QUANTIFICATION OF PHOTOSYNTHETIC PIGMENTS OF PLANTS, WATER AND SEDIMENT SAMPLES IN CHIRACKAL AND KATTIPARAMBU OF ERNAKULAM DISTRICT, KERALA

ABSTRACT

Aims : The present study intended to investigate the pigment composition of four selected mangrove plants viz., *Avicennia officinalis, Excoecaria agallocha, Rhizophora mucranata and Sonnaratia alba* and water and sediment samples. And to quantify the concentration of various pigments found in the above samples.

Place and Duration of Study: The samples were collected from the mangrove creeks of Chirackal and Kattiparambu of Ernakulam district, Kerala. Duration of the study was from 2013 December to 2015 December.

Methodology: The estimation of the total pigments, chlorophyll a, chlorophyll b and carotenoid concentration of the biotic samples, water and sediments were done using standard methods in Spectrophotometer.

Results: Plants showed high pigment concentration compared to water and sediments. High chlorophyll 'a'(2 mg/g), chlorophyll 'b'(0.8 mg/g) and total chlorophyll(2.74 mg/g) were observed in *Excoecaria agallocha* of Kattiparambu and carotenoids (0.72 mg/g) observed in *Rhizophora mucranata,* Chirackal. In sediment samples, high chlorophyll 'a'(0.85mg/g), total chlorophylls(1.31mg/g) and carotenoids (0.725 mg/g) were observed in Chirackal area and chlorophyll 'b'(0.595 mg/g) obtained in Kattiparambu. Chlorophyll 'b'(0.6 mg/g) and carotenoids(0.86 mg/g) were reported high in the water samples of Kattiparambu region and chlorophyll 'a'(0.61 mg/g) and total chlorophylls(0.86 mg/g) in Chirackal. In Pearson's correlation coefficient studies, the content of KEA-chlorophyll was found to have a strong positive correlation among other mangrove species and some sediment samples.

Conclusion: Seasonal changes and local geological conditions are the major factors for variations in pigment concentrations in plants, water and sediment samples. Sediment pigments proved to be good indicators of lake-ecosystem response to climate change and long-term variability in the photo trophic community.

Keywords: Chlorophyll, Carotenoids, Pigments, Sediments, Mangroves, Correlation.

1. INTRODUCTION

Total leaf pigment includes chlorophyll-a(chl.a), chlorophyll-b(chl.b) and carotenoids that are necessary for photosynthesis process. Variation in leaf pigments (chlorophylls and carotenoids) and its relation can be due to internal factors and environmental conditions. Chlorophyll and carotenoids content varied with microclimatic conditions in species (1). The ratio of chl.a and chl.b in terrestrial plants has been used as an indicator of response to light shade conditions (2). The small proportion of chlorophyll a/b is considered as sensitive biomarker of pollution and environmental stress (3). Acetone gives very sharp chlorophyll absorption peaks and has great merit as the solvent for assay of chlorophylls (4). Chlorophyll is a pigment that has a clear impact on the spectral responses of plants, mainly in the visible spectrum portion. N is a key element in chlorophyll, therefore is usually a high correlation between them (5).

Previous studies indicated that chlorophyll pigments have antioxidant, anti inflammatory and wound healing properties. It has been observed that chlorophyll pigments contain chlorophyllin which is responsible for increasing the number and activity of dominant immune cells like Bcells, T- cells and macrophages essential to human health (6,7). Photoactive pigments such as chl.a cause distinct changes in the color of water by absorbing and scattering the light incident on water. In deep ocean waters, phytoplankton is usually the predominant constituent and the concentrations of other constituents covary with chl.a concentration. Thus, the optical properties of these waters are dominated by phytoplankton and the observed spectral features in the reflected light can be directly related to chl.a concentration (8,9). In most island, estuarine, and coastal waters, constituents such as suspended solids and dissolved organic matter occur in abundance and their concentrations do not co-vary with chl.a concentration (10,11). This study was designed to investigate the pigment composition of selected mangrove species and water and sediment samples.

