Journal of the Persian Gulf
(Marine Science)/Vol. 6/No. 22/December 2015/12/1-12

Stress Response of Dominant Forest Tree Species South of the Caspian Sea in Relation to Soil from Coast to Upland

Zare-Maivan, Hassan*; Lotfi Fard, Farnoosh; Tayebi, Zahra

Department of Plant Biology, Tarbiat Modares University. Tehran, IR Iran

Received: March 2015 *Accepted: October 2015*

Abstract

Distribution and growth of plant species is affected by many factors including abiotic (topography, altitude and soil) and biotic (root symbiosis) factors. In light of global warming, recent findings advocate microhabitats and micro-conditions of the root zone and canopy ambiance as determining factors in sustaining of plant populations. Although, occurrence of ectomycorrhizal (ECM) and non-mycorrhizal plants in forests south of the Caspian Sea have been studied extensively, stress response of tree species in relation to altitude and soil (texture and contents of heavy metals) has not been investigated much. This research investigated the antioxidant stress response of three dominant tree species (*Alnus subcordata* C.A.Mey, *Acer velutinum* Boiss. *and Carpinus betulus* L.) of three selected hyrcanian forests south of the Caspian Sea (Javaherdeh, Dalkhani and Tonekabon) at 4 altitudes (sea level, 500, 1000 and 1500 m above sea level). Results showed some non-enzymatic antioxidants, such as flavonoids and anthocyanins contents correlated with ectomycorrhizal root colonization, differed amongst trees in corresponding altitudes and was generally greater in upper altitudes. Activity of antioxidant enzymes like catalase and peroxidase and total protein content were affected by soil physicochemical factors. *A.velutinum* grew better in sandy soils while *A.subcordata* dominanted clay soils. Although, nutrient status of the soil was different within each soil type, cation competition between Ca and Mg and high concentration of Fe in soil most likely affected root performance and consequentially pigment content in leaves, particularly in upper altitudes where temperatures were low and UV radiation was strong. UV radiation induces anthocyanin biosynthesis which mainly protects the DNA from damage. In this research, Chllorophyll/ Carotenoid ratio reduced with increasing altitude perhaps because of photoprotection.

Keywords*: Stress response, Caspian forests, Ectomycorrhiza, Altitude*

1. Introduction

Plants growing in terrestrial ecosystems and in particular, many tree species occupy a wide range of latitudes and altitudes in forest ecosystems. Research has shown, not only edaphic but also topographic as well as climatic conditions and biotic factors affect plant growth and development (Kialashaki and Shabani, 2011). Heavy metals, as an example of edaphic factors, are distributed in many forest ecosystems, particularly in forests disturbed for mining

* *E-mail: zare897@yahoo.com*

or industrial development or are chronically present as a result of natural processes (Liu, et al., 2012; Zare-Maivan, 2013) and may leach in run-offs after rain (Gisbert et al., 2003).

Heavy metals as well as geographical characteristics cause production of extra amounts of reactive oxygen species (ROS) in plant cells depending on the duration of exposure, type and quantity of the heavy metal, the stage of the plant life cycle and the root zone characteristics (Polle and Rennenberg, 1993; Foyer et al., 1994; Smirnof, 1998; Stockwell et al., 1997; Chkhubianishvili et al, 2011). ROSs are produced normally at proper quantities throughout the biological processes of plant cells (Asada, 1994; Schutzendubel and Polle, 2002; Zolfaghari et al., 2010) but, in higher quantities, ROSs damage cells through oxidation of membrane lipids and proteins, DNA mutation, disruption of some metabolic pathways and so on (Ames et al, 1993; Semane et al, 2010; Rastgoo and Alemzadeh, 2011; Liu et al, 2012). Extra amounts of ROS as an indication of increased stress on plants growing in an ecosystem, activate feedback and defensive mechanisms of plant cells and forces plants to activate their antioxidant systems and produce scavenger compounds to eliminate ROSs and as such, measuring scavenger elements has been used in quantifying level of stress on plant parts (Teichamann, 2001; Bolwell et al., 2002). For example, H_2O_2 scavengers, such as catalase and peroxidases (in peroxisomes and mitochondria) eliminate the hydrogen peroxide generated during heavy metal stresses and decrease the cellular oxidative damage (Anderson et al, 1995; Asada, 1999; Michalak, 2006; Zolfaghari et al., 2010). Beside antioxidant enzymes, many phenolic compountds, such as flavonoids and lignans can reduce ROS levels in plant tissues (Olga et al., 2003).

