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Comparative Assessment of Biochemical Parameters of Plants in Industrial and Non-Industrial Areas of Western Odisha, India

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ABSTRACT

Industrialization being the main force of development has caused many changes not only in the global phenomena but also on a regional level through its ill effects on plants and animals. The present study was thus undertaken to assess the biochemical alterations in plants subjected to polluted (industrial) and non-polluted (control) environments. The results revealed that all the studied biochemical parameters (ascorbic acid, protein, carbohydrate, total chlorophyll, catalase, and peroxidase activities) showed significant variation with respect to sites ($p < 0.05$). Excepting the peroxidase activity, all other biochemical parameters showed a decline in their concentration in the polluted environment as compared to their counterparts in a non-polluted environment. The highest concentration of biochemical parameters in plants of polluted sites were: ascorbic acid (4.85 mg/g), carbohydrate (0.905 mg/g), protein (28.07 mg/g), total chlorophyll (1.13 mg/g), catalase (0.394 µmoles/H_2O_2 decomposed/min/g) and peroxidase (433.76 µmoles/GDHP/min/g) while that in the control site, the highest value of all the biochemical parameters were: ascorbic acid (8.97 mg/g), carbohydrate (1.283 mg/g), protein (48.68 mg/g), total chlorophyll (1.17 mg/g), catalase (0.434 µmoles/H_2O_2 decomposed/min/g) and peroxidase (271.25 µmoles/GDHP/min/g) respectively. Hence, it can be concluded that plants do undergo physiological stress when exposed to polluted environments and their biochemical synthesis is severely altered by pollution. However, they develop an inbuilt mechanism to counter the pollution and protect themselves in polluted or stressed environment. In the present study, peroxidase activity was primarily responsible to protect the plant in the stressed environment.

INTRODUCTION

Industries not only play an important role in the economic development of a nation but also produce both traditional pollutants (organic substances, sulphur dioxide, particulates, nitrogen oxide, etc.) and newly recognized pollutants (dioxins, furans and other toxic substances). These pollutants in various forms can cause serious damage to the biosphere.

Environmental pollution due to industries has led to a steep increase in various illnesses and it continues to affect on a daily basis. With so many small, mid and large-scale industries coming up, air pollution has taken a toll on the health of the people and the environment. With the rise in industrial pollution, global warming has been increasing at a steady pace. Melting of glaciers, extinction of polar bears, floods, tsunami, hurricanes coupled with rising in sea level, migration of species, invasion of diseases and change in oceanic currents are some of the effects of global warming.

The components of environment and living organisms nearby ecosystems are being badly affected by massive environmental pollution. It is a well-known fact that pollution creates a negative impact on human and plants. The harmful effects of pollution on vegetation have been studied by several workers (Agrawal et al. 1991, Dasgupta et al. 2002). Plants being the initial acceptor to pollutants, experience many adverse consequences like chlorosis, bleached leaves, stunted growth, necrosis, lack of pigmentation, destruction of guard cell in the stomatal region, premature defoliation etc. Besides these, ozone has been reported to decrease the photosynthetic rate and injure the intermediate-aged leaves. While SO_2 damage the plants through interveinal bleaching of leaves, fluoride creates toxicity, bleaching of the tips and margins of leaves (Caldwell 1970, Rhimi et al. 2016).

Air pollutants induce morphological, physiological and biochemical changes in plants (Seyyednejad & Koochak 2011, Areington et al. 2015). Biochemical synthesis mainly includes protein synthesis, carbohydrate synthesis, catalase activity, peroxidase activity etc. involving plant biochemistry associated with the molecular mechanism of the plant cell (Okano et al. 1985, Soda et al. 2000, Vollenweider et al. 2003, Chaudhary et al. 2008). Plants have a very important role in combating the level of air pollution in the at-

mosphere by taking up gaseous pollutants and particulates. Primarily, most of the particulate matters are deposited on the upper surface of the leaves. This deposition leads to the reduction in photosynthetic pigment like chlorophyll and carotenoids ultimately affecting the total productivity of the plant. Pollution creates a stress condition in the plant body which leads to a change in biochemical synthesis. The negative effect on the biochemical synthesis causes deterioration in the tolerance of plants.