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2. MATERIALS AND METHODS

Collection and preparation of Samples : Fresh leaf samples were collected from the mangrove creeks of Chirackal and Kattiparambu of Ernakulam district, Kerala, India from 2013 December to 2015 December and washed thoroughly first with tap water followed by distilled water in the laboratory, kept to dry in room temperature and ground using mortar and pestle (12). Then analyzed for the determination of chlorophylls (Chl.a and Chl.b) and carotenoids content using spectrophotometer. Water sample were collected from three locations of Kattiparambu and Chirackal areas in clean sampling bottles. For chlorophyll estimation, sample was collected from the sub surface water in sampling bottle and add 1 ml saturated $MgCl_2$ per liter of sample and kept in chilled condition, then used for analysis. Sediments also collected in polythene bags from three locations of these two areas, then they were dried, powdered and then used for the analysis.

Estimation of pigments

The amount of chlorophyll present in the leaves was estimated by the standard method. Five hundred milligrams of leaf tissues were ground well using mortar and pestle with 10 ml of 80% acetone and the homogenate was centrifuged at 3000 rpm for 15 minutes and the supernatant was used for pigment analysis. Pigments in water and sediment samples were extracted by adding 10 ml of 90% acetone to the samples and mixed well and kept for overnight at low temperature under dark condition. Then the supernatant was centrifuged at 2000 to 3000 rpm to get clear solution and the solution was used for analysis. Absorbance of the samples was measured at 645nm, 663nm and 480nm in a spectrophotometer. The chlorophylls and carotenoid contents were determined by using following formulas in fresh weight basis,

$$\text{Chlorophyll a} = \frac{12.7 \times A663 - 2.69 \times A645}{a \times 1000 \times W} \times V$$

$$\text{Chlorophyll b} = \frac{22.9 \times A645 - 4.68 \times A663}{a \times 1000 \times W} \times V$$

$$\text{Total Chlorophylls} = \frac{20.2 \times A645 + 8.02 \times A663}{a \times 1000 \times W} \times V$$

$$\text{Carotenoids} = A480 + (0.114 \times A663) - 0.638 \times A645 \ (13)$$

Where,

A - Absorbance at respective wave length

a - Path length of the cell

W - Fresh weight of the sample (g)

V - Volume of the extract (ml)

3. RESULTS AND DISCUSSION

Chlorophyll a (chl a) is a ubiquitous pigment and can be used as a global biomass indicator (14). In Angiosperms (most land plants) there are typically two types of chlorophyll (chl) molecules, namely, chl a and chl b. Both of these pigments absorb photons of light in the blue and red spectral regions, but the specific wavelengths of light they absorb are different. These natural pigments exhibit various beneficial biological activities such as antioxidant, anticancer, anti-inflammatory, anti-obesity, anti-angiogenic and neuroprotective activities(14). Therefore, various natural pigments isolated from plants have attracted much attention in the fields of food, cosmetic and pharmacology(15).In the present study, pigment level of plants gave good results when compared to water and sediments. *E. agallocha* in Kattiparambu showed high range of chl.a and chl.b (2.01 mg/g and 0.804 mg/g) contents. Total chlorophylls were found to be higher in *E. agallocha* (2.74 mg/g) of Kattiparambu, and lower in *E. agallocha* (1.09 mg/g) of Chirackal. Similarly, carotenoids were higher in leaves of *A. officinalis* (0.72 mg/g) and *E. agallocha* (0.76 mg/g) of Chirackal and Kattiparambu respectively, minimum levels of caretenoid was present in *R. mucranata* (0.48 mg/g) of Chirackal compared to other plants (Figure-1). Acetone is known to have a lower extractability of chlorophylls from the protein matrix (16).The change in the carotenoids and tocopherols during seed maturation of *Cassia* species is studied (17). Water and sediment samples of Chirackal showed high chl.a (0.61 mg/g and 0.83 mg/g) and total chlorophyll (1.074 mg/g and1.31 mg/g) contents. High range of chl.b in water (0.61 mg/g) and sediment (0.85 mg/g) was reported from Kattiparambu. High range of carotenoids (0.86 mg/g) reported in Kattiparambu water and sediment carotenoids (0.73 mg/g) from Chirackal (Figures 2 and 3). Chlorophyll capture sunlight and make it available to plant system for photosynthesis (18). Chlorophyll a/b ratio is an index for determining the photosynthetic efficiency of the mangrove plants (19). But, in this study, we claim that ratio between the bound and free forms of chlorophylls can be used as an index for determining the photosynthetic efficiency of the mangrove species. Similar reports have been made earlier in mangrove species, such as *R. apiculata, R. mucronata* and *Avicennia marina* (20,21,22) and pine species (23). Higher content of chlorophyll in reaction centre might enhance the light-induced photosynthetic activity of the chloroplast, thereby high energy transfer (24) and energy production could be assumed.