Apart from antioxidants in plants cells and their roll in stress reduction, ectomycorrhizal fungi (ECM) as biological agents have shown to facilitate the plant life by providing stable environmental conditions (Rivera-Becerril et al, 2002; Abdel Latef, 2013) and reducing of negative effects of stress caused by nutrition deficiency or lack of sufficient organic matter (Pringle et al., 2009). Although, it has been suggested that ECM fungi provide insufficient tolerance to plants in metal-polluted sites (Meharg and Cairney, 2000), there are reports to indicate some Ascomycetes and Basidiomycetes taxa could exclusively occupy metal-contaminated ecosystems as mycobiont (Gorfer et al, 2009; Jourand et al, 2010; Colpaert et al, 2011) of tree species.

Baghvardani and Zare-Maivan (2000) showed presence of different heavy and radioactive metals as well as adsorption of metals by spores of arbuscular mycorrhizae (AM) and ECM roots in a Caspian forest dominated by plant species such as ash, betula, maple and oaks. Similarly, Vafadar and Zare-Maivan (2006) and Zare-Maivan, et al., (2014) indicated the prevalence of heavy and radioactive metals in grass species *Lolium prenne L*. in the same forests.

There has been a general principal that topography and in particular altitude from sea level affects distribution of plant species and many plant species are adapted for particular habitats within each biome or vegetation zone (Zare-Maivan et al. 2015). However, recent findings advocate that microhabitats and micro-conditions of the root zone and canopy ambiance as determining factors in establishing, persistence and perseverance of plant populations (Stockwell et al., 1997; Chkhubianishvili et al., 2011; Naqinejad, et al., 2013, 2015; Vinton and Burke, 1995; Kialashaki and Shabani, 2011) and might influence the evolutionary and ecological processes of populations in the environment in the long run (Nagajyoti et al., 2008; Umadevi and Avudainayagam, 2013). Although, Zare-Maivan (2013) discussed effects of heavy metal toxicity on mycorrhizal and non-mycorrhizal plants in selected ecosystems of the Caspian forests, he did not elaborate on the distribution of tree species in altitude nor identified the response of plants to the presence of naturally occurring heavy metals, especially whether plants of the same species occurring at the same altitude but distantly apart, showed similar physiologic characteristics. Hence, objectives of the present study were to examine some biochemical parameters (total protein, flavonoids, anthocyanins, chlorophylls, carotenoids and malondialdehyde (MDA)

contents) and antioxidant enzymes (catalase, peroxidase) activities of common ectomycorrhizal trees of selected forests by the southern coast of the Caspian Sea in relation to edaphic factors (soil texture and metals content).

2. Materials and Methods

2.1. Soil and Plant Materials

Soil and plant samples were collected from 4 altitudes (Sea coast, 500, 1000 and 1500 m above sea level) from 3 selected forests; Javaherdeh (J); Dalkhani (D) and Tonekabon (T) located at south of the Caspian Sea, Iran (Table 1). Roots and leaves of dominant trees were sampled and transferred to the laboratory in a cooler packed with dry ice. Data of frequent tree species were collected on three random 10x10 m quadrates at each altitude. Soil as well as root samples were collected from top 30 cm of the soil beneath the trees using non-metallic polyvinyl borer (10cm diam.). Soil samples were treated for soil physical, chemical and texture analysis and tree root pieces examined for ectomycorrhizal colonization.

