International Journal of Botany Studies
ISSN: 2455-541X, Impact Factor: RJIF 5.12
www.botanyjournals.com
Volume 2; Issue 4; July 2017; Page No. 43-48

Morpho-anatomical adaptations in some herbs growing near Ulhas River polluted with industrial effluent

[1] Shital Gharge, *[2] Dr. Geetha Menon

[1] Research student, Botany Department, R.K.T. College, Mumbai University, Ulhasnagar, Maharashtra, India
[2] Associate Professor, Botany Department, R.K.T. College, Mumbai University, Ulhasnagar, Maharashtra, India

Abstract
Ulhas River in the district of Thane flows across six cities and several small villages before joining the Thane creek. The study investigated the influence of water polluted with industrial effluents on the morphology and anatomy of four herbs selected *viz. Alternanthera*, *Amaranthus*, *Chenopodium* and *Eclipta* growing in Ambernath MIDC. Physicochemical parameters of the water from the study site analyzed in two seasons, monsoon (August-September) and winter (January-February) revealed higher values of electrical conductivity (512 and 736 s/m), total hardness (118.15 and 169.44mg/L), chloride (90.19 and 103.3 mg/L), sulphate (253.10 and 494.8 mg/L), nitrate-nitrogen (868.8 and 1198 mg/L) and low DO (0.96 and 0.72 mg/L) respectively. Heavy metals like Lead and Zinc content in the water from the study site (SS) were analyzed using ICP-AES and was found to be very high (Pb -31ppm and Zn-1278 ppm). The changes in external morphology and in the anatomical structures of the plants at the site were significant at $P < 0.05$. In plants from study site features like the appearance of stone cell in the cortical region of both stem and root of *Amaranthus*, sclerenchymatous cell among the chlorenchyma in the hypodermal region of *Chenopodium* stem, may indicate the ability of the plants to adapt to the stress in the environment and eventually can act as bioindicator of heavy element load in the environment. Among the four herbs studied *Amaranthus spinosus* showed greater variation in both morphological and anatomical structures thus indicating best adaptation to the stressful environment.

Keywords: alternanthera, amaranthus, anatomy, chenopodium and eclipta, heavy metals, morphology, stone cells, Ulhas River

Introduction

Population explosion, industrialization and urbanization produces large amount of wastewater in our country. The Maharashtra Industrial Development Corporation (MIDC) has created 265 industrial estates resulting in high GDP and revenues at the cost of environmental degradation, particularly due to the massive concentration of the chemicals industry. India is facing the twin problems of Population explosion and of effluent disposal generated by industries. The effluents are either released into water body or directly on to the lands, which are agricultural. These effluents contain heavy metals as well as nutrients, which affect plants and soil in variety of ways [1]. Water pollution may result from municipal, agricultural or industrial wastes containing organic and inorganic chemical substances, dissolved or suspended solid [2]. More so plants respond to pollution stress and metabolize pollutants differently by varying mechanism of uptake, translocation and accumulation [3].

For the present study, Ambernath MIDC was taken as the study site (SS). The chemical industries release the effluents into the Ulhas River water body flowing across the cities. Ambernath city in the district of Thane, state of Maharashtra, is one of the fast growing and populous city. Ambernath west is in an industrial zone designated as the MIDC (Maharashtra Industrial Development Corporation) which houses hundreds of small and large chemical units. Visit to the site attracted our attention to the reduced size of the plants and of its leaves as compared to the plants of the same kind growing in the vicinity away from the river side. The study was conducted during July 2013 to September 2015 to assess the impact of polluted water on herbs which were growing in the industrial area.

Material and Method

Sample

For the experiment ten quadrates (1 m x 1 m) were laid to record the annual herbs and to select the abundantly growing annual herbs as the experimental plants *viz. Alternanthera sessilis* (L.)Br (Al). *Amaranthus spinosus* Linn. (Am), *Chenopodium album* Linn. (Ch) and *Eclipta alba* (L) Hassk (Ec). The study was conducted for two season *viz.* monsoon (August-September) and winter (January-February) from July 2013 to September 2015 and compared with the same species grown in the Botanical Garden of R. K. Talreja College, Ulhasnagar, as control (C). The experimental samples are abbreviated as Al-SS and Al-C for *Alternanthera sessilis*, Am-SS and Am-C for *Amaranthus spinosus*, Ch-SS and Ch-C for *Chenopodium album*, Ec-SS and Ec-C for *Eclipta alba* collected from study site and control site respectively.