Figure 1 :Pigment content in Plant Samples(mg/g)

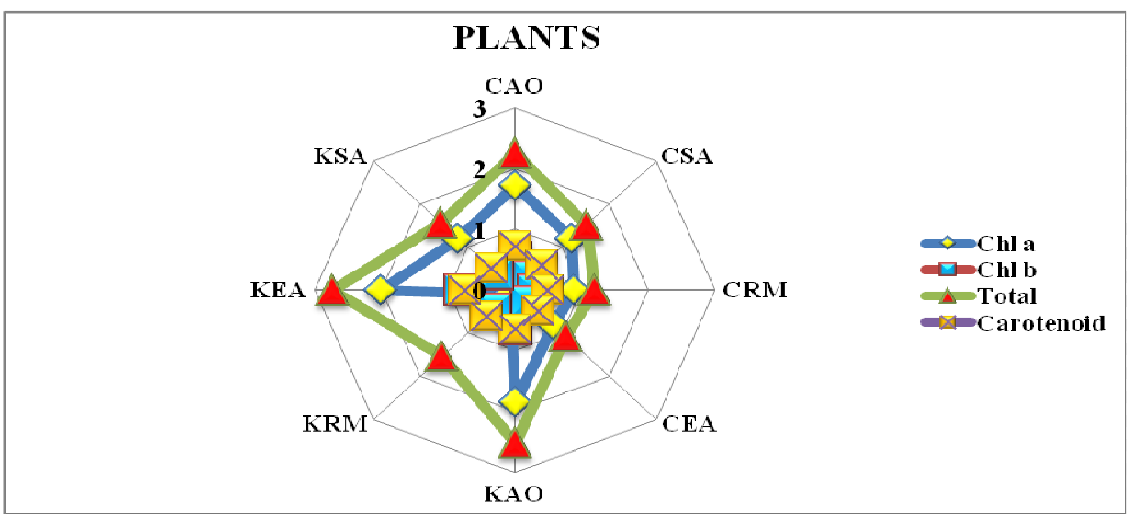

Figure 2 :Pigment content in Water Samples(mg/g)

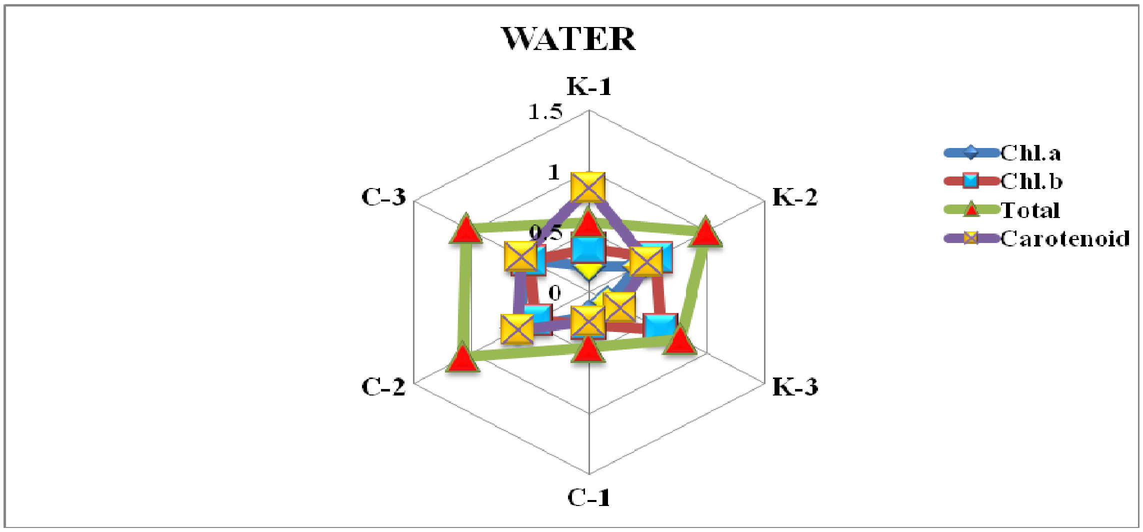

Figure 3 :Pigment content in Sediment Samples (mg/g)