Table 1: Geographical coordinates and altitude from sea level of study areas

Station	Altitude (m)	N	E
J_1*	-6	36°54.188	50°39.699
J_2	408	36°53.572	50°34.623
J_3	968	36°52.361	50°32.654
J_4	1419	36°52.035	50°30.392
D_1	-13	36°52.904	50°44.673
D_2	471	36°49.016	50°41.100
D_3	921	36°49.133	50°33.706
D_4	1408	36°37.687	50°37.050
T_1	-12	36°47.828	50°53.965
T_2	529	36°39.565	50°43.347
T_3	NS	NS	NS
T_4	1385	36°33.889	50°44.253

* Distance between locations (J, D and T) 10 Km
NS: Not sampled because of severe disturbance

2.2. Biochemical Analysis

For biochemical analysis, leaf cell pigment content, total protein content, MDA content, flavonoids and anthocyanins contents as well as enzyme activities of catalase and peroxidases were measured in triplicate. Contents of chlorophylls a and b and carotenoids were measured as described by Lichtenthaler and Wellburn (1983) using following coefficients:

Chl a (?mg/ml)=12.21(A_{663})-5.03 (A_{646})

Chl b (?mg/ml)=20.13 (A_{663})-2.81(A_{646})

Carotenoid (?mg/ml)= (1000A_{470})-3.27 [Chla]- 104 [Chlb]/227(A_{646})

Catalase activity was determined according to Arrigoni et al. (1992). Peroxidase activity was assayed by the method of Ermakov (1987). Total protein content of leaves was measured using the method of Bradford (1976). Malondialdehyde (MDA) was measured based on Thiobarbituric acid (TBA) reaction (Heath and Packer, 1968). Flavonoids and anthocyanins contents were assayed according to Krizek et al. (1998) and Wagner (1979), respectively.

2.3. Soil Mineral Analysis

To analyze the mineral content in each soil sample, X-ray fluorescence (XRF) technique was used. The soil samples were labeled and analyzed at Tarbiat Modares XRF laboratory following standard operational procedure.

Soil type and chemical characteristics were determined at the ecology laboratory of the Tarbiat Modares University.

3. Results

There were 3 dominant common tree species which occurred from sea level to 1500 m as follows: *A. velutinum* Boiss., *C. betulus L.* and *A. subcordata C.A.Mey*. Plant species occurred in a range of altitudes with many other common species both within stations and within each altitude. For example, *A.velutinum* Boiss. ranged from about 300 to 1000 m in Javaherdeh and at 1000 and 1500 m at Dalkhani and Tonekabon forests, respectively. *A.velutinum* Boiss. showed the

widest range of distribution. In contrast, *A.subcordata* C.A.Mey was limited to lower altitudes in Dalkahni forest near water soaked soils and in Tonekabon forest in areas with greater clay and silt contents at altitudes up to 1000 m. *C.betulus L*. occupied altitudes ranging above 1000 m in both Dalkhani and Tonekabon forests, but was absent from sampling plots in Javaherdeh forest. Other tree species with scattered distribution, particularly in higher altitudes included *Quercus macranthera* Fisch. & *C.A.Mey*. ex Hohen., *Fagus orientalis* Lipsky, and *Corylus avellana* L. *Crataegus* microphylla *C. Koch* and *Pronus* sp. were dominant shrubs (up to 3m height) occupying disturbed and marginal areas in the forest. Except for river banks, other areas near coast were devoid of naturally occurring common forest trees as signs of disturbance, both natural and man-made, were visible all around.

3.1. Soil pH and Electric Conductivity (EC)

Soil type for all coastal stations was sand and other stations sandy loam except for J_{1500} and T_{500} and T_{1500} which were clay loam. pH ranged between 7.00 at J_{1000} to 7.75 at T_{1500}. Greatest EC was measured at T_{1500} (0.065ds/cm) and the lowest at D_{1000} and J_{1000} (0.026 ds/cm). There was no strong correlation between fungal density and soil elements as well as pH and EC, except with contents of Na and clay at lower elevations which correlated negatively with (Table 2).

Soil pH and EC followed similar patterns (Table 2) declining towards altitudes up to 1000 m above sea level and increasing at stations located at 1500 m above sea level (stations J_4, D_4 and T_4). Soil texture and type of each station is presented in Table 2.

