World Applied Sciences Journal 17 (5): 557-565, 2012
ISSN 1818-4952

Physiological Characters of *Populus deltoids* Marsh at Different Seasons in North of Iran

[1]*Mozhgan Farzamisepehr,* [2]*Mahlagha Ghorbanli and* [1]*Mahboobeh Mohammadi*

[1]Department of Biology, Saveh Branch, Islamic Azad University, Saveh, Iran
[2]Department of Biology, Gorgan Branch, Islamic Azad University, Gorgan, Iran

Abstract: *Populus deltoids* Marsh is a fast growing tree which is on high demands as there is an ever-increasing need for wood and its byproducts in the modern society where there is an awareness not to overexploit forests. Seasonal changes can affect physiological and reproduction processes in plants. In the present study, in order to analyze seasonal physiological and biochemical changes in *Populus deltoids* trees in Langaroud region in north of Iran, leaf and root samples of the plant were collected in 3 replicates during spring, summer and autumn 2010. The findings suggested that the features under study changed with the season. In summer leaves, with an increase in the temperature and dryness, there was a significant difference in chlorophyll a and b contents, soluble sugars and catalase and poly phenol oxidase enzymes activities as compared with spring samples. The difference in peroxidase activity was not significant though. In summer root samples, no significant difference was observed in soluble sugars content and catalase and peroxidase enzymes activities compared with spring samples. However, poly phenol oxidase enzyme activity significantly increased during summer in root. In spring, proline content of leaves and roots was significantly increased compared to the summer. Iron, cupper and manganese contents of leaves and roots were significantly higher in spring samples. Finally, iron and manganese concentrations in roots were higher than in leaves while the leaves cupper concentration in spring and summer was higher than that in roots.

Key words: *Populus deltoids* Marsh · Seasonal changes · Proline · Peroxidase · Poly phenol oxidase · Soluble sugars · Catalase · Chlorophylls · Iron · Cupper · Manganese

INTRODUCTION

Seasonal temperature cycle, daylight duration, moisture and wind can have controlling effects on physiological and reproductive processes in plants. External factors including temperature, light, access to water and nutrients together with internal factors such as the level of carbohydrates, hormones and enzymes activities have a bearing on the seasonal growth cycle of trees [1]. The plant growth, productivity and dispersion are strictly controlled by environmental stresses such as frost, drought, salt and generally any factor disturbing water balance in cells. Various plants show different adaptation strategies to reduce harmful effects of these stresses [2]. One of these strategies is reserving osmosis controlling agents such as sugars. Carbohydrates are direct products of photosynthesis process which are used as a source of energy and provide the required carbon for producing new tissues. There are some research studies reporting the effect of seasonal changes on some plants [3]. Stewart and Bannister [4] showed that carbohydrates contents of *Vaccinium* cultivars change with the season as for instance in *Vaccinium myrtillus* and *Vaccinium vitis-idaea,* carbohydrates contents began to increase in early spring and decrease in early winter.

In another study, Kramer and Kozlowski [5] studied seasonal changes in carbohydrates of some temperate region deciduous plants and reported that general hydrocarbon contents reached a peak during the autumn while they shed their leaves and then a minimum during springtime while there is an increase in respiration. Also Sivaci [3] found a cut in the overall carbohydrates contents of 3 varieties of apple trees during springtime believing that the decrease was due to developing the buds and forming new leaves when the reserved carbohydrates turn into structural carbohydrates.

Corresponding Author: Mozhgan Farzamisepehr, Department of Biology, Saveh Branch, Islamic Azad University, Saveh, Iran.

Exposed to unfavorable conditions, plants will experience oxidative stress which due to generation of reactive oxygen species (ROS) will affect their growth [6]. Through the activity of enzymatic antioxidant systems including superoxide dismutase, peroxidase, ascorbat peroxidase, catalase, poly phenol oxidase and glutathione reductase, plants fight against stress [7]. Activity of peroxidase and poly phenol oxidase enzymes in pistachio trees increases in winter when trees are dormant due to the cold weather and its resulting oxidative stress. This increase helps the trees to tolerate the cold and resist it. The activity of these enzymes decreased to the minimum during the late winter and early spring [8].