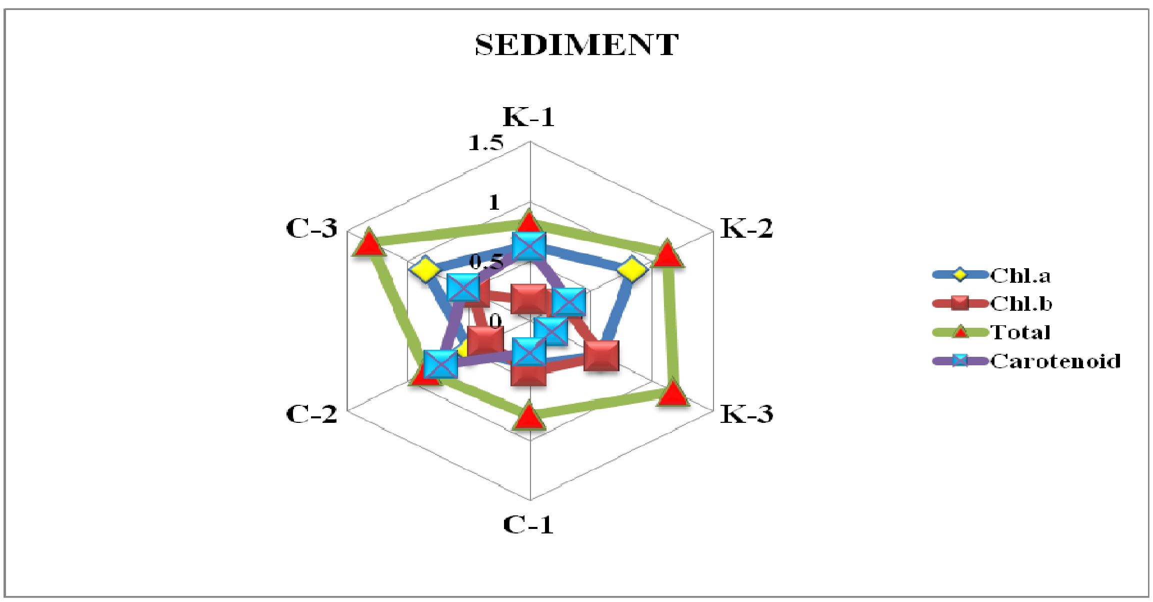

Table 1: Correlation Analysis of plants, water and sediments

	CAO	CSA	CRM	CEA	KAO	KRM	KEA	KSA	SK1	SK2	SK3	SC1	SC2	SC3	WK1	WK2	WK3	WC1	WC2	WC3
CAO	1																			
CSA	0.994**	1																		
CRM	0.994**	0.998**	1																	
CEA	0.984*	0.994**	0.997**	1																
KAO	0.995**	0.978*	0.981*	0.964*	1															
KRM	0.536	0.518	0.575	0.588	0.545	1														
KEA	0.993**	0.975*	0.979*	0.962*	1.000***	0.562	1													
KSA	1.000***	0.994**	0.995**	0.985*	0.995**	0.536	0.993**	1												
SK1	0.793	0.851	0.851	0.886	0.731	0.522	0.726	0.795	1											
SK2	0.999**	0.987*	0.989*	0.975*	0.999**	0.545	0.998**	0.998**	0.763	1										
SK3	0.787	0.720	0.751	0.716	0.838	0.713	0.850	0.785	0.357	0.815	1									
SC1	0.745	0.689	0.732	0.710	0.786	0.861	0.801	0.743	0.425	0.769	0.970*	1								
SC2	0.563	0.613	0.644	0.692	0.510	0.792	0.514	0.565	0.865	0.539	0.342	0.513	1							
SC3	0.970*	0.958*	0.975*	0.969*	0.970*	0.724	0.974	0.970*	0.787	0.972*	0.855	0.861	0.677	1						
WK1	-0.224	0.159	-0.123	0.058	0.282	0.458	0.275	-0.221	0.316	0.251	0.295	0.056	0.680	0.063	1					
WK2	0.612	0.569	0.624	0.618	0.643	0.966*	0.660	0.611	0.431	0.631	0.862	0.959*	0.645	0.780	0.217	1				
WK3	0.247	0.169	0.230	0.205	0.314	0.789	0.336	0.244	0.061	0.284	0.758	0.830	0.252	0.437	0.078	0.874	1			
WC1	0.589	0.552	0.609	0.607	0.615	0.981*	0.633	0.589	0.450	0.606	0.827	0.939*	0.684	0.765	0.283	0.998**	0.862	1		
WC2	0.846	0.845	0.879	0.890	0.837	0.892	0.846	0.847	0.802	0.845	0.783	0.869	0.848	0.945*	0.249	0.882	0.543	0.886	1	
WC3	0.763	0.745	0.790	0.795	0.769	0.955*	0.781	0.763	0.672	0.769	0.829	0.926*	0.798	0.897	0.264	0.958*	0.698	0.961*	0.980*	1