3.2. Soil Metal Content

XRF analysis showed oxides of 13 metals and organic combustible materials existed in all forest soils (Table 3). Si, Fe and Al comprised the most abundant and frequent elements in soil and S, Mn, Zr and Sr occurred in fewer stations in minimal quantities. For example, except for station J1, S was absent in all other stations. Frequency of occurrence of micronurient Mn or rare metals, Zr and Sr, was inconsistent and did not follow any specific pattern. There were trace elements such as Ni, Cu, Ag and As which were not detectable via XRF analysis (not reported in Table 3). Distribution of some elements correlated with altitude. For example, Ti increased consistently and Ca decreased generally with increasing altitude in all forests except site D_1 which the greatest Ti content was recorded from. In case of Mg, this correlation (decreasing with hiking altitude) was seen at Tonekabon forest. Fe, Al and Sr were present at concentrations that could be considered as contamination in the ecosystem. There was also strong correlation between soil clay content and contents of Na, Al, Si, Ti and K at sites where *A.velutinum* Boiss. and *C.betulus L*. grew (Table 4). Soil oganic content was lower at higher altitudes.

Table 2: Soil physiochemical characteristics and percent ectomycorrhiza of three forest tree species south of the Caspian Sea sampled during October, 2013.

Station	Species	Ectomycorrhiza colonization (%)	pH	EC (ds/m)	Sand %	Silt %	Clay %	Soil type
J_0	No trees	-	7.56	0.06	66	23.6	10.4	Sandy -loam
J_{500}	*A. velutinum* Boiss	50	7.24	0.04	55	29	16	Sandy-loam
J_{1000}	*A. velutinum* Boiss	60	6.97	0.030	69	15	16	Sandy -loam
J_{1500}	*A. velutinum* Boiss	55	7.68	0.05	43	29	28	Sandy –clay-loam
D_0	*A. subcordata* C.A.Mey.	80	7.75	0.07	90	5	5	Sand
D_{500}	*A. subcordata* C.A.Mey.	35	7.60	0.06	66	18	16	Sandy-clay-loam
D_{1000}	*A. velutinum Boiss&C. betulus* L.	86 & 77	7.16	0.02	47	42	11	Sandy -loam
D_{1500}	*C. betulus L.*	36	7.69	0.06	64	25	11	Sandy -loam
T_0	*A. subcordata* C.A.Mey.	-	7.73	0.06	45	38.4	16.6	Loam
T_{500}	*A. subcordata* C.A.Mey.	22	7.46	0.05	26.6	35.4	38	Clay -loam
T_{1500}	*A. velutinum Boiss&C. betulus* L.	37 & 14	7.75	0.06	32	35	33	Clay- loam

Table 3: Metal percentage (g/Kg) of soil of forests south of the Caspian Sea sampled during October, 2013

Station	LOI*	Na	Mg	Al	Si	P	K	Ca	Ti	Fe	Mn	Zr	Sr
G1	127	12.084	23.898	77.507	253.442	0.704	20.575	24.933	5.641	43.638	0	0.50	0
G2	134	7.074	50.082	65.137	228.109	0.770	9.146	44.510	6.028	58.030	0	0	0.23
G3	243	3.870	9.288	68.730	239.338	2.217	10.624	6.919	6.905	41.741	1.609	0.18	0
G4	100	4.824	10.434	89.008	284.256	0.484	12.458	4.54	8.307	48.636	1.201	0.23	0.24
D1	62	8.680	35.616	42.908	284.904	0.677	7.544	59.04	8.797	53.55	0	0.22	0.23
D2	456	0	77.85	32.351	84.318	1.271	4.457	98.87	2.003	17.57	0	0	0
D3	420	4.684	7.908	48.770	188.596	1.271	9.661	7.96	5.375	23.65	1.37	0	0
D4	270	4.832	15.792	66.424	222.319	0.730	10.665	15.80	6.140	38.47	0	0.15	0.25
T1	230	12.202	23.088	61.734	214.244	1.117	12.674	48.10	5.977	35.46	0	0	0
T2	303	4.454	11.646	54.775	222.756	2.085	10.980	12.146	7.017	33.75	0	0.22	0.26
T4	92	8.746	7.548	81.869	298.257	0.470	18.060	5.392	8.574	37.71	0	0	0