Proline is a compound that concentrates in plants under biological and non-biological stresses such as low temperature, maintaining osmotic pressure and keeping the membrane and protein structure in the plant. Activities of antioxidant enzymes such as catalase and peroxidase together with proline content of the leaf in the Sabina evergreen woody plants increased as a result of the low temperature in the winter and the increase is a prerequisite of their adaptation to winter cold [9]. As a photosynthesis pigment, chlorophyll is an important compound in plants' photosystem. There is a positive relationship between photosynthesis and chlorophyll content of the plants. In fact, higher chlorophyll content in the plant can absorb and transport more energy from the light and therefore increase the plant's photosynthesis efficiency. Change in photosynthesis pigments content is directly associated with biomass production [10]. The study of chlorophyll content in 4 plant varieties in Ziarat region in Pakistan showed a reduction during the dry months which may be attributed to higher decomposition ratio of chlorophyll to its synthesis under drought conditions [11].

Heavy metals are defined as elements with metallic properties (ductility, heat and electricity conductivity and stability), atomic numbers higher than twenty and masses more than 5 $gr.cm^3$. Some of these elements, namely, Fe, Mn, Mo, Ni, Zn, Cu and Co are essential micronutrients engaged in redox reactions, electron transportation and other important metabolic processes in natural growth. Some other heavy metals such as Hg, Cr, Cd and Pb are classified as nonessential metals which have generally higher toxic effect on the plants [12]. These pollutants generally pass into the environment through urban, agricultural and industrial activities [13]. Heavy metals hinder physiological processes such as respiration, photosynthesis, lengthwise growth of the cell, water connection of the plant, Nitrogen metabolism and mineral nutrition [14]. Zalesny *et al*., [15] suggested that *Populus deltoids* Marsh because of consuming much water; growing fast and its deep root system is an ideal plant for phytoremediation. Assimilation and reserving heavy metals by plants depends on [1] the nature and properties of the metal, [2] properties of the soil and [3] properties of the plant itself [16]. Heavy metal concentration can also be associated with seasonal changes. Martin and Couphtrey [17] measured the highest and lowest heavy metal concentrations during the spring and winter respectively. Brekken and Stennes [18] on the other hand recorded the highest and lowest contents of metals such as Zn, Si and Pb in autumn and spring respectively.

Poplar trees are dioecious deciduous broadleaf trees that belong to a big family of trees called Salicaceae. There are 25-35 species mostly found in the northern hemisphere. *Populus deltoides* Marsh is one of these species that original habitat of which is around Mississippi River in North America and deltas with soil and weather conditions similar to those around the Sefidrud River in Gilan. This is why these trees are one of the clones adapted to the weather conditions in Gilan Provinse in Southern Caspian Sea region. In the present study, in order to delve into the physiological tolerance of poplar trees under temperature and climatic changes due to the seasonal variations, physiological and biochemical changes of *Populus deltoides* Marsh plants in Langarud region in north of Iran was studied.

MATERIALS AND METHODS

Leaf and root samples of *Populus deltoides* Marsh tree were randomly collected from 4 points of a farmland with 2000 m^2 in Qazi Mahalleh, Shalman, located in central region of Langarud, Iran during spring (May), summer (August) and autumn (early October before the leaves change color) 2010. After the samples were washed a part of them were oven dried at 60 °C for 48 h and the other part were kept in-19 °C for laboratory analysis. Soil samples were also collected from 20 cm below the surface of the same points of the farmland and sent to soil laboratory for relevant analyses (Table 1). Table 2 shows the climatic features of the region under study at different seasons.

Measuring Chlorophylls Contents [19]: According to this method, 0.2 g of fresh leaf was weighted and it was ground in Chinese mortar containing 80% acetone. Then 5 ml Acetone was added to it and solution volume was reached to 15 ml. 3 ml of this solution was poured in cuvette and its absorption intensity was read in

Table 1: Soil characteristics of the region in three seasons

Soil characteristics	Test Type	Test Method	Spring	Summer	Autumn
EC dS⁄m	electrical conductivity	Saturated extract	1.2	0.7	0.77
pH	acidity	Total saturation	7	7.2	7.1
T. N. V. %	neutralizable substances	titration	9.9	4.7	8.5
O.C. %	organic carbon	Walky-Black	1.2	0.76	0.88
Total N %	total nitrogen	Kjeldahl method	0.1	0.07	0.08
P (ppm)	absorbable phosphorus	Olsen	25.4	23.3	54.5
K (ppm)	absorbable potassium	Flame photometer	384	362	371
Clay %	clay	Hydrometer	17	14	15
Silt %	silt	Hydrometer	25	24	25
Sand %	sand	Hydrometer	58	62	60
texture	-	Hydrometer	fine sand	fine sand	fine sand
Fe (ppm)	ferrous	Atomic	30.1	30.9	33.2
Cu (p pm)	cupper	Atomic	1.82	2.6	1.8
Mn (ppm)	manganese	Atomic	4.54	3.84	4.22