The width of root and stem of the selected plants were measured and the leaf area was calculated (Graphical method) [4]. Anatomical changes were also studied by taking the transverse section (T.S) of root and stem staining with safranin and observing it under compound light microscope at 15X magnification. The result was later analyzed statistically. The water samples were collected in clean containers during working hours and directly taken to laboratory for further analysis of physico-chemical characteristics [Color, pH, Electrical conductivity, Turbidity (Turbidometric method), Total acidity, Total alkalinity (Titrimetric method), DO (Winkler's Iodometric Method), BOD, COD (Titrimetric method), Total hardness (EDTA Titrimetric method), Chlorinity, Salinity (Argentometric method), Sulphate (Turbidometric method) and Nitrate-Nitrogen] using standard methods [5]. Similarly, the soil sample was collected from the rhizosphere zone of the plants and analyzed for soil pH, $CaCO_3$, moisture content and chloride [5] and organic C- content using Standard kit KRANTI provided by BARC. Tap water and garden soil were treated and used as a control samples.

Results and Discussion

The area around the MIDC zone of Ambernath was considered as the study site (SS), Quadrates were laid and the herbaceous plants growing in the vicinity were recorded *viz. Tridax procumbens, Alternanthera sessilis, Amaranthus viridis, Amaranthus spinosus, Cleome viscosa, Cleome burmani, Cyanadon dactylon, Euphorbia hirta, Chenopodium album, Portulaca oleracea, Eclipta alba,* etc. Some plant species were more abundant than the others like *Alternanthera sessilis* L. Br., *Amaranthus spinosus* Linn., *Chenopodium album* Linn. And *Eclipta alba* (L.) Hassk. (Table -1).

The morphological aspects of the plants are a true indicator of its adaptation to surrounding. The average width of the root, stem, leaf area and height of different plants from Study site (SS) and Control (C) site for two seasons (monsoon and winter) were recorded (n=25). (Fig. No. 1, 2 and 3). It was observed that most of study samples showed decreased size of root, stem and leaf area as compared to the control plants from the garden indicating stressful environment in which the plant were growing. Student 't' test was used to evaluate the significance of the morphological data. The width of root in in Al-SS during monsoon (Aug.-Sep.) season and in winter (Jan-Feb) was less than half the size of that seen in the control plants though in the monsoon season, the differences were comparatively lower. The roots of Am-SS, Ch-SS and Ec-SS also showed the similar variation. The roots of Ec-SS were of smaller width than the roots of other plants. (Fig. No.1). The width of root in control plants are considered as normal indicating the basic growth of the plant while the decreased width of root in plants collected from (SS) may indicate stressed environment. This may also account for decreased absorptive capacity by the roots under the stress condition. The variation observed in the roots of Al-SS, Am-SS, Ch-SS and Ec-SS were highly significant at $P < 0.05$ (p value at 0.05%= 2.447) (Table No. 2).

The width of stem also showed variation among the plants of same species collected from SS and from the garden C. Al-SS collected during August-September showed width of stem 40% lesser than Al-C, though variation was comparatively lower (< 16%) in samples collected during winter (January-February). In Am-SS plants showed 41-48% decreased stem width as compared to Am-C collected during Aug-Sep and during winter (January-February) (Fig 1). Similar, observations were noted in Ch-SS stem. Though, in Ec-SS variation observed were small as compared to other plants. All values were highly significant at $P < 0.05$ (p value at 0.05%= 2.447) (Table no. 2). The width of the stem in control plants were treated as normal indicating favorable environment for the growth of the plant. Regular supply of water, minerals, sunlight helps the plants to produce more biomass indicating growth [6].

The leaf area of a plant gives the direct relation to the productivity of the plant. The leaf area in Al-SS did not show much variation when compared to Al-C and also when compared with samples collected during monsoon and winter. Though the leaf area in plants like Am-SS, Ch-SS and Ec-SS was one third the size of leaves of Am-C, Ch-C and Ec-C collected both during monsoon and winter. (Fig. 2). The leaf area is a cumulative effect of the environmental stress. Less leaf area indicates poor supply of the basic requirements like water, minerals for the growth of the plant. Similarly, all plants from Control site and study site plants. In general the height of the plants collected from study site was 20-40% shorter than those collected from garden as control. (Fig. 3). It indicated that most of the samples collected from study site irrespective of the plants species showed decreased growth in terms of the root, shoot (stem) and the leaf. These observations support our assumption that the plants were growing in the stressful environment. The study site from where the plants were collected had many chemical and pharmaceutical industries within the diameter of 5 km. These industries release their effluents into the Ulhas River and the site selected is closer to the stream of the polluted water and continuously irrigated by the polluted water. Hence, it shows the probable chance of the load of various chemical and pharmaceutical compounds in the soil that would pose stress on to the plants to a greater extent. Moreover, shorter height of plant also projects the stressful effect on the metabolism in the plants (Fig.3). The foliar energy is resultant of several reversible and irreversible physiological and biochemical changes.