* *loss on ignition*

Ectomycorrhizal colonization of roots showed negative correlation with clay content of soils while soils with more organic content showed greater ECM colonization. Where soil contained greater clay content such as the sites occupied with *A.subcordata* C.A.Mey (Table 2) less ectomycorrhiza existed. Similarly, ectomycorrhizal colonization of trees was lower at sites in which soil contained greater quantities of Al and Ti.

Total protein content of leaves of all sampled plant species generally increased with increasing altitude (Table 4). All tress showed peroxidase and catalase activity with varying degrees depending on the plant species and altitude. However, trees in Dalkhani (D) forest showed the greatest and the least peroxidase and catalase activities, respectively. Correlation analysis revealed that activity of peroxidase correlated negatively with the activity of catalase. Furthermore, activity of peroxidase decreased and catalase increased as altitude hiked in each forest (e.g. *A.velutinum* Boiss. grew at Javaherdeh) (Table 4). Catalase and peroxidase activity of all examined species indicated there were significant differences among trees at different altitudes. *A.subcordata C.A.Mey*. showed similar peroxidase activity at the same altitudes. There was a strong correlation between peroxidase activity and ectomycorrhizal colonization in *C.betulus L.* and *A.subcordata* C.A.Mey. MDA, flavonoids and anthocyanins contents usually increased with hike in altitude. *A. subcordata* contained more MDA than other tree species and *A. velutinum* showed lower MDA content in Dalkhani and Tonekabon forests (Table 4). Degree of fluctuations in MDA content varied for each species at different altitudes of the same forest and similar altitudes of different forests. *A.velutinum* Boiss. showed the least variation within stations and within forests. Differences in MDA content were greatest in Dalkhani forest at 1000 m above sea level.

Trees species in Tonekabon forest generally showed significantly greater flavonoids and anthocyanins contents than trees in other forests (Table 4).

Pigment contents, chlorophylls and carotenoids, showed significant differences between species and *C. betulus* showed greater carotenoids content than other tree species (Table 5).

A.velutinum leaves showed greater chlorophylls a and b and total chlorophyll than *A.subcordata* and *C.betulus L. Although*, chlorophyll a, b and total chlorophyll of the leaves of the *C.betulus* declined from 1000 to 1500 m above sea level, it seemed there was no apparent correlation between chlorophyll a and b and total chlorophyll contents and altitude (Table 5).

Carotenoids were also present in different quantities in all species. No general trend was observed between carotenoids content of leaves and altitude. However, total Chlrophyll/carotenoid content ratio decreased with altitude. Soil texture and nutrient quality affect plant metabolism including chlrophyll content of leaves differently. For example, chlorophyll content correlated strongly with P content and in *A.velutinum*, *A.subcordata* and *C.betulus L.* with sand, clay and silt contents of soil, respectively (Tables 3 and 5).

Table 4: Antioxidant enzymes (Peroxidase, catalase) activity, antioxidant compounds (flavonoids, anthocyanins), total protein and MDA contents in leaves of dominant forest tree species south of the Caspian Sea sampled during October, 2013*