Table 2: Evaporation, precipitation and wind speed in the region under study (2010)

Month	Total Evaporation (mm)	Total Precipitation (mm)	Total Wind Speed (km/h)	Average Temperature (°C)
October	27.3	220	222	18.8
November	11	94.5	301	15
December	9.2	140.5	330	8.9
January	11	27.5	334	10
February	9.6	94.5	481	7.1
March	14.2	101	667	9.8
April	34.1	73	700	11.9
May	40.9	84.5	526	16.6
June	115.3	0	476	24.8
July	132.5	71	535	27.8
August	148.7	21	561	28.2
September	69.7	63	395	24.3
annual	623.5	990.5	5527	16.9

663 and 647 nm by Spectrophotometer. For regulating spectrophotometer, 80% Acetone was used as control. Pigments contents were determined in terms of mg/g leaf fresh weight.

Proline Assay: The method suggested by Bates *et al*. [20] was used to measure the proline content. In brief, 100 mg of frozen plant material was homogenized in 1.5 ml of 3% sulphosalicylic acid and the residue was removed by centrifugation. Two ml of glacial acetic acid and 2 ml of acid ninhydrin (1.25 g ninhydrin warmed in 30 ml glacial acetic acid and 20 ml of 6 M phosphoric acid until dissolved) were added to 100 µl of the extract for 1 h at 100 °C and the reaction was then completed in an ice bath. The reaction mixture was added 1 ml toluene. The mixture was warmed to room temperature and its optical density was measured at 520 nm. The amount of proline was determined from a standard curve in the range of 20-100 µg.

Enzyme Extraction: Two grams of frozen (liquid nitrogen) cress leaves were homogenized in cold 0.05 M phosphate buffer at pH 7 containing 0.5 g polyvinylpolypyrrolidone (PVP) in a 1:5 tissue to buffer ratio using an ice cold mortar and pestle. The extracts were used for assaying the activities of Peroxidase and Catalase. The homogenates were mixed on a vortex mixer and insoluble materials separated by centrifugation at 14,000g for 20 min at 4°C. All extracts were stored at-70 °C until assayed.

Peroxidase and Polyphenoloxidase Assay: 20 µl of the extract from each sample was added to 3 ml of assay mixture, consisting of solution of 0.1 M sodium phosphate buffer (pH 6.0), 1 mM hydrogen peroxide and 0.1 mM O-methoxyphenol (guaiacol). The mixture was mixed thoroughly and the increase in absorbance was monitored at 470 nm using a spectrophotometer (Spectronic_ 20 GenesysTM) for 1 min. Peroxidase activity was expressed as change in absorbance min^{-1} g^{-1} of fresh tissues.

The activity of polyphenol oxidase was determined spectrophotometrically in a reaction mixture (3 mL) containing 20 mmol L^{-1} sodium phosphate (pH 6.5) with 25 mmol L^{-1} pyrocatechol and the enzymatic extract (0.01 mL). The change in absorbance was monitored at 410 nm.

Catalase Assay (Cat): The catalase activity was assayed by Chance and Maehli [21] method with the following modification: 5 ml of assay mixture for catalase activity contained: 300 µM of phosphate buffer (pH 6.8) 100 µM of H_2O_2 and 1 ml of the twice diluted enzyme extracted. After incubation at 25°C for 1 min, the reaction was stopped by adding 10 ml of 2% (v/v) H_2SO_4 and the residual H_2O_2 was titrated against 0.01 N of $KMnO_4$ until a faint purple color persisted for at least 15 sec. One unit of catalase activity is defined as the amount of enzyme which breaks down 1 µ mol of H_2O_2/min under the described assay condition.

Soluble Carbohydrate Determination: Soluble carbohydrate analysis was performed using a method described by Kochert [22]. Dry weight of shoot and root parts of collected samples were used for separation of carbohydrate. 40 ml ethanol (80%) was gradually added to about 0.1 g plant material and the mixture was heated up in water bath for 1 hour then centrifuged at 2500 rpm for 10 min. About 2 ml of the prepared sample was vigorously shaken with 2 ml of phenol solution (5%) and after addition of 5 ml concentrated Sulphuric acid, optical density of the sample was measured at 700 nm.