There were distinct changes observed in the anatomy of the plants too (Plate 1). In the T.S of Al-C stem (Plate 1, Fig A) the hypodermal region shows comparatively more amount of chlorenchymatous cells than that found in the T.S of Al-SS stem. (Plate 1, Fig B). The diameter of cell of the parenchymatous pith was also more in the control plant than that in the stem from study site. There was marked difference in the complete volume of the cells in the T.S of Al-SS root, more volume of xylem vessels as compared to that of Al-C (Plate 1, Fig C and D). Similarly, in the T.S of Am- SS stem and root numbers of stone cells were observed in the section, though not seen in the Am-C stem. (Plate 1, Fig E,F, G and H). This can be attributed to as adaptive features developed by the plant towards the stress in the environment. Studies carried on Okra revealed similar results [7].

T.S. of Stem of Ch-SS showed the sclerenchymatous hypodermal region much thicker layer with reduced cortical zone. (Plate 1, Fig I and J).Also in the T. S. of root of Ch-SS the volume of the cortical zone and the vascular element were lesser as compared to the control plants.(Plate 1, Fig K and L). In the T. S. of Ec-C stem (Plate 1, Fig M) cortical region showed parenchymatous water storage cells though in Ec- SS sample some sclerenchymatous cells were seen among the parenchymatous cortex and the width of the cortex was found to be slightly decreased.(Plate 1, Fig N). Moreover in the Ec-SS root xylem vessels were smaller in size as compared to that observed in Ec-C. (Plate 1, Fig O and P). This can be correlated to the different width of root and stem found in study site and control (Fig. No.1).

Transverse section of stem as well as root of all the plants under study (Plate 1) *viz Alternanthera*, *Amaranthus*, *Chenopodium* and *Eclipta* from study site showed a clear anatomical variation to that of control. The anatomical variations seem to be correlated with the morphological differences observed in the specimen collected from study site and control. Such variation may be considered as the cumulative effect of the pollutants in the environment around the plants. Among the four plants studied vivid variation in both morphological and anatomical structures were observed in *Amaranthus sps* than the other three plants from study site. This variation from the control plant in the *Amaranthus* could be considered as an adaptive measure of the plant to the external stressful environment.

The physico-chemical analysis of water (Table No -3) collected during monsoon (Aug- Sep) and winter (Jan-Feb) period showed very high conductivity (512 and 736 s/m) which is a clear reflection of large number of metals and salts in the water polluted with effluents. Also the acidity was very high (42 and 48mg/L).The water was highly turbid containing huge amount of total solids. (705.4 and 816 FTU). Total hardness (118.15 and 169.44 mg/L), the sulphate content (253.10 and 494.8 mg/L) and the nitrate-nitrogen content (868.8 and 1198 mg/L) were recorded to be very high. These components of the water contribute to the plant nutrients and also help in the growth of the plants. DO of the polluted river water was very low (0.96 and 0.72 mg/L) during the time of collection (Aug-Sept and Jan-Feb) which confirms their highly polluted status and deteriorating condition. Similarly, Chloride (90.19 and 103.3 mg/L) and Salinity (154.07 and 185.93mg/L) were also found to be very high in these study site samples. The pH of the effluent laden soil was recorded to be in acidic range around 6.3-6.5. The Calcium Carbonate percentage was very low (5.4 and 6.8%) than that of control garden soil. This could be attributed to the influence of water that was recorded to be highly acidic on to the soil. The carbon content of the soil is the reflection of suitable soil for cultivation and in the soil from the study site the carbon content was very low. Thus, indicating that the soil being unsuitable for plant growth and in poor condition. Many workers have reported the effect of effluent on the germination of various crop seeds. Mohammad and Khan (1985) have reported adverse effect of textile effluent on the germination of *Phaselous aureus* and *Ablemoschus esculentus* seeds [8]. Dhanam (2009) [9] has documented increased percentage germination of *Paddy* in low concentration of effluent, though higher concentrations were injurious to crop seeds [9].