Species	Station	Peroxidase (U/mg protein)	Catalase (U/mg protein)	Total protein (mg/g FW)	MDA (nmol/g)	Flavonoids (µg/g F.W)	Anthocyanins (µg/g F.W)
Acer velutinum	J_2	118.11 ± 5.39	10.77 ± 0.57	31.2 ± 1.40	1.52 ± 0.03	22.06± 1.88	19.6 ± 0.9
Acer velutinum	J_3	15.90 ± 1.10	49.52 ± 2.16	23.71 ±0.54	1.85 ± 0.03	60.31± 3.30	22.95 ± 1.0
	D_3	151.89 ± 3.52	15.51 ± 0.82	11.57± 0.74	1.29 ± 0.02	47.51 ± 2.22	22.70 ± 0.8
	T_4	73.48 ± 2.80	32.43 ± 2.13	56.42± 0.93	1.19 ± 0.03	80.18 ± 3.71	30.11 ± 2.2
	D_1	48.55 ± 4.15	42.57 ± 2.04	28.25 ± 0.58	1.42 ± 0.03	132.4 ± 4.00	19.43 ± 1.0
Alnus	D_2	2.34 ± 0.21	51.66 ± 2.13	48.90 ± 0.70	3.16± 0.06	22.313 ± 1.32	30.50 ± 1.4
subcordata	T_2	1.43 ± 0.07	66.38 ± 2.73	47.23 ± 1.33	1.32 ± 0.05	97.420 ± 3.31	17.03 ± 0.9
	D_3	24.99 ± 2.17	13.73 ± 0.67	7.36 ± 0.95	1.83 ± 0.02	173.14 ± 4.01	22.07 ± 0.9
	D_4	17.22 ± 1.05	6.72 ± 0.55	11.71 ± 1.25	1.40 ± 0.02	92.62 ± 3.46	30.4 ± 2.61
Carpinus	T_4	2.95 ± 0.12	51.66 ± 2.66	17.71 ± 0.42	2.17 ± 0.05	278.60 ±6.04	32.3 ± 2.2 0
betulus							

Data present only occurrence of enzymes without statistical inference of variations between species nor locations.

Table 5: Pigment content in leaves of dominant forest tree species south of the Caspian Sea sampled during October, 2013

Species	Station	Chlorophyll a (mg/g F.w)	Chlorophyll b (mg/g F.w)	Chlorophylls (a+b)	Carotenoids (mg/g F.w)	Chl/Car
Acer	J2	1.02 ± 0.02	0.43 ± 0.01	1.46 ± 0.01	1.18 ± 0.37	1.23
velutinum	J3	1.44 ± 0.01	0.56 ± 0.02	2.01 ± 0.03	1.76 ± 0.04	1.13
	D3	1.14 ± 0.03	0.45 ± 0.00	1.59 ± 0.02	1.26 ± 0.08	1.26
	T4	1.00 ± 0.01	0.40 ± 0.00	1.40 ± 0.01	1.48 ± 0.02	0.94
Alnus	D1	0.64 ± 0.02	0.20 ± 0.00	0.85 ± 0.02	0.68 ± 0.02	1.24
subcordata	D2	1.02 ± 0.01	0.65 ± 0.01	1.67 ± 0.03	1.33 ± 0.01	1.25
	T2	1.40 ± 0.03	0.63 ± 0.00	2.03 ± 0.04	1.77 ± 0.02	1.14
Carpinus	D3	0.965 ± 0.01	0.40 ± 0.01	1.37 ± 0.02	1.62 ± 0.05	0.84
betulus	D4	0.288 ± 0.01	0.10 ± 0.01	0.40 ± 0.01	1.06 ± 0.02	0.37
	T4	0.934 ± 0.0	0.36 ± 0.02	1.30 ± 0.03	1.58 ± 0.01	0.82

4. Discussion

Recent activities of human have caused changes in global climate regime and as such, worldwide shifts in the distribution and geography of vegetation is noticed. Shifts in vegetation patterns are detected through different means, but physiological changes, such as response to stress caused by global warming or local disturbances are not easily detected unless specific investigations are carried out. Altitudinal gradients are strongly affected by changes in climate regime and in turn, distribution of plants and their symbiotic counterparts, ectomycorrhizal fungi (Bahram et al., 2012.) are both affected in time (i.e. evolve) and space (e.g. migrate). Many researchers have shown the relative effects of altitude, temperature, precipitation, host community and soil nutrient concentrations on species richness and community composition of tree species and ECM fungi in many forest ecosystems (Vafadar and Zare-Maivan, 2006; Griffiths et al. 1996; Bahram et al., (2012). It is commonly believed that altitude, mean annual temperature and precipitation determine tree species distribution as well as defining ECM development in line with their respective hosts. However, effects of soil are usually overlooked and

less attention is given to chemical and physical aspects of immediate rhizosphere soil.

