

Morphological and biochemical differentiations of plants in polluted and nonpolluted environments



Sk K. Basar, Amal K. Mondal

UGC-DRS-SAP, Department, Department of Botany and Forestry, Vidyasagar University, Midnapore 721102, West Bengal, India

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Corresponding Author:

Sk. Khairul Basar
Email: basarkhairul444@gmail.com

ABSTRACT

Environmental pollution is a severe threat in both developed and developing countries. Plants growing in the industrial areas absorb the pollutants at their foliage surface. The present study was to investigate the alterations of total plant morphology and biochemical parameters induced by water and soil pollutants generated by carbo and TATA Metalics factory combined with the human activities. In this investigation, five major plants species, *Pistia stratiotes*, *Nelumbo nucifera*, *Eichhornia crassipes*, *Trapa natans*, *Solanum lycopersicum* along with some other plants were selected for study purpose. These plants species have been identified to be able to absorb, detoxify and tolerate high levels of pollution. The tolerance degree is indirectly correlated with the intensity of injuries which occur in plant structure such as, morphology. According to quantitative analysis of morphological parameters, it shows that decrease in size of plant grown in polluted areas. The results of the present study indicated that the biochemical parameters of plants, total chlorophyll, ascorbic acid, leaf pH and relative water content for all species and are the most significant and determining factors on which the tolerance depends. Thus in future these plant species play an important role in phytoremediation and green development in urban area.

1. Introduction

Environmental pollution is caused by rapid industrialization, urbanization, population explosion and other human activities like modern agriculture and de-forestation etc. It injects diverse pollutants into environment as byproducts (<https://www.conserve-energy-future.com/causes-effects-solutions-urbanization.php>). Pollutants are generally grouped into two classes: biodegradable and nonbiodegradable. Biodegradable pollutants are broken down by the activity of biological agents primarily microorganisms and enter into the biogeochemical cycles, for example - domestic waste products, urine and faecal matter, sewage, agricultural residue, paper, wood and cloth etc. Nonbiodegradable pollutants are not easily broken down into simpler and harmless products. These include various pesticides, insecticides, herbicides, metals (mercury, lead arsenic and aluminum), plastics, e-wastes, medical wastes, radioactive waste etc.

Water, soil and air of environment are polluted by above mentioned pollutants generated from various point and

nonpoint sources (Bhakta et al., 2009). Numerous manufacturing plants discharge undiluted corrosives, poisons, and other noxious byproducts to water and soil. The construction industry discharges slurries of gypsum, cement, abrasives, metals and poisonous solvents. The mining industry also presents persistent water pollution problems. Hot water discharged by factories and power plants causes so called "thermal pollution" by increasing water temperatures. Such increases change the level of oxygen dissolved in a body of water, thereby disrupting the water ecological balance, killing off some plants and animals while encouraging the overgrowth of others. Towns and municipalities are also major sources of water, soil and air pollution. In many public water systems, pollution exceeds safe levels. One reason for this is that much groundwater has been contaminated by wastes pumped underground for disposal or by seepage from surface water (Bhakta et al. 2016). When contamination reaches underground water tables, it is difficult to correct and spreads over wide areas. Discharge of untreated or only partially treated sewage into the waterways threatens the health of their own and

neighbouring population as well. Along with domestic wastes, sewage carries industrial contaminants and a growing tonnage of paper and plastic refuse. Additionally most sewage treatment does not remove phosphorus compounds, contributed principally by detergents. Various solid wastes (paper, clothes, plastics, rubber, leather, brick, sand, metal, etc.) from household and industries also pollute land and degrade the physical, chemical and biological qualities of soil.

However, all kinds of pollution can cause severe environmental and human health hazards. Thus, environment pollution has appeared as an immense problem during last few decades worldwide, since it perturbs the environmental homeostasis and poses severe threats to life on earth.

Plants and aquatic animals are primarily affected by the environmental imbalance induced by pollution especially in water and soil environments, since they are directly associated with the water and soil environments (<https://greentumble.com/effect-of-pollution-on-plants/>).

Pollutants are responsible to change the morphological and biochemical characteristics of plants. According to Mapaure et al. (2011), mine dump pollution has significantly reduced woody plant densities, species richness and diversity in the affected area due to disappearance of pollution-sensitive species and preponderance of tolerant ones. The studies regarding the morphological and biochemical changes in plants due to the environmental pollution are scanty. The present study focused on the investigation of the alterations of

total plant morphology and biochemical parameters induced by the water and soil pollutants generated by carbo and TATA Metalics factory combined with the human activities.