In the present study, all the observed variations in all sssplants, both in the morphological and anatomical structures may be due to effect of effluent laden soil on the plants at the study site. All the variations noted by the authors were highly significant at P< 0.05. (standard p value at 0.05%= 2.447) when compared to respective plants grown as control. Thus confirming the possibility of positive correlations between physical growth of the plants and the anatomical changes observed to the quality of effluents and its composition (Table No. 2)

Tables and Figures

Table 1: Annual herbs growing at the Study site

S. No.	Name of Plant	Quadrate					Frequency (%)	Density
		I	II	III	IV	V		
1	Tridax procumbens	5	6	3	5	5	100	4.8
2	Alternanthera sessilis	11	10	12	15	10	100	11.6
3	Amaranthus spinosus	15	15	10	5	5	100	10
4	Cleome viscosa	10	5	3	6	2	100	5.2
5	Cynadon dactylon	6	2	3	5	4	100	4
6	Euphorbia hirta	2	5	4	2	3	100	3.2
7	Chenopodium album	8	12	15	15	16	100	13.2
8	Portulaca oleracea	0	1	2	3	4	66	2
9	Eclipta alba	14	10	11	14	15	100	12.8
10	Cleome burmanii	1	2	5	3	4	100	3

Table 2: Statistical analysis of the variation in the root, stem and leaf area of plants from the Study site

S. No.	Plant Name	Calculated p values					
		Aug-Sep			Jan- Feb		
		Root	Stem	Leaf Area	Root	Stem	Leaf Area
1	Alternanthera sessilis (L.)Br.	2.9680	2.4670	2.4905	3.9487	3.5123	2.5936
2	Amaranthus spinosus Linn.	2.5230	2.5015	2.6850	2.7157	2.7231	2.9237
3	Chenopodium album Linn.	2.5052	2.6535	2.7105	2.8953	3.7043	2.8763
4	Eclipta alba (L.) Hassk.	2.6235	2.4545	2.6022	2.7839	2.9739	2.8686

(standard p value at 0.05%= 2.447)

Table 3: Physicochemical Aspect of Water sample collected from the Study Site and Tap Water as control.

S. No	Parameters	Aug-Sept.		Jan-Feb.		CPCB Std. for effluent
		Study Site	Normal	Study Site	Normal	
1)	Color	Light Grey	Colorless	Colorless	Grey Black	-
2)	pH	6.3±0.22	7±0	6±0.5	7±0	6.5-8.5
3)	EC (s/m)	512±0.6	0.66±0.02	736±0.8	0.64±0.006	5.00

4)	Turbidity (FTU)	705.4±6.76	32±1.87	816±77.97	42.4±1.81	-
5)	Acidity(mg/L)	42±2.05	19.90±1.73	48±1.44	28.31±4.90	15.55
6)	Alkalinity(mg/L)	Zero	56±0.74	Zero	64±1.42	20.30
7)	DO (mg/L)	0.96±0.018	5.14±0.02	0.72±0.018	5.03±0.01	-
8)	BOD (mg/L)	0.96±0.02	4.29±0.20	0.4±0.15	3.12±0.61	100
9)	COD (mg/L)	152.6±23.0	4.46±0.42	192.1±1.21	5±1.58	250
10)	Hardness (mg/L)	118.15±4.8	31.21±1.44	169.44±44	41.63±0.03	120
11)	Chloride (mg/L)	90.19±6.07	24.99±2.95	10.3.1±0.0	33.35±0.02	250
12)	Salinity(mg/L)	154.07±29.38	44.65±3.50	185.93±0.46	60.24±0.02	254
13)	Sulphate (mg/L)	253.10±28.75	32.46±0.58	494.8±2.38	50.2±0.01	< 1000
14)	NO_3-N(mg/L)	868.8±10.79	146.08±3.79	1198±3.96	200±1.82	2

The readings are average of 10 observations.