Keeping this in view, work was undertaken to assess the biochemical parameters of plants in an industrial and non-industrial area of Western Odisha, India to analyse the impact of stress induced by the pollution due to industrial activities.

MATERIALS AND METHODS

Geographical description of the study area: The study area (experimental/polluted industrial site), selected for the present investigation, was near Hindalco aluminium smelter plant in Hirakud of Sambalpur district located at the geographical coordinates between 21.5138° N and 83.3508° E at an elevation of 161 m above MSL. The control site (non-polluted) was chosen to be the campus of Sambalpur University located at an aerial distance of 15 km from the polluted site and found within the geographical coordinates between 21.5789° N and 83.4483° E situated at an elevation of 180 m above MSL.

Sampling and selection of plant species: The study was carried out during the month of July 2018. Five evergreen plants based on their commonness to both the sites (polluted and non-polluted) were chosen for the investigation, the details of which are presented in Table 1. Leaves were carefully cut from the base of their petiole in triplicates from each plant species and wrapped in a polythene bag. The leaves present at least 6 ft above the ground were only considered for the present study. Then it was quickly transported to the laboratory for further analysis after keeping inside an ice-box. All the biochemical parameters were determined for each species taking three replicates.

Biochemical analysis of leaf: The ascorbic acid content of leaves was analysed by DCPIP (2,6-dichlorophenol indophenol) method given by Keller & Schwager (1977). Similarly, the carbohydrate and protein content of the leaves was analysed following the anthrone reagent method (Yemm & Wills 1954) and Folin's reagent method (Lowry et al. 1951) respectively. The total chlorophyll content of the leaves was analysed by the acetone method given by Arnon (1949) while the catalase and peroxidase activities in leaves were determined by the methods given by Aebi (1974) and Putter (1974) respectively.

RESULTS AND DISCUSSION

Results

The results of various biochemical parameters of plants from the control (non-industrial) and experimental (industrial) sites are presented in Table 2. It is evident from the table that ascorbic acid content ranged between 0.61 ± 0.04 (*F. religiosa*) and 8.97 ± 0.35 (*F. benghalensis*) mg/g in the control site while that in the experimental site, it ranged between 0.30 ± 0.27 (*F. religiosa*) and 4.85 ± 0.12 *(M. indica*) mg/g. The carbohydrate content ranged between 0.524 ± 0.069 *(M. indica)* and 1.283 ± 0.32 *(A. indica)* mg/g in the control site, and between 0.295 ± 0.066 *(M. indica)* and 0.905 ± 0.029 *(A. indica)* mg/g in the experimental site. The protein content of various plant species ranged between 28.48 ± 2.04 *(A. indica)* and 48.68 ± 6.3 *(F. religiosa)* mg/g in the control site, and between 10.99 ± 0.15 *(A. scholaris)* and 28.07 ± 0.7 *(F. religiosa)* mg/g in the experimental site. The chlorophyll content of various plant species, on the other hand, was observed with the highest value of 1.17 ± 0.01 and 1.17 ± 0.03 mg/g in *F. benghalensis* and *A. indica* respectively, and lowest value of 0.083 ± 0.006 mg/g in *F. religiosa* in the control site. The same in the experimental site, ranged between 0.33 ± 0.0006 *(F. religiosa)* and 0.63 ± 0.013 *(M. indica)* mg/g.

Similarly, the catalase and peroxidase activities of various plant species were found to be in the range of 0.183 ± 0.001 *(A. scholaris)* - 0.434 ± 0.004 *(F. religiosa)* μmoles/

Table 1: Description of the evergreen plant species selected for the present study.

Sl. No.	Species Name	Common Name	Family
1.	*Ficus benghalensis* (L)	Bara	Moraceae
2.	*Ficus religiosa* (L)	Peepal	Moraceae
3.	*Mangifera indica* (L)	Amba	Anacardiaceae
4.	*Alstonia scholaris* (L)	Saptaparni	Apocyanaceae
5.	*Azadirachta indica* (A. Juss)	Neem	Meliaceae

Table 2: Biochemical parameters of plants in industrial and non-industrial sites of western Odisha.