Measuring Fe, Cu and Mn Content: Dried samples of root and leaf (1g each) were separately put into Crucible Porcelain and then kept in an oven at 400 °C for 5h to obtain ashes. Afterwards, they were moved out of the oven to cool. The ashes were then dissolved in 5 ml Hcl (2N) and passed through paper filter. Fifty ml of the filtered solution was then obtained to measure its Iron, Cupper and Manganese contents using atomic absorption equipment Biotech (Model Phoenix 986). One way ANOVA was used as the statistical test with SPSS to analyze the data. Difference in Means were also compared through LSD test ($p<0.05$) and the relevant graphs were drawn with Microsoft Excel.

RESULTS

The highest and lowest chlorophyll a contents were observed in summer and spring samples respectively. The difference was significant ($p<0.05$). The highest chlorophyll b content was however recorded in autumn which was not meaningfully different from that in summer samples ($p<0.05$). As with chlorophyll a, the lowest chlorophyll b content was observed in spring which was meaningfully different from summer and autumn samples (Table 3).

The highest leaf soluble sugars contents were observed in summer which was meaningfully different from those in the spring and autumn samples ($p<0.05$). In roots however, the highest and lowest soluble sugars contents were recorded in the autumn and spring samples

Table 3: The contents of leaf chlorophylls of *Populus deltoids* Marsh in different seasons

seasons Chl contents (mg g^{-1}FW)	Spring	Summer	Autumn
Chl a	0.425±0.032[b*]	1.037±0.060[a]	1.007±0.025[a]
Chl b	0.160±0.009[b]	0.459±0.025[a]	0.489±0.022[a]

*Different letters in each row indicate significant difference at P<0.05

FW: Fresh Weight

Table 4: The soluble sugars and proline contents of *Populus deltoids* Marsh in different seasons

seasons Sugars and proline	Spring	Summer	Autumn
Soluble sugars (µM g^{-1}FW) leaf	39.94±2.775[c*]	72.44±5.189[a]	30.15±0.726[c]
Soluble sugars (µM g^{-1}FW) root	15.822±0.96[c]	20.01±1.119[ac]	23.695±3.227[a]
Proline (mg g^{-1}FW) leaf	41.055±1.181[b]	25.682±0.765[c]	32.257±1.224[a]
Proline (mg g^{-1}FW) root	54.202±1.22[b]	33.97±1.315[c]	45.59±0.818[a]

*Different letters in each row indicate significant difference in level P<0.05

FW: Fresh Weight

Table 5. The enzyme activity of *Populus deltoids* Marsh different organs in different seasons

Seasons Enzyme activity (unit mg^{-1} protein)	Spring	Summer	Autumn
Catalase leaf	13.49±1.38 [b*]	22.65±1.93 [a]	16.27±1.26[b]
Catalase root	5.51±0.80 [b]	4.23±0.19 [b]	4.92±0. 25 [b]
Peroxidase leaf	13.16±1.89 [b]	16.3±0.81[b]	13.81±0.52[b]
Peroxidase root	4.95±0.463 [a]	4.36±0.13 [ab]	3.46±0.12 [b]
Poly phenol oxidase leaf	5.22±0.48 [b]	7.58±0.19[a]	11.47±1.01 [c]
Poly phenol oxidase root	3.79±0.44 [b]	4.86±0.24 [a]	3.25±0.18[b]
Catalase leaf	13.49±1.38 [b]	22.65±1.93[a]	16.27±1.26 [b]
Catalase root	5.51±0.80[b]	4.23±0.19[b]	4.92±0. 25 [b]
Peroxidase leaf	13.16±1.89 [b]	16.3±0.81[b]	13.81±0.52[b]

*Different letters in each row indicate significant difference in level P<0.05

Table 6: The elements content at different organs of *Populus deltoids* Marsh in different seasons

Seasons element (mg g^{-1} DW)	Spring	Summer	Autumn
Fe leaf	7.54±0.273 [c*]	5.06±0.085 [d]	4.18±0.255 [e]
Fe root	37.32±0.475 [a]	27.74±0.521[b]	23.24±0.71 [b]
Cu leaf	2.587±0.129 [f]	1.16±0.053 [g]	1.40±0.086 [g]
Cu root	2.002±0.065 [f]	1.08±0.046 [g]	1.76±0.099 [g]
Mn leaf	1.889±0.129 [h]	0.54±0.033[i]	1.16±0.066 [h]
Mn root	2.108±0.099 [h]	0.78±0.054 [i]	1.22±0.12[h]

*Different letters in each row indicate significant difference in level P<0.05.