2. Materials and methods

2.1. Study area

The present intersection was carried out within the different area in midnapore (22.424°N and 87.319°E), kharagpur (22.330239°N and 87.323653°E) and kolaghat (22.43°N and 87.87°E) zones, West Bengal, India (Fig. a and b) with a tropical climate during the time period of February to June 2018. The sites selected for the present study includes agriculture land area and industrial area. Rapid industrialization in the cities increases the density of vehicles which further increases the load of vehicular concentration in the roads which directly affects the road side plants and also the plant are present in populated area which remains in direct contact with these types of pollutants. Therefore, plant species selected in the studies are as follows: *Trapa natans* (Trapaceae), *Lycopersicon esculentum* (Solanaceae), *Pistia stratiotes* (Araceae), *Eichhornia crassipes* (Pontederiaceae) and *Nelumbo nucifera* (Nelumbonaceae) (Fig. 2).

The investigation was conducted in polluted (P) and control (C) sites considering the common plants as above mentioned.



Fig. 1 Study sites- Tatametals, Kharagpur, West Bengal (a) and Kolaghat, Thermal Power Project ,Midnapur (b)

Fig. 2 Plant species selected in the studies for investigating the pollution impacts



Trapa natans

Lycopersicon esculentum



Pistia stratiotes



Eichhornia crassipes



Nelumbo nucifera

2.2. Morphological characterization

Morphological characteristics of above mentioned plants were performed on the basis of observation of the plants of both polluted and control sites. Fresh leaves of plants were collected from both polluted and control experimental sites for the study of leaf morphology. The observation on morphology of overall plant body, such as root length, shoot length, leaf length, leaf width number of leaf venation, petiole length, intermodal length, number of nodes was also done in both sites. Leaves sample were dried in hot air oven at 80°C till they attained constant weight and dry weight was recorded. Specific leaf weight (SLW) was calculated by taking the ratio of leaf dry weight to the total leaf area by using the method of Pearce et al. (1968).

2.3. Biochemical analysis

The plant species were randomly selected from experimental sites. Aqueous extract of fully matured fresh leaves were prepared and used for biochemical study according to the standard prescribed method.

Leaf extract pH – 5g of the fresh leaves was homogenized in 10 ml deionised water, filtered and the pH of leaf extract was determined using pH meter (HI 98130) (Agbaire and Esiefarienrhe, 2009)

Total chlorophyll content – 3g of fresh leaves were blended and extracted in 10 ml 80% acetone. After 15 mins of thorough extraction, the liquid portion was poured into another test tube and centrifuged at 2500 rpm for 3 mins.

The supernatant was then collected and the absorbance was then taken at 645 nm and 663 nm using systronics UV spectrophotometer (Sharma and Tripathi, 2008).

Hence, Chlorophyll- a = $12.7dx663 - 2.69 dx V/1000w$ mg/g

Chlorophyll- b = $22.9d x 645 - 4.68d x 663 x V / 1000$ mg/g.

TCH (Total chlorophyll content) = chlorophyll a + b mg/g

Where, Dx = Absorbance of the extract at the wave length in nm, V = total volume of the chlorophyll solution (ml), and W = weight of the tissue extract (g).

Relative water content of leaf (RWC)– Fresh leaves were weighted and then immersed in water over night, blotted dry and then weight to get the turgid weight. Then, the leaves were dried over night in a hot air oven at 70° c and re weighted to obtain the dry weight (Singh, 1983).

$RWC = [(FW-DW)/(TW-DW)] \times 100$

Where, FW= fresh weight, DW= dry weight, TW= turgid weight

Ascorbic Acid (AA) content – 1g of the leaf sample was measured into a test tube and oxalic acid – EDTA extracting solution was added. Then, 1 ml of orthophosphoric acid followed by 1 ml 5% tetra oxosulphate acid, 2 ml of ammonium molybdate and then 3ml of water added. The solution was then allowed to stand for 15 mints, after which the absorbance at 760 nm was measured with systronics UV spectrophotometer 1800 240V. The concentration of ascorbic acid in the leaf samples were analysed comparing with the standard ascorbic acid curve (Dipu and Thanga, 2014).

3. Results and discussion

3.1. Morphological characterization

Table 1 represents the morphological properties of *Nelumbo nucifera*, *Eichhornia crassipes*, *Rorpipa indca*, *Trapa natans* and *Pistia stratiotes* plants in control (C) and polluted (P) zones investigated.

Root length, shoot length, leaf length, leaf breadth, number of leaf and length of petiole (cm) of all plants (*Nelumbo nucifera*, *Eichhornia crassipes*, *Rorpipa indca*, *Trapa natans* and *Pistia stratiotes*) of polluted areas showed reduced growth compared to that the plants collected from control area (Table 1).

Table 1. Total morphological comparison of *Nelumbo nucifera*, *Eichhornia crassipes*, *Rorpipa indca*, *Trapa natans* and *Pistia stratiotes* plants in control (C) and polluted (P) zones investigated.