Parameters	Sites	FB	FR	MI	AS	AI
Ascorbic acid (mg/g)	Control	8.97 ± 0.35	0.61 ± 0.04	7.94 ± 0.48	4.33 ± 0.03	4.66 ± 0.04
	Experimental	2.90 ± 0.01	0.30 ± 0.27	4.85 ± 0.12	3.14 ± 0.01	4.56 ± 0.04
Carbohydrate (mg/g)	Control	0.916 ± 0.024	0.773 ± 0.029	0.524 ± 0.069	0.865 ± 0.013	1.283 ± 0.32
	Experimental	0.870 ± 0.140	0.551 ± 0.016	0.295 ± 0.066	0.457 ± 0.049	0.905 ± 0.029
Protein (mg/g)	Control	37.94 ± 5.4	48.68 ± 6.3	37.32 ± 6.3	30.36 ± 2.4	28.48 ± 2.0
	Experimental	20.82 ± 0.2	28.07 ± 0.7	12.50 ± 0.6	10.99 ± 0.8	12.07 ± 0.8
Total Chlorophyll (mg/g)	Control	1.17 ± 0.001	0.083 ± 0.006	1.12 ± 0.006	0.88 ± 0.01	1.17 ± 0.003
	Experimental	1.03 ± 0.002	0.74 ± 0.0006	1.004 ± 0.013	0.73 ± 0.009	1.13 ± 0.001
Catalase (μmoles/H_2O_2 decomp./min/g)	Control	0.214 ± 0.0008	0.434 ± 0.0016	0.237 ± 0.0021	0.183 ± 0.0014	0.189 ± 0.0021
	Experimental	0.169 ± 0.0021	0.394 ± 0.0044	0.211 ± 0.0016	0.174 ± 0.0028	0.163 ± 0.0016
Peroxidase (μmoles/ GDHP/min/g)	Control	172.55 ± 5.95	271.25 ± 37.85	268.03 ± 8.44	219.44 ± 26.79	215.93 ± 22.93
	Experimental	205.56 ± 4.81	433.76 ± 59.56	349.21 ± 13.74	283.8 ± 17.87	343.48 ± 37.11

FB = *Ficus benghalensis*, FR = *Ficus religiosa*, MI = *Mangifera indica*, AS = *Alstonia scholaris*, AI = *Azadirachta indica*

H_2O_2 decomposed/min/g protein, and 172.55 ± 5.95 *(F. benghalensis)* - 271.25 ± 37.83 *(F. religiosa)* μmoles/ GDHP/min/g respectively in the control site while that in the experimental site, it was found to be in the range of 0.163 ± 0.002 *(A. indica)* - 0.394 ± 0.001 *(F. religiosa)* μmoles/H_2O_2 decomposed/min/g protein and 205.56 ± 4.81 *(F. benghalensis)* - 433.76 ± 59.56 *(F. religiosa)* μmoles/ GDHP/min/g respectively.

The two-way ANOVA computed for various biochemical parameters between species and sites is presented in Table 3. It is evident from the table that significant variation was exhibited, both with respect to species and sites in case of the carbohydrate, protein, total chlorophyll content and catalase activity of the plants ($F_1 \geq 9.02$, $F_2 \geq 15.68$; $p < 0.05$) while, ascorbic acid content and the peroxidase activity exhibited significant variation only with respect to sites ($F_2 \geq 9.03$; $p < 0.05$).

The Pearson correlation matrix worked out between various biochemical parameters of plants in control and experimental sites is presented in Tables 4 and 5 respectively. The values marked with an asterisk (*) in the tables suggests a strong correlation at 0.05 level of significance. It is evident from the table that, in the control site, significant positive correlation was found between ascorbic acid and total chlorophyll; protein content and catalase activity; and catalase and peroxidase activity ($r \geq +0.626$; $p < 0.05$) while, significant negative correlation was observed between ascorbic acid and catalase activity; carbohydrate

Table 3: Two-way ANOVA for various biochemical parameters between various species in different sites.