DW: Dry Weight

respectively where the differences were meaningful at (p<0.05). Results also revealed that soluble carbohydrate contents in leaves were higher than those in roots. As for proline, the highest and lowest contents in leaves and roots were observed in spring and summer, respectively and the differences between the samples of all 3 seasons were statistically meaningful (p<0.05). Additionally, proline content in roots was higher than that in leaves during the 3 seasons of study (Table 4).

Similarly, antioxidant enzymes activities showed seasonal variations and for peroxidase and catalse enzymes, the highest and lowest activities in leaves were recorded in summer and spring samples. While the difference between the highest and lowest catalase contents was meaningful, in the case of peroxidase the difference was not statistically significant. In roots, no meaningful difference was observed between catalase activities in different seasons. Peroxidase enzyme activities were also not meaningfully different in spring and summer samples. However, there was a meaningful reduction in peroxidase activities in autumn samples. Also, polyphenol oxidase activity in leaves showed a meaningful increase from spring to autumn. The highest polyphenol oxidase activities in roots were also observed in summer (Table 5).

Finally, ferrous, cupper and manganese contents in spring samples showed meaningful increase in leaves and roots. As for ferrous, the lowest content both in leaves and roots were recorded in autumn. On the other hands, the lowest cupper and manganese contents in both leaves and roots were observed in summer. Ferrous and manganese contents in roots were generally higher than in leaves and concentration of cupper in spring and summer leaves were more than roots in the same seasons (Table 6).

DISCUSSION

Chlorophyll content is an important factor for photosynthesis capability in plants [23]. This study showed that chlorophyll content in poplar trees changes with the season so that the highest and lowest contents of chlorophyll a were observed in summer (August) and spring (May). As Table 3 suggests, during the months when samples were collected, August and May had maximum and minimum temperatures respectively. This means that temperature, as the one of main factors, increased in summer, so did the chlorophyll a content in leaves and again as temperature fell in autumn (October), the chlorophyll content also declined. However, the drop

in chlorophyll a level was not statistically meaningful. The maximum level of chlorophyll a content recorded for summer in this study is in line with Kaleem *et al.* [24] who showed similar trends in sunflower plant. Similar trends were also reported by Kingston *et al*. [25] who found that leaves of the maize plants grown in lower temperature had less chlorophyll content than those grown in higher temperature. Chlorophyll a and b contents were lowest in May when the average temperature was at its lowest point.

During the cold, electron transportation from photo system II to photo system I and the main electron recipient ($NADP^+$) is disturbed. At this point, high chlorophyll content boosts production of active oxygen (ROS) which has destructive effects on chloroplast and the cells. One of the strategies adopted by plants to reduce ROS is increasing the activities of an enzyme called chlorophyllase which decomposes chlorophyll and results in a drop in the plants' chlorophyll content [26].

As for chlorophyll b, the highest content was recorded in autumn although the difference with summer samples was not meaningful. This is similar to the study on 4 plant species reported by Aziz [11] who found that chlorophyll synthesis increases during the wet season when the moisture in the soil is high (As Table 2 shows, rainfall in October is higher than in May).

The soluble sugars content in the study also varied with the change of season as the highest sugar content in leaves were observed in summer with maximum temperature and minimum rainfall in the region (the dry season). Drought has severe effects on the plants' photosynthesis process. It slows down electron transportation and disrupts formation of the substances needed for photosynthesis, e.g., it affects carbohydrate contents of the plants. During the drought, complex carbohydrates decompose into simple ones and as a result soluble sugars are increased. Soluble sugars play an important role in retaining osmotic balance of the cells under drought and low temperature circumstances [27]. The finding of this study, i.e., the rise in simple carbohydrates during dry summer season was similar to a study on *Lonicera japonica* [28] that showed soluble sugar contents increases under dry conditions. This is also in line with Sircelj *et al*. [29] who found that soluble sugars content in two apple cultivars increased under average dry conditions.

