lowest ascorbic acid content was recorded in sample 1 (1.78±0.17). (Table 9). Ascorbic acid plays a significant role in the light reaction of photosynthesis. It activates a defense mechanism (Singh *et al.*, 1991), and under stress conditions, it can replace water from light reaction II (Krishnaveni & Kiran Kumar, 2017). Due to its multiple roles in the metabolism and defense of plants, ascorbic acid is used as a reliable parameter to indicate the tolerance level of plants against various conditions, including pollution stress (Michael, 2015) (Table 9). Ascorbic acid plays a role in cell wall synthesis, defense, and cell division and is a reducing agent that protects chloroplasts against pollutants. Thus, plants maintaining high ascorbic acid under pollutant conditions are considered tolerant to air pollution. Due to its multiple roles in the metabolism and defense of plants, ascorbic acid is used as a reliable parameter to indicate the tolerance level of plants against various conditions, including pollution stress.

Carotenoid estimation: The highest carotenoid content was observed in sample 3 (**0.9 ± 0.0**) followed by sample 5 (0.6 ± 0.0), sample 1 (0.4 ± 0.0), sample 4(0.2 ± 0.0), sample 6 (0.19 ± 0.0). The lowest content was found in sample 2 (**0.1 ± 0.0**) (Table 10). Carotenoids are used for the synthesis of isomers. It has molecule content of oxygen such as lutein and zeaxanthin, known as xanthophyll. The polluted plants had highly carotenoid content. Amino acids have various prominent functions in plants. Besides their usage during protein biosynthesis, they are also building blocks of several other biosynthesis pathways. In the present study, sample 4 had the highest amount of amino acids (Table 10). Carotenoids exist in plant tissues, capturing the light and protecting the cells against photooxidative processes and essential structural compounds as accessory pigments of photosystems. In most of the studies, plant species subjected to air pollution showed a decrease in carotenoid contents (Joshi & Swami, 2009).

Relative water content: The highest relative water content was observed in sample 3 (86.2±0.02), followed by sample 1 (82.5±0.05), sample 4(80.3±0.13), sample 5 (78.9±0.03) and sample 2(76.4±0.08). The lowest relative water content was

recorded in sample 6 (72.8±0.05) (Table 11). Higher relative water content was advantageous for drought resistance (Roy et al., 2012). The transpiration rate remains very high under pollution stress and might lead to desiccation. Therefore, the maintenance of relative water content by the plant might decide the close tolerance of plants to air pollution (Roy et al., 2012) (Table 11).

Relative water content is associated with protoplasmic permeability in cells which causes loss of water and dissolved nutrients, resulting in early senescence of leaves. More water in a leaf will help to maintain its physiological balance under the stress conditions of air pollution. Higher relative water content was advantageous for drought resistance. However, the transpiration rate remains very high under pollution and might lead to desiccation.

AIR POLLUTION TOLERANCE INDEX (APTI)

The highest air pollution index value was scored by sample 4 (11.30±0.03), followed by sample 3 (11.05±0.02), and sample 2 (9.91±0.03). The lowest APTI value was observed in sample 1 (8.65±0.02) (Table 12). APTI value represented the following characteristics of plants. APTI value was <1; the plant had very sensitive to pollution it can easily damage. APTI value was 1 to 6 the plant had sensitive to pollution. APTI value was 17 to 29; the plant had intermediated for pollution. APTI value was 30 to 100 plants with tolerant character; they can easily survive polluted environments (Table 12). Thus, with cell pH, ascorbic acid also plays a significant role in determining the air pollution sensitivity of plants (Roy et al., 2012). A combination of these four parameters represents the best index to determine the tolerance level of plants to air pollution (Phisitkul, 2021; Roy et al., 2012). Krishnaveni and Kiran carried out a similar study on the air pollution tolerance index on commonly available tree species in Vijayawada city. They observed physiological and biochemical changes in selected species, and their sensitivity to pollutants was expressed in APTI. It gives a practical value for the tolerance level of plants to air pollution and is an indicator of air pollutants. APTI value was <1; the plant had very sensitive to pollution it can easily damage. APTI value was 1 to 6 the plant had sensitive to pollution. APTI value was 17 to 29; the plant had intermediated for pollution. APTI value was 30 to 100; plants had a tolerant character they can easily survive the polluted environment.

REFERENCES

Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts. Polyphenol oxidase in Beta vulgaris. *Plant Physiology*, **24**(1), 1.

Bashan, Y., Holguin, G., & De-Bashan, L. E. (2004). Azospirillum-plant relationships: physiological, molecular, agricultural, and environmental advances (1997-2003). *Canadian journal of microbiology*, *50*(8), 521-577.

Béthoux, J. P., Morin, P., & Ruiz-Pino, D. P. (2002). Temporal trends in nutrient ratios: chemical evidence of Mediterranean ecosystem changes driven by human activity. *Deep Sea Research Part II: Topical Studies in Oceanography*, *49*(11), 2007-2016.

Gill, S. S., & Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant physiology and biochemistry*, *48*(12), 909-930.

Henson, I. E., Mahalakshmi, V., Bidinger, F. R., & Alagarswamy, G. (1981). Genotypic variation in pearl millet (Pennisetum americanum (L.) Leeke) in the ability to accumulate abscisic acid in response to water stress. *Journal of Experimental Botany*, 899–910.

Joshi, P. C., Swami, A. (2009). Air pollution induced changes in the photosynthetic pigmets of selected plant species. *J. Environ. Boil., 30*, 295-298.

Khan, M. A., Ansari, R., Ali, H., Gul, B., & Nielsen, B. L. (2009). Panicum turgidum, a potentially sustainable cattle feed alternative to maize for saline areas. *Agriculture, ecosystems & environment*, *129*(4), 542-546.

Krishnaveni G. and Kiran Kumar K., (2017). Air pollution tolerance index of selected plants in Vijayawada city, Andhra Pradesh. *International Journal of Green Pharmacy, 11*(4), 877-881.

Michael, Q. (2015). *Assessment of Bioactivity of Selected Plants against Pests and Microbes from Agro-pastoral Communities in Mbulu District, Tanzania* (Doctoral dissertation, The Open University of Tanzania).

Phisitkul, V. D. (2021). A Review of Electromagnetic Radiation Impacts on Heterojunction Intrinsic Thin Layer Solar Cells. *International Journal of Innovative Research in Applied Sciences and Engineering, 4*(9), 879-884.

Roy, A., Bal, S. S., Fergany, M., Kaur, S., Singh, H., Malik, A. A., ... & Dhillon, N. P. S. (2012). Wild melon diversity in India (Punjab state). *Genetic resources and crop evolution*, *59*(5), 755–767.

Singh, S. K., Rao, D. N., Agrawal, M., Pandey, J., & Naryan, D. (1991). Air pollution tolerance index of plants. *Journal of Environmental Management*, *32*(1), 45–55.

Singh, S. N., & Verma, A. (2007). Phytoremediation of air pollutants: a review. In *Environmental bioremediation technologies* (293–314). Springer, Berlin, Heidelberg.

Tripathi, A.K. and M. Gautam, (2007). Biochemical parameters of plants as indicators of air pollution. *J. Environ. Biol., 28*, 127–132.

Villanueva, R., Nozais, C., & Boletzky, S. V. (1997). Swimming behaviour and food searching in planktonic Octopus vulgaris Cuvier from hatching to settlement. *Journal of Experimental Marine Biology and Ecology*, *208*(1-2), 169-184.

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