Parameters	Source of Variation	SS	df	MS	$F_{tab\ at\ 0.05}$	$F_{crit\ at\ 0.05}$	S/NS
Ascorbic acid	Between Species	40.98	4	10.24	4.24	6.39	NS
	Between Sites	21.78	1	21.78	9.03	7.71	S
Carbohydrate	Between Species	0.54	4	0.13	12.87	6.39	S
	Between Sites	0.16	1	0.16	15.68	7.71	S
Protein	Between Species	448.54	4	112.13	20.10	6.39	S
	Between Sites	966.88	1	966.88	173.33	7.71	S
Total Chlorophyll	Between Species	0.14	4	0.04	9.02	6.39	S
	Between Sites	0.76	1	0.76	187.62	7.71	S
Catalase	Between Species	0.081	4	0.02	204.89	6.39	S
	Between Sites	0.002	1	0.002	21.45	7.71	S
Peroxidase	Between Species	30380.62	4	7595.15	5.69	6.39	NS
	Between Sites	21658.78	1	21658.78	16.24	7.71	S

and peroxidase activity; and total chlorophyll and catalase activity ($r \geq -0.566$; $p < 0.05$). Similarly, in the experimental site, significant positive correlation was found between ascorbic acid and total chlorophyll content; protein and catalase activity; and catalase and peroxidase activity ($r \geq +0.774$; $p < 0.05$), while significant negative correlation was observed between ascorbic acid and protein; ascorbic acid and catalase activity; protein and total chlorophyll; and total chlorophyll and catalase activity ($r \geq -0.576$; $p < 0.05$).

The percent reduction in biochemical parameters of plant leaves is given in Table 6. From the table, it was observed that only peroxidase showed increment in its activity in the plant species of the polluted site as compared to the same species in control site while all other parameters showed a reduction in concentration. In the case of ascorbic acid, *F. religiosa* showed an insignificant reduction ($p > 0.05$) while all other studied species (*F. benghalensis, M. Indica, A. scholaris, A. indica*) showed a significant reduction. Similarly, in case of carbohydrate, *F. benghalensis, M. indica* and *A. scholaris* showed an insignificant reduction ($p > 0.05$), whereas, that of protein and chlorophyll showed a significant reduction in all of the studied species ($p < 0.05$). On the other hand, catalase exhibited an insignificant reduction in *A. scholaris* only ($p > 0.05$).

Discussion

Ascorbic acid has been reported to behave as a stress reducer in plants. It is an anti-oxidant and has multiple functions

Table 4: Correlation matrix between various parameters of plants at control site.

	AA	Carbo	Prot	TC	Cat	Perox
AA	1					
Carbo	-0.151	1				
Prot	-0.367	-0.536	1			
TC	0.796*	0.292	-0.449	1		
Cat	-0.670*	-0.348	0.928*	-0.601*	1	
Perox	-0.523	-0.566*	0.481	-0.486	0.626*	1

'*' $p < 0.05$, AA- Ascorbic acid, Carbo- Carbohydrate, Prot- Protein, TC-Total Chlorophyll, Cat- Catalase, Perox- Peroxidase

Table 5: Correlation matrix between various parameters of plants at the experimental site.

	AA	Carbo	Prot	TC	Cat	Perox
AA	1					
Carbo	-0.008	1				
Prot	-0.882*	0.157	1			
TC	0.876*	-0.045	-0.576*	1		
Cat	-0.818*	-0.295	0.807*	-0.634*	1	
Perox	-0.347	-0.371	0.318	-0.354	0.774*	1

'*' $p < 0.05$, AA- Ascorbic acid, Carbo- Carbohydrate, Prot- Protein, TC-Total Chlorophyll, Cat- Catalase, Perox- Peroxidase

Table 6: Percent reduction in biochemical parameters of plant leaves.