Plants	Root length (cm)		Shoot length (cm)		Leaf length (cm)		Leaf breadth (cm)		Number of leaf		Length of petiole (cm)	
	C	P	C	P	C	P	C	P	C	P	C	P
<i>Nelumbo nucifera</i>												
Replicates	24	21	18.2	17.7	12.5	11.6	9	8.1	4	4	16.2	15.1
	23	22	21.4	16.4	14.3	10.3	8.7	8.6	3	2	15.6	14.2
	25	20	22.3	15.3	11.2	11.5	8.5	8.4	4	3	14.3	13.3
Mean	24	21	20.63	16.46	12.6	11.1	8.7	8.3	3.6	3	15.3	14.2
<i>Eichhornia crassipes</i>												
Replicates	6.3	5.7	10.4	9.7	4.3	4.1	3.7	3.8	6	5	2.6	2.7
	5.2	6.1	8.6	10.1	5.4	4.3	4	3.7	5	6	3.5	3.1
	7.2	6.3	9.8	7.4	5.6	5.3	3.9	3.6	6	4	4	3.5
Mean	6.23	6.0	9.6	9.06	5.1	4.5	3.8	3.7	5.6	5	3.3	3.1
<i>Rorpipa indica</i>												
Replicates	5.2	3.2	8.4	7	4.4	4	2.5	2.6	9	8	1.5	1.4
	4.5	4.7	9.8	7	4.2	3.5	2.7	2.2	7	6	1.3	1.2
	4.7	3	10.4	8.3	4.7	3.6	2.9	2.3	10	8	1.2	1
Mean	4.7	3.6	9.5	7.4	4.4	3.7	2.7	2.4	8.7	7.3	1.3	1.2
<i>Trapa natans</i>												
Replicates	7.8	5.2	9	7.5	4.8	3.8	3.7	3	8	6	3.6	2.8
	6.9	6.7	8.6	6.3	5	4.1	3.5	4	7	7	2.9	3
	5.6	4.9	7.3	8.2	4.9	4.6	4	3.2	8	7	3	3.1
Mean	6.7	5.6	8.3	7.3	4.9	4.1	3.7	3.4	7.6	6.6	3.1	2.9
<i>Pistia stratiotes</i>												
Replicates	3.4	3	4	3	2.5	2.5	1.9	1.6	6	7	0.6	0.7
	4.2	4.1	3	3.4	2.2	2.3	1.7	1.4	7	5	0.8	0.3
	3.7	3.5	3.2	3.7	2.6	2	1.6	1.3	6	6	0.5	0.5
Mean	3.7	3.5	3.4	3.3	2.4	2.2	1.7	1.4	6.3	6	0.6	0.5

Table 2. Leaf extract pH of the selected plant species with graphical presentation.

Plant species	Leaf extract pH	
	C	P
<i>Eichhornia crassipes</i>	6.4	5.5
<i>Nelumbo nucifera</i>	5.4	5.6
<i>Trapa natans</i>	4.7	5
<i>Pistia stratiotes</i>	5.3	6.2
<i>Rorpipa indica</i>	6.5	6.4
<i>Solanum nigrum</i>	6.2	6.4
<i>Solanum lycopersicon</i>	6.3	6.2
<i>Nicotianaplumbaginifolia</i>	6.0	5.5

Accordance to results, it is inferred that pollutants in the polluted area inhibits the growth process of plants. Mine dump pollution has significantly reduced woody plant densities, species richness and diversity in the affected area due to disappearance of pollution-sensitive species and preponderance of tolerant ones (Mapaure et al., 2011). Leaf Morphology Study Accumulation of dust particles depends on internodal distances, petiole length, leaf area, orientation, margin, folding and arrangement, hair density, hair type and length (Varshnay and Mitra, 1993).

3.2. Biochemical analysis

Leaf extract pH – The pH value of leaf extracts of all tested plants in polluted (P) area is higher than that of the plants of control area (C) with some exception (Table 2). The pH value of some other plants, *Syzygium cuminiis* - 4.8 (C) and 5.4 (P), *Eucalyptus globulusis* - 5.5 (C) and 6.2 (P),

*Swietenia mahagoni*s - 6.3(C) and 6.4(P) also showed similar results. It indicates that pH is increased in plants of high pollutant resistant.

Total chlorophyll content – The chlorophyll content of leaf extracts of all tested plants in C area is higher than that of the plants of polluted area (Table 3). It is concluded that the chlorophyll is lost in presence of pollutants, that means that the synthesis rate of chlorophyll in plants of polluted area is lower than that of the control plants.

Table 3. Total chlorophyll (mg/gm) of the selected plant species with graphical presentation.