Souza *et al.,* [30] observed the highest soluble carbohydrates in a variety of grass in Brazil in July (summer) which was again in line with the findings of the present study. However, the summer rise in soluble carbohydrates levels in deciduous poplar trees observed in this study, disagrees with an earlier report by Kramer and Kozlowski [5]. Studying seasonal variations in carbohydrates contents of some deciduous trees in mild climate, Kramer and Kozlowski [5] observed that total carbohydrates levels in trunks and branches were at their peak in autumn as the trees were shedding leaves. On the other hands, the spring reduction in soluble carbohydrates content observed in the present study, agrees with Kramer and Kozlowski [5] and also Sivaci [3] who worked on the trunks of 3 apple varieties. While Kramer and Kozlowski attributed reduction of carbohydrate contents in spring to increased respiration and consumption of these carbohydrates for the growth of new tissues, Sivaci [3] associated this with opening the buds and formation of new leaves. The highest and lowest soluble carbohydrates in roots were observed in autumn and spring respectively. Moreover, there was more reduction in summer soluble carbohydrates content in roots than leaves as compared with spring and autumn samples. In dry circumstances, the root system dehydrates the surface layers of the soil (90% of the extractable water at 30 cm below the surface is absorbed by roots in summer). As a result, water is absorbed from deeper layers of the soil and roots close to the surface die away [31].

As for the proline content, the findings showed meaningful variation with the change in season in both roots and leaves. The highest proline content was observed in spring (May) when the temperature was lowest compared with August and October. Also the lowest proline content was recorded in summer (August) as the temperature was at peak. Proline is a compound that accumulates in plants during abiotic stresses such as low temperature. In fact, plants are equipped with various defensive mechanisms to cope with various stresses. Under stress, e.g. cold, high concentrations of compatible solutes build up. These compounds while do not change physiological pH and are not toxic, are highly concentrated and maintain osmotic pressure and at the same time stabilize the structure of protein and the membrane under stress. As such, they play an important role in the adaptation of cells to various stresses. Proline is an example of such compounds whose synthesis is remarkably increased under cold stress [32]. The findings of this study agrees with Alia and Saradhi [33] and Ashraf and Foolad [32] who also reported that proline content remarkably increased under cold stress.

Antioxidant enzymes activities in this study also showed variations with the change in season. As it was mentioned before, the highest temperature and lowest precipitation in the region under study occur in summer. Accordingly, the findings suggested that antioxidant enzymes activities increased in summer as the region experienced dry conditions. In these circumstances, when there is maximum radiation, closing stomata as a reaction to water or temperature stress reduces CO_2 fixation while photo reaction and electron transportation is still carried out normally. In this state, there would be a deficit in NADP to receive electrons. Therefore, oxygen can act as an electron recipient agent [34]. This would result in production of Reactive Oxygen Species (ROS) [35] which in turn damage many cellular compounds such as lipids, proteins, carbohydrates and nucleic acids [36]. Prochazkova *et al*. [7] argued that in order to mitigate the oxidative damages of drought stress, plants increase the activity of their enzymes such as catalase, peroxidase and polyphenol oxidase. Sairam and Srivastave [37] showed a strong relationship between environments induced oxidative stress tolerance and the increase in antioxidant enzymes activities in photosynthesizing plants. Sharma and Dubey [38] reported that imposing a mild drought on rice plant increased peroxidase enzyme activities. This was also the case with a study on wheat plant [39] which was an increase in the activities of antioxidative enzymes such as catalase and peroxidase under drought stress. Similarly, Egert and Tenini [40] observed that in *Allium schoeenoprasum* 9 days after irrigation was cut, peroxidase enzyme activites increased and this is in line with findings of the present study showing that antioxidant enzymes' activities increased in dry summer. However, the same researchers found that activities and contents of antioxidant enzymes increased with a decrease in temperature and this was counter to findings of Chen *et al.,* [9].

Finally, this study showed that ferrous, cupper and manganese contents in poplar trees also vary with the season. The highest concentrations of all these elements both in roots and in leaves were observed in spring. This is in line with the study reported by Martin and Couphtrey [17] who also found that the highest heavy metals contents were seen in spring. Cupper can highly affect plant cells, namely, it can affect chlorophyll synthesis and electron transportation and also it has a toxic effect on early photosynthesis reactions [41]. This study which showed a cut in chlorophyll contents during the spring when cupper content was maximum agrees with Yruela [41]. Also low catalase and peroxidase activities in the present study during spring agrees with the study reported by Chaoui and Ferjani [42] who argued that an increase in cupper content hinders the activities of these enzymes.