Hyrcanian (Caspian) forests are one the oldest and richest Eurasian temperate forests in the north of Iran (Marvie Mohadjer, 2006). Although, the forest is influenced by weather fronts, both from west (Mediterranean) and North (Siberian), because of spatial variations, plant communities are diverse and distributed along gradients in the mountains and plains (Jackson and Caldwell, 1993; Kialashaki and Shabani, 2011). Results of this investigation showed that although, altitude might affect tree species distribution as well as their biochemical stress responses to environmental conditions, it was mostly the soil chemical characteristics and texture that affected plant species distribution and function and subsequently, shaped physiological responses of plants to elements of environmental stress as indicated by Naqinezhad, et al. (2011, 2012).

Soil physical and chemical characteristics differed among forest types in particular, Tonekabon forest which had more clay. These characteristics contributed to prevalence of *A. subcordata* C.A.Mey in the region. Soil type in lower altitudes in all forests closer to the Caspian Sea was sandy-loam and where adequate moisture existed, *Phragmites australis* (Cav.) Trin. ex Steud. and *Juncus* sp. occurred densely. Only in Tonekabon forest, *A.subcordata C.A.Mey*. grew in the river banks near shore where no ECM was observed in roots collected from soaked soil. Since, all trees grew on soil types containing sand and silt with various amount of clay, it seems clay content is a determining factor for successful establishing of plants; for example, *A.subcordata* distinctly occurred in soil with high clay and moisture contents. Soils with high clay content usually correlate negatively with ECM colonization.

Generally, soil texture as well as metal content affects ectomycorrhizal development and distribution in the rhizosphere (Zare-Maivan, 2013). For example, there was a negative correlation with mycorrhizal colonization and Al at sites occupied by *A.velutinum* Boiss. and *C. betulus L*. Al competes and disrupts the uptake and transport of nutrient and cations, like Ca and Mg (Silva et al., 2005) and potentially affects DNA synthesis, alters properties of the cell wall and protoplasm and restricts cell division (Kabata-Peudias, 1992) as well as plant growth, thus limiting ECM development as well (Stankevičienė and Pečiulytė, 2004; Kim et al., 2003; Vilkka,1990).

Ca and Mg concentrations decreased with hike in altitude as a result of precipitation which washed them away to lower altitudes. Clay particles due to their negative charge attract cationic elements. In other words, clay has higher cation exchange capacity compared to other soil particles (Aprile, 2012). Strong correlation existed between clay and Na, Al, Si, Ti and K in many stations; Ability of *A.velutinum*, *C.betulus* and *A.subcordata* to grow in wider areas or where clay content of soil was greater could be a contributor to greater distribution of these species in different altitudes and in different forests. On the other hand, greater clay content translates into lower oxygen availability to roots and aerobic communities, including mycorrhizal fungi (Read and Armstrorng, 1972; Bougher and Malajczuk, 1990). This problem is improved by increased organic content of soil, particularly in upper 10 cm of the rhizosphere under canopy where majority of mycorrizal roots are scattered. In this study, organic material content of soil was lower in soil collected from upper altitudes than in lower altitudes. Mycorrhizal percentages of *C.betulus and A.subcordata* in upper altitudes of forests were lower which confirmed negative effects of greater silt (and partially clay) content and lower organic matter in these soils (Slankis, 1974, Harley & Smith, 1983, Adams and Bioeng, 1995; Naqinezhad et al., 2011, 2012).

Ectomycorrhizal fungi because of their saprophytic nature usually grow better in soil with high levels of humus. It contains abundant soil nutrition as well as moisture which are suitable substrates for most ectomycorrhizal fungi (Harvey et

al., 1979; Barr, 1930; Vogt et al., 1981; Bougher and Malajczuk, 1989). However, these are in contrast to some other studies, such as that done by Baar and Braak