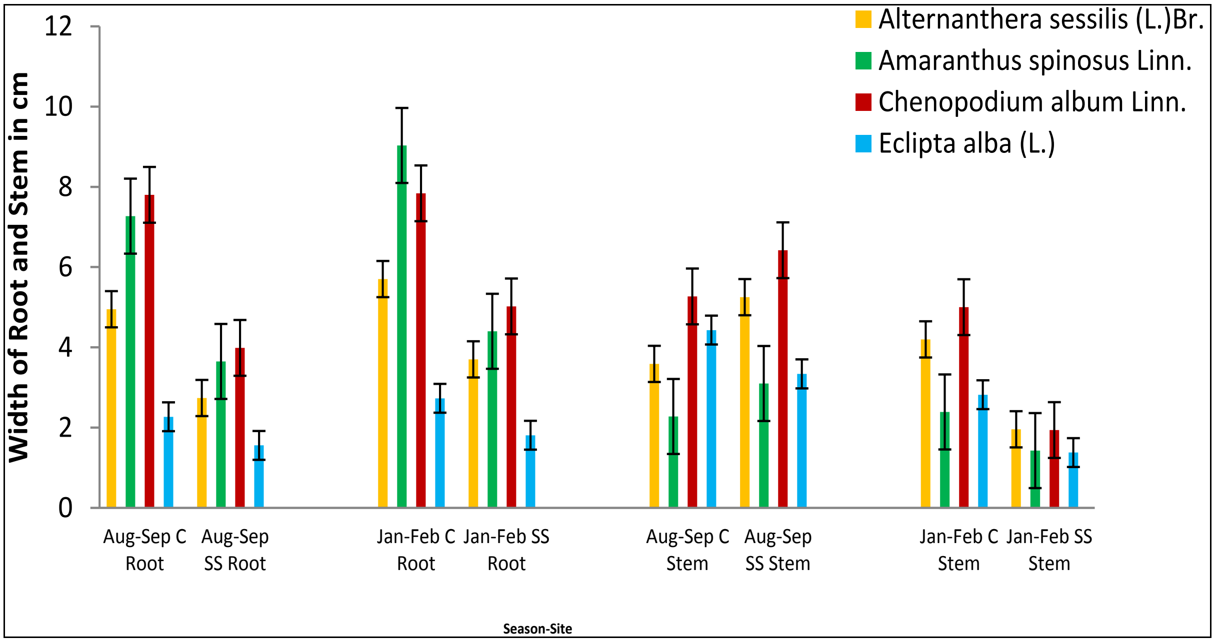

Fig 1: Width of Root and stem of the Plants collected from the Study Site and Garden as Control. (n= 25)

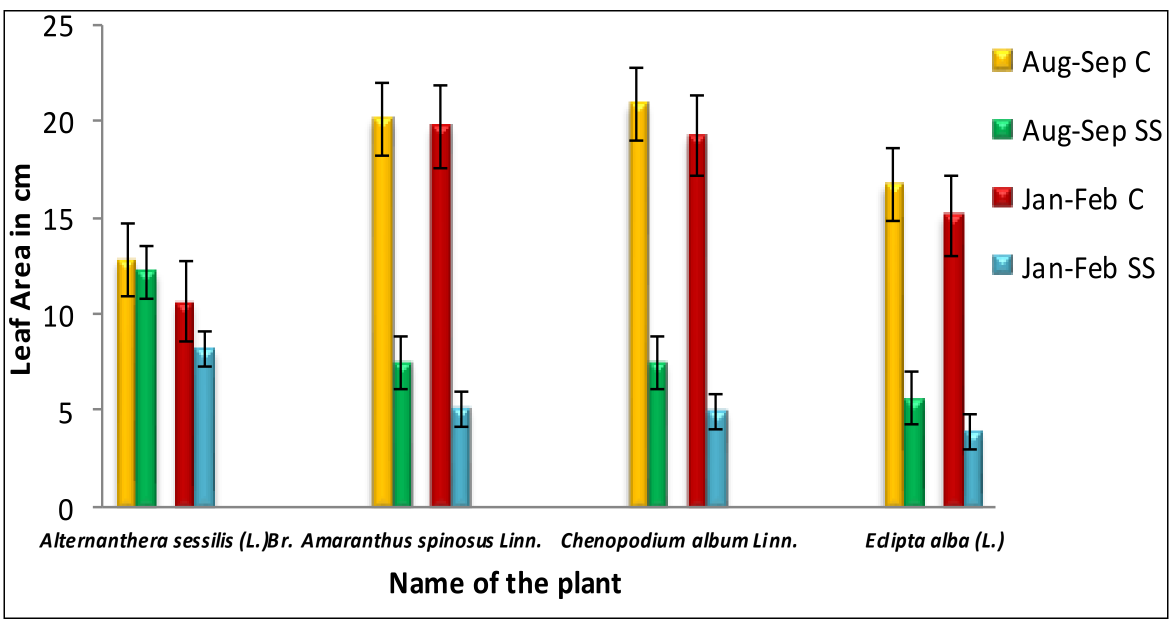

Fig 2: Leaf Area of the Plants collected from the Study Site and Garden as Control. (n= 25)