Correlation studies

The result of Pearson's correlation coefficient studies conducted between the pigment contents in Plants, Water and Sediments in Table 1. Plants showed strong positive correlation among themselves, with water and sediment samples and also very strong correlation (0.998 and 0.997) with sediment between the two media implying common source of plants and sediments. The content of KEA-chl was found to have a high positive correlation with the photosynthetic efficiency of mangrove species. There was a strong negative correlation between water and plants (-0.224,-0.221 and -0.123). This suggests that in plants there is less production of pigments in the presence of certain sediments and water or vice versa in a particular condition. Statistical analysis showed significant decrease in chl.a, chl.b and carotenoids of plants by increasing stress conditions of water and soil. The decrease in the photochemical activities of chloroplast caused by water stress can be correlated with the decrease in the accumulation of chlorophyll. A decrease in net photosynthetic rate under water stress is also related to disturbances in biochemical processes of plants, caused by oxidation of chloroplast lipids and changes in the structure of pigments and proteins (25).

Abbreviations

CAO- Chirackal *Avicennia officinalis*

CRM-Chirackal *Rhizophora mucranata*

CEA – Chirackal *Excoecaria agallocha*

CSA – Chirackal *Sonneratia alba*

KAO – Kattipapambu *Avicennia officinalis*
KRM – Kattiparambu *Rhozophora mucranata*
KEA – Kattiparambu *Excoecaria agallocha*
KSA – Kattiparambu *Sonneratia alba*
SK-1 – Sediment of Kattiparambu-1
SK-2 - Sediment of Kattiparambu-2
SK-3 - Sediment of Kattiparambu-3
SC-1 - Sediment of Chirackal-1
SC-2- Sediment of Chirackal -2
SC-3 - Sediment of Chirackal -3
WK-1 – Water of Kattiparambu-1
WK-2 - Water of Kattiparambu-2
WK-3 - Water of Kattiparambu-3
WC-1 - Water of Chirackal -1
WC-2 - Water of Chirackal -2
WC-3 - Water of Chirackal -3
K-1 - Kattiparambu 1
K-2 - Kattiparambu 2
K-3 - Kattiparambu 3
C-1 - Chirackal -1
C-2 - Chirackal -2
C-3 - Chirackal -3

4. CONCLUSION

Results from the above analysis clearly indicate that extraction of photosynthetic pigments depend on chemical nature of bio-molecules (cholorophyll-a, chlorophyll-b and carotenoids). The pigment content was influenced by environmental parameters. Temporal and seasonal changes and local geological conditions may be the reasons for variations in pigment concentrations in plants, water and sediment samples. Sediment pigments proved to be good indicators of lake-ecosystem response to climate change and long-term variability in the photo trophic community, which is needed for predicting possible effects of future climate change. It was also recognized that the quality of the pigment record is highly dependent on the preservation regime in the sediment and water. Increase of pigment concentration accelerate the performance of photosynthesis and carbohydrate metabolism, which help to maintain the balance of ecosystem and the rejuvenation of life as a whole. Therefore further study in this context is recommended.

REFERENCES

1. Shaikh S. D and Dongare M. Analysis of photosynthesis pigments in *Adiantum lunulatum,* Burm. At different localities of Sindhu durg District (Maharastra). *Indian Fern J*. 2008; 25: 83–86.
2. Vicas S. I, Laslo V, Pantea S. and Bandict G. E. Chlorophyll and carotenoids pigments from Mistletoe (*Viscum album*) leaves using different solvents. *Fascicula Biol.* 2010; (2): 213–218.
3. Tripathi A. K and Gautam M. Biochemical parameters of plants as indicators of air Pollution. *J. Environ. Biol*. 2007; 28: 127–132.
4. Ritchie R. J. Consistent sets of spectrophotometric chlorophyll equations for acetone, methanol and ethanol solvents. *Photosynth. Res.* 2006; 89: 27–41.
5. Schlemmer, M. R, Francis, D. D, Shanahan, J. F & Schepers, J. S. Remotely measuring chlorophyll content in corn leaves with differing nitrogen levels and relative water content. *Agronomy Journal*. 2005; *97*(1): 106–112.