Plant species	Total chlorophyll (mg/g)	
	C	P
<i>Eichhornia crassipes</i>	30	27.07
<i>Nelumbo nucifera</i>	25.24	19.03
<i>Trapa natans</i>	24.50	20.52
<i>Pistia stratiotes</i>	16.60	11.45
<i>Rorpipa indica</i>	21.39	18.45
<i>Solanum nigrum</i>	39.65	34.38
<i>Solanum lycopersicon</i>	40.37	36.59
<i>Nicotianaplumbaginifolia</i>	27.78	24.68

Chlorophyll estimation is one of the important criteria for pollution measurement. Loss in total chlorophyll content of plant depends on the degree of pollution. It is established that degradation of photosynthetic pigment i.e., chlorophyll indicates different level of air pollution (Aarti et al. 2011).

Relative water content of leaf (RWC) – The RWC is an essential factor for the transportation of food, minerals. So, Plants with relatively high water content are highly resistant to pollution (Tanaka et.al. 1982).

The amount of relative water content which we have observed for different plant sample is summarized in table 4. The result it can be concluded that control plants showed relatively high RWC compared to plants of polluted area.

The relative water content of some other control plant and pollutant plant sample i.e *Mangifera indica* is 2.65 (C) and 2.60 (P), *Syzygium cumini* is 3.24 (C) and 3.02 (P), *Eucalyptus globulus* is 3.16 (C) and 2.30 (P) and *Swietenia mahagoni* is 3.00 (C) and 2.50 (P) respectively.

Table 4. RWC (per g leaf) of the selected plant species.

Plant species	Relative water content (g)	
	C	P
<i>Eichhornia crassipes</i>	3.20	3.11
<i>Nelumbo nucifera</i>	2.60	2.55
<i>Trapa natans</i>	3.14	2.30
<i>Pistia stratiotes</i>	2.26	2.33
<i>Rorpipa indica</i>	4.33	4.30
<i>Solanum nigrum</i>	4.60	4.52
<i>Solanum lycopersicon</i>	4.38	4.32
<i>Nicotianaplum baginifolia</i>	4.65	4.55

Ascorbic Acid (AA) content – The AA content of plant is shown in table 5. It is higher in control plant than that of the polluted plants investigated (Table 5).

The AA content of some other control plant and pollutant plant samples (*Mangifera indica* 0.173 (C) and 0.159 (P), *Syzygium cumini* 0.055 (C) and 0.032 (P), *Eucalyptus globulus* 0.184 (C) and 0.106 (P) and *Swietenia mahagoni* 0.094 (C) and 0.039 (P)) also showed similar results as table 5. From our result it shows that in *Manifera indica* the difference of ascorbic acid content is very low between control plant and pollutant plant. So, this plant has high defensive mechanism for tolerating pollution.

The AA content estimation is one of the important criteria for pollution measurement as seen earlier. Ascorbic acid being a strong reductant protects chloroplast against sulphur dioxide induced H₂O₂, O⁻ and OH accumulation. Similarly, it protects the enzymes of CO₂ fixation cycle and chlorophyll from inactivation. (Kovacic 2005). Defence mechanism in plants cause increased level of ascorbic acid. It indicates that pollution can suppress the AA synthesis.

Table 5. AA Content of the selected plant species.

Plant species	AA content (mg/g)	
	C	P
<i>Eichhornia crassipes</i>	0.165	0.154
<i>Nelumbo nucifera</i>	0.057	0.034
<i>Trapa natans</i>	0.187	0.109
<i>Pistia stratiotes</i>	0.088	0.041

4. Conclusion

The observations recorded in the present study clearly indicated that pollutants emitted from the industry and automobile exhaust exercised a decisive influence on plant anatomy. The statistical analysis also corroborated the same. From the present data it is also become apparent that the pollutants inhibit and suppress the growth of plants (Raina and Bala, 2011). It is difficult to estimate the effects of pollutants because the organisms are exposed to a wide range of uncontrolled variables (parasites, weather conditions, complex mixture of pollutants). On the morphological and anatomical point of view, the plants from polluted sites present important changes especially regarding their colors, shapes, length, width, area and petiole length. However, despite of these changes, plants were survived well at the polluted environment of Kolaghat Thermal Power Plant. These results showed the importance of morphological data for precocious diagnosis injury and to determine the sensitivity of different plant species to the action of air pollutants. After this study, we can consider that there is still a serious lack of knowledge of the impact of pollution quality on vegetation in the urban areas. Overall, the study reveals that all the plant species growing in the polluted environment of the city are badly affected by auto-emission. There is a need to set limits on how much of a pollutant is allowed in the pollution. The exchange of experience and information from the developed countries on this aspect of pollution impact on plants might be useful. Our goal must be

to have clean air for flora and fauna. We should take necessary steps to get rid of the ever increasing pollution.

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