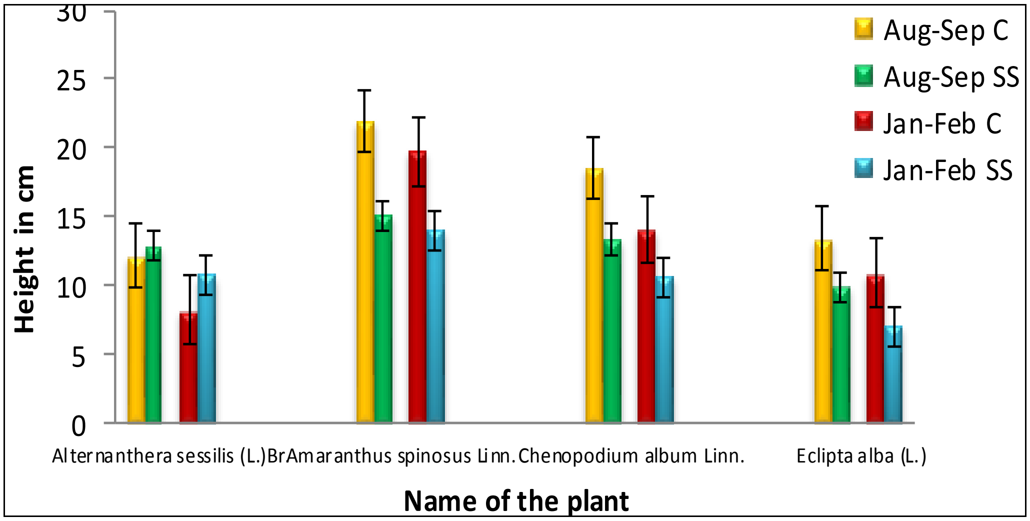

Fig 3: Height of the Plants collected from the Study Site and Garden as Control. (n= 25)