	F. benghalensis	*F. religiosa*	*M. indica*	*A. scholaris*	*A. indica*
Ascorbic acid	67.67*	50.82	38.92*	27.48*	2.15*
Carbohydrate	5.02	28.72*	43.70	47.17*	29.46
Protein	45.12*	42.34*	66.51*	63.80*	57.62*
Chlorophyll	55.56*	60.24*	43.75*	53.41*	55.56*
Catalase	21.03*	9.22*	10.97*	4.92	13.76*
Peroxidase	-19.13*	-59.91	-30.29*	-29.33	-59.07*

'*' $p < 0.05$

through cell wall synthesis, cell division, and protection of plants against the reactive oxygen species (ROS) besides acting as an enzyme cofactor in photosynthesis (Hippeli & Elstner 1996). The present study observed a decline in the concentration of ascorbic acid in the industrial site as compared to the counterparts in the control site. This observation was against the findings reported by many workers who opined that ascorbic acid tends to increase in polluted areas since plants develop a tolerance mechanism when exposed to polluted air primarily attributed to the synthesis of ascorbic acid (Liu & Ding 2008, Rai et al. 2013). This suggests that all the selected plant species in this study have not responded well enough with respect to ascorbic acid synthesis to tolerate the air pollution and hence are likely to be affected due to the reactive oxygen species formed in the air of the polluted site.

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. The present result suggests that the plants were severely affected due to the polluted environment thereby causing a reduction in their carbohydrate content.

Protein is the most important bio-molecule responsible for the growth and development of plants. The present study also observed a significant reduction in protein content of plants in the polluted site than the control site in all the studied species. The possible cause could have been the denaturation and breakdown of existing protein complex to amino acids assisted by enzyme activities responsible for polypeptide breakdown during the stress period (Dohmen 1990). Decrease in protein content of leaves has also been reported to cause senescence of leaves (Kar & Mishra 1976). This further suggests that the plants in polluted environment are likely to suffer from early leaf fall.

Chlorophyll content is not only associated with food production but also is closely related with ascorbic acid synthesis and therefore plays an important role in the defence mechanism of plants. Reduction in the chlorophyll content leads to yellow coloration of leaf that ultimately causes leaf senescence (Kar & Mishra 1976). The observation of a significant reduction of chlorophyll content in the studied plants could have been due to the impact of dust or particulate matter which is a common pollutant in the industrial environment. Several studies have supported the view that chlorophyll content undergoes rapid degradation under the influence of dust pollution. Our study was consistent with the findings of Prusty et al. (2005) who also reported the negative effects of dust on chlorophyll content of leaves.

Catalase and peroxidase accelerate the decomposition of hydrogen peroxide to water and oxygen. These enzymes have anti-oxidative properties mainly responsible to protect the plants from any oxidative damage caused by ROS. These enzymes have been considered as good parameters to indicate air pollution (Khan et al. 1990). Catalase activity is variable within species. Some species show an increased activity nearing senescence while others exhibit decreased activity. The present study observed an increase in the activity of peroxidase but a decrease in catalase activity. Although, both the enzymes have the same prosthetic group (iron-porphyrin), the variation in their activities has been previously reported by other workers. The increase of peroxidase enzyme activity suggests that the plant protection against the pollution stress in the industrial area was chiefly managed by this particular (peroxidase) enzyme when other protecting bio-molecule like ascorbic acid and catalase activity failed to cope up to the polluted environment. Peroxidase activity bearing a non-significant negative correlation with chlorophyll content was consistent with the findings reported by Kar & Mishra (1976) while the antagonistic relation between peroxidase activities and stress (both biotic and abiotic) is also supported by Bharwana et al. (2016).

CONCLUSION

The present investigation tried to make a comparative assessment of the biochemical parameters of plants in the industrial area of Hindalco, Hirakud against a control area located at an aerial distance of around 15 km i.e. the campus of Sambalpur University. The results clearly highlight the fact that the plants growing near the industrial area are facing serious stress condition which is reflected from the depletion in their biochemical synthesis. Their defence mechanism (primarily the peroxidase activity in this case) against the polluted environment has led to their sustenance till date. It can be inferred from the study that plants develop their own strategy to counter the pollution stress by either reducing or enhancing some of their biochemical synthesis.

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