6. Rajalakshmi K and Banu N. Antioxidant capacity of chlorophyll in from *Mimosa pudica* by formation of a phosphomolybdenum complex. *International Journal of Frontiers in Science and Technology*. 2014; 2: 1-14.
7. Durgadevi, M and Banu, N. Study of antioxidant activity of chlorophyll from some medicinal plants. Paripex Indian Journal of research. 2015; 4(2): 6-8.
8. Morel A and Prieur L . Analysis of variations in ocean color *Limnol. Oceanogr.* 1977; 22 709–22
9. O'Reilly J E. *SeaWiFS Postlaunch Calibration and Validation Analyses* (*Part 3. NASA Tech. Memo. 2000-206892*) vol 11 (MD: NASA Goddard Space Flight Center). 2000; 49.
10. Carder K L, Chen F R, Cannizzaro J P, Campbell J W and Mitchell B G. Performance of the MODIS semi-analytical ocean color algorithm for chlorophyll-*a Adv. Space Res.* 2004; 33 1152–9
11. Dall'Olmo G and Gitelson A A. Effect of bio-optical parameter variability on the remote estimation of chlorophyll-*a* concentration in turbid productive waters: experimental results *Appl. Opt.* 2005; 44 412–22
12. Kupper, H, Kupper, F and Spiller, M. *In situ* detection of heavy metal substituted chlorophylls in water plants. *Photosynthesis Res.*1998; 58: 123–133.
13. Arnon D. I. Copper enzymes in isolated chloroplasts. Polyphenol oxidase in Beta vulgaris. Plant. Physiol.1949; ,24 : 1-5.
14. Rehman, A.M, Mohamed, M.I. Effect of cement dust deposition on physiological behaviors of some halophytes in the salt marshes of red sea. Egyptian Academic Journal of Biological Sciences. 2012; 3(1): 1-11.
15. Pangestuti, R., and S-K. Kim, (2011). Biological activities and health benefit effects of natural pigments derived from marine algae. J. Func. Food., 3(4): 255-266.
16. Nakamura, A., T. Watanabe, (2001). Separation and determination of minor photosynthetic pigments by reversed-phase HPLC with minimal alteration of chlorophylls. Anal. Sci. 17: 503–508.
17. Zako S. M, W. Akht A. R, Khan S.A and Bhathy M.K. Characterization of *Cassia* seed oil. Proc. Pakistan Acad. Sci. 1986; 23 : 167-172.
18. Rao, A.V. and Rao, L.G. Carotenoids and human health. *Pharmacological Research.*2007; 55: 207-216.
19. Kathiresan, K. & L. Kannan.. Photosynthetic productivity in species of Rhizophora. In: The Mangroves. Proc. Natl. Symp. Biol. Util. Cons. Mangroves Shivaji university, Kolhapur, India. 1985; 262-265.
20. Kathiresan, K. & P. Moorthy. Influence of different irradiance on growth and photosynthetic characteristics in seedlings of Rhizophora species. Photosynthetica. 1993; (29): 143-146.
21. Kathiresan, K. & P. Moorthy. Photosynthetic responses of *Rhizophora apiculata* Blume seedlings to a long chain aliphatic alcohol. Aquat. Bot. 1994; 47: 191-193.
22. Kathiresan, K. & P. Moorthy.. Hormone-induced physiological responses of a tropical mangrove species. Bot Mar. 1994a ;37: 139-141.
23. Krivosheeva, A., S.A. Shavnin, V.A. Kalinin & P.S. Venedikov. Effect of industrial pollutants on seasonal changes of chlorophyll content in scotch pine seedlings. Fiziol. Rastenii. 1991; 38: 162-168.
24. Moorthy, P.& K. Kathiresan. Physiological responses of a mangrove seedling. Biol. Plant. 1993; 35: 577-581.
25. Marcinska, I., Czyczyo-Mysza, I., Skrzypek, E., Filek, M., Grzesiak, S., Grzesiak, M.T., Janowiak, F., Hura, T., Dziurka, M., Dziurka, K., Nowakowska, A., Quarrie, S.A. Impact of osmotic stress on physiological and biochemical characteristics in drought- susceptible and drought-resistant wheat genotypes. Acta Physiol. Plant. 2013; 35, 451–461.