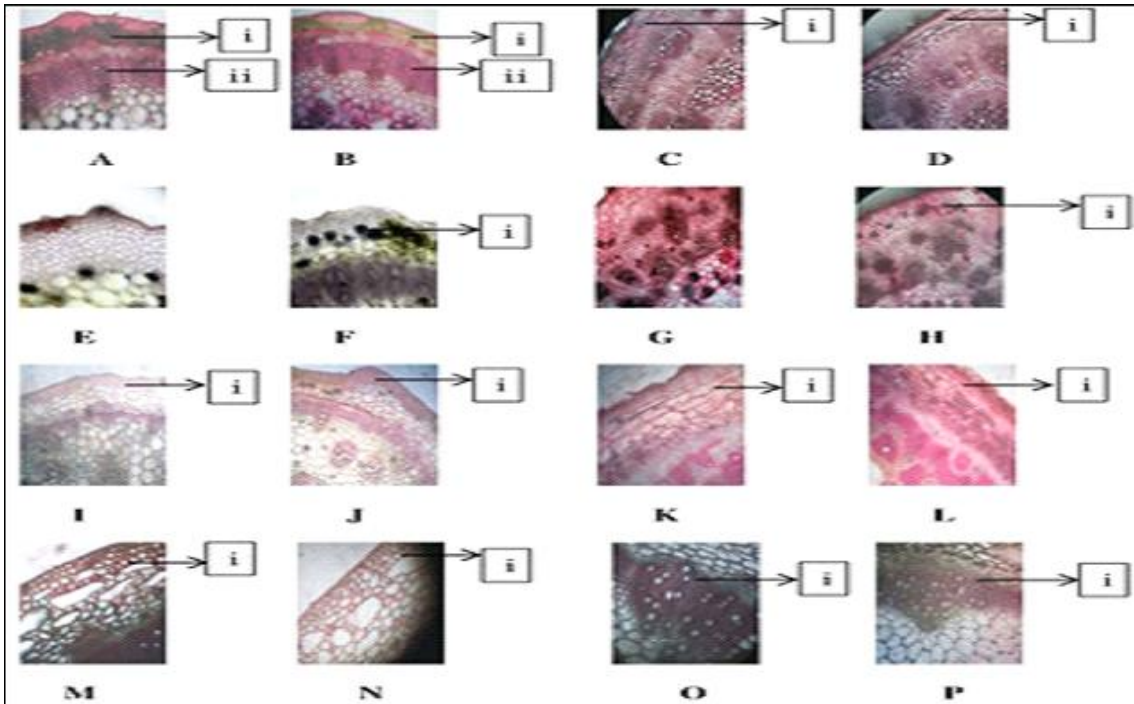

Plate 1. Transverse Section (Stem and Root) of Selected Plants

Fig A- Stem of *Alternanthera sessilis* (Control i-More Chlorenchyma, ii- Large Xylem vessels); **B-Stem of *Alternanthera sessilis*** (Study Site i-Less Chlorenchyma, ii- Small Xylem vessels); **C- Root of *Alternanthera sessilis*** (Control i-Thick Hypodermis); **D-Root of *Alternanthera sessilis*** (Study Site i-Thin Hypodermis);

E- Stem of *Amaranthus spinosus* (Control); **F- Stem of *Amaranthus spinosus*** (Study site i-Stone cells); **G- Root of *Amaranthus spinosus*** (Control); **H- Root of *Amaranthus spinosus*** (Study site i-Stone cells);

I- Stem of *Chenopodium album* (Control i- Thick Scelerenchymatous layer); **J- Stem of *Chenopodium album*** (Study Site1 i- Thin Scelerenchymatous layer); **K- Root of *Chenopodium album*** (Control i- Thick Cortical Zone); **L- Root of *Chenopodium album*** (Study Site1 i- Thin Cortical Zone);

M-Stem of *Eclipta alba* (Control i- Large water storage tissue); **N- Stem of *Eclipta alba*** (Study site i- Small water storage tissue); **O-Root of *Eclipta alba*** (Control i- Large Xylem Vessels); **P- Root of *Eclipta alba*** (Study site i- Small Xylem Vessels)

Fig 4

Conclusions

In study site, prevalence of some weed growing abundantly as dominant species could be related to their tolerance to the pollutant present in their environment. These plants *viz. Alternanthera sessilis* L. Br., *Amaranthus spinosus* Linn., *Chenopodium album* Linn. And *Eclipta alba* (L.) Hassk seems to have developed an adaptive feature towards their survival against the external environmental stress. These plants can be considered as indicators of metal stress and as good model for phytoremediation.

Acknowledgments

We wish to thank the Principal and the staff of Department of Botany, R.K.T. College for the support extended to conduct this work and also express thanks to RSIC, IIT, Powai, Mumbai for extending the facility of ICP-AES to analyze the samples.

References

1. Devagi P, Oblisami G. Effect of paper mill effluent on germination of agricultural crops. J Ecobiol. 2000; 12(4):243-249.
2. Moeller DW. Liquid Waste. Environmental Health, 3rd ed, Harvard University Press, Cambridge, MA, 2004. ISBN-10; 0-674-01494-4:192-214.
3. Rehman A, Bhatti HN, Athar HR. Textile Effluents Affected Seed Germination and Early Growth of Some Winter Vegetable Crops: A Case Study. Water Air soil Pollution. 2008; 198:153-160.
4. Patil S, Bodhe SK. Betel Leaf Area Measurement Using Image Processing. International Journal on Computer Science and Engineering (IJCSE). ISSN: 0975-3397. 2011; 3:2656-2660.
5. APHA. American Public Health Association, 19th edition, 1015 Fifteenth street NW, 1998.
6. Marshall RH. Effects of atmospheric pollutants on the spruce-fir forests of the Eastern United States and the Federal Republic of Germany. Fort Koegh Research Symposium. 1988, 27-36.
7. Uaboi-Egbenni PO, Okolie PN, Adejuyitan OE, Sobande AO. Akinyemi Oa. Effect of industrial effluents on the growth and anatomical structures of Abelmoschus esculentus (okra). African Journal of Biotechnology. 2009; 8(14):3251-3260.
8. Mohammad A, Khan AU. Effect of a textile factory effluent on soil and crop plants. Environmental Pollution. Series A. 1985; 37:131-148.
9. Dhanam S. Effect of dairy effluent on seed germination, seedling growth and biochem parameters of paddy. Bot. Res. Int. 2009; 2:61-63**.**