REFERENCES

1. Forshey, C.G. and D.C. Elfving, 1989. The relationship between vegetative growth and fruiting in apple trees. Hort. Rev., 11: 229-287.
2. Palva, E.T., S. Thtiharju, I. Tamminen, T. Puhakainen, R. Laitinen, J. Svensson, E. Helenius and P. Heino, 2002. Biological mechanisms of low temperature stress response: cold acclimation and development of freezing tolerance in plants. JIRCAS Working Report No.23, Genetic Engineering of Crop Plants for Abiotic Stress (Iwanaga M, ed.) pp: 9-15.
3. Sivaci, A., 2006. Seasonal changes of total carbohydrate contents in three varieties of apple (*Malus sylvestris* Miller) stem cuttings. Scientic Hort., 109: 234-237.
4. Stewart, C.R. and P. Bannister, 1973. Seasonal changes in carbohydrate content of tree. *Vaccinium* sp. With particular reference to *V. uliginosum* and its distribution in the British Isles. Flora, 162: 134-155.
5. Kramer, P.J. and T.T. Kozlowski, 1979. Physiology of woody plants. Academic press, London, pp: 258-274.
6. Mittler, R., S. Vanderauwera, M. Gollery and F.V. Breusegem, 2004. Abiotic stress series.Reactive oxygen gene network of plant. Trends Plant Sci., 9(10): 490-498.
7. Prochazkova, D., R.K. Sairam, G.C. Srivastava and D.V. Singh, 2001. Oxidative stress and antioxidant activity as the basis of senescence in maize leaves, Plant Sci., 161: 765-771.
8. Pakkish, Z., M. Rahemi and A. Baghizadeh, 2009. Seasonal changes of peroxidase, polyphenol oxidase enzyme activity and phenol content during and after rest in Pistachio (*Pistacia vera* L.). flower buds. World Applied Science Journal, 6(9): 1193-1199.
9. Chen,Y., M. Zhany, T. chen, Y. Zhang and L. An, 2006. The relationship between seasonal changes in anti-oxidative system and freezing tolerance in the leaves of evergreen woody plants of sabina. South African Journal of Botany, 72: 272-279.
10. Jaleel, C.A., P. Manivannan, A. Wahid, M. Faroog, R. Somasundaram and R. Pannerselvam, 2009. Drought stress in plants: A review on morphological characteristics and pigments. composition. Int. J. Agric. Biol., 11: 100-105.

11. Aziz, I., 2007. Seasonal flux in water potential, chlorophyll and proline content in plants at Ziarat Valley Balochistan, Pakistan. Pak. J. Bot., 39(6): 1995-2002.
12. Sebastiani, L., F. Scebba and R. Tongetti, 2004. Heavy metal accumulation and growth responses in poplar clones Eridano (Populus deltoides x maximowiczii) and I-214 (P. x euramericana) exposed to industrial waste.Environmental and Experimental Botany, 52: 79-88.
13. Clements, S., 2001. Molecular mechanism of plant metal tolerance and homeostasis. Planta, 212(4): 475-486.
14. Zornoza, P., S. Vazquez, E. Esteban, Fernandez-M. Pascual and R. Carpena, 2002. Cadmium-.stress.in nodulated with lupine: strategies to avoid toxicity. Plant Physiology and Biochemistry, 40: 1003-1009.
15. Zalesny, R.S., H. Wiese, E.O. Bauer and D.E. Riemenschneider, 2006. Sapflow of hybrid poplar (*Populus nigra* L.×P. Maximowiczii A. Henry 'NM6') during phytoremediation of land fill leachate. Biomass and Bioenergy, 30(8-9): 784-793.
16. Baragagli, R., 1998. Chemical elements and plant life. In: R. Baragagli, (Ed.), Trace elements in Terrestrial Plants-An Ecophysiological approach to Biomonitoring and Biorecovery. Springer, New York, pp: 324.
17. Martin, M. and P. Couphtrey, 1982. Biological monitoring of heavy metal pollution. Applied sciences publications, London/New York.
18. Brekken, A. and E. Steinnes, 2004. Seasonal concentration of cadmium and zinc in native pasture plants. Consequences for grazing animals. Science of the Total Environment, 326(1-3): 181-195.
19. Arnon, O.I., 1949. Copper enzyme in isolated chloroplast, polyphenol oxidase in *Beta vulgaris*. plant physiology, 24: 1-15.
20. Bates, L., P.P. Waldren and J.D. Teare, 1973. Rapid determination of the free proline of water strees studies.plant soil, 39: 201-205.
21. Chance, B. and A. Maehley, 1995. Assay of catalase and peroxidase. Method Enz., 2: 764-774.
22. Kochert, G., 1978. Carbohydrate determination by the phenol sulfuric acid method *In*: J.A. Helebust and J.S. Craig, (ed.): Hand book of phycologia method, pp: 56-97. Cambridge University Press. Cambridge.
23. Jiang, Y. and B. Huang, 2001. Drought and Heat Stress Injury to Two Cool-Season Turfgrasses in relation to Antioxidant Metabolism and Lipid Peroxidation. Crop Sci., 41: 436-442.
24. Kaleem, S., F.U. Hassan and A. Saleem, 2009. Influence of environmental variations on physiological attributes of sunflower. African J. Biotechnol., 8: 3531-3539.
25. Kingston, S.A.H., J. Harbinson, and C.H. Foyer, 1999. Acclimation of photosynthesis, H_2O_2 contents and anti oxidants in maize growth at sub-optimal temperatures. Plant Cell Environ., 22: 1071-1083.
26. Adeniyi., O.T., S.O. Akparobi and I.J. Ekanayake, 2004. Field studies on chlorophyll a fluorescence for low temperature tolerance testing of cassava (*Manihot esculenta* Crantz.). Food, Agriculture and Environment, 2(1): 166-170.
27. Joshi, S.C., S. Chandra and L.M.S. Palni, 2007. Differences in photosynthetic characteristics and accumulation of osmoprotectants in saplings of evergreen plants grown inside and outside a.glasshouse during the winter season. Photosynthetica, 45(4): 594-600.
28. Xu, Y.C., J.B. Zhang, Q.A. Jiang, L.Y. Zhou and H.B. Miao, 2006. Effects of water stress on. the growth of *Lonicera japonica* and quality of honeysuckle. Zhong Yao Cai., 29(5): 420-423.
29. Sircelj, H., M. Tausz, D. Grill and F. Batic, 2005. Biochemical responses in leaves of two apple tree cultivars subjected to progressing drought. Journal of plant physiology, 162(12): 1308-1318.
30. Souza, A., C.Z. Sandrin, M.F.A. Calio, S.T. Meirelles, V.R. Pivello and R.C.L. Figueiredo-Riberio, 2010. Seasonal variation of soluble carbohydrates and starch in *Echinolaena inflexa*, a native grass species from the Brazilian Savanna and in the invasive grass Melinis minutiflora. Braz. J. Biol., 70(2): 1-11.
31. Fulai, L., R.J. christain and N.A. Mathias, 2001. Drought stress effect on carbohydrate. concentration in soybean leaves and pods early reproductive development. Field crops research, 86: 1-3.
32. Ashraf, M. and M.R. Foolad, 2007. Roles glycine betaine and prolin in improwing plant abiotic stress resistance. Environmental and Experimental Botany, 59: 206-216.
33. Alia, P. and P. Saradhi, 1991. Proline accumulation under heavy metal stress. J. Plant Physiol., 138: 554-558.
34. Egneus, H., H. Heber and M. Krik, 1975. Reduction of oxygen by the electron chain of chloroplast during assimilation of carbon dioxide. Bioch. Biophys. Acta. 408: 252-268.

35. Bowler, C., M. Van Montagu and D. Inze, 1992. Superoxide dismutase and stress tolerance. Ann. Rev. Plant Physiol., 43: 83-116.
36. Halliwell, B., 1982. The toxic effects of oxygen on plant tissue. In: L.W.oberley (Ed.). Superoxide dismutase. 1: 89-123. CRC press Inc., Boca Raton. USA.
37. Sairam, R.K. and G.C. Srivastava, 2002. Changes in antioxidant activity in Sub-cellular fractions of tolerant and susceptible wheat genotypes in response to long term salt stress. Plant Science, 162: 897-904.
38. Sharma, P. and R.S. Dubey, 2005. Drought induces oxidative stress and enhances the activities of antioxidant enzyme in growing rice seedling. Plant Growth Regul., 46: 209-221.
39. Zhang, G., K. Tanakamaru, J. Abe and S. Morita, 2006. Influence of water logging on some anti-oxidative enzymatic activities of two barley genotypes differing in anoxia tolerance. Acta Physiologia Plantarum, 29(2): 171-176.
40. Egert, M. and M. Tevini, 2002. Influence of drought on some physiological parameters. symptomatic for oxidative stress in leaves of chires (*Allium schoeenoprasum*). Environmental and Experimental Botany, 48: 43-49.
41. Yruela, I., 2005. Copper in plants. Brazilian Journal of plant physiology, 17: 145-1.
42. Chaoui, A. and E.E. Ferjani, 2005. Effects of cadmium and Copper on antioxidant capacities, lignification and auxin degradation in leaves of pea (*Pisum sativum* L.) seedlings. Plant Biology and Pathology, 328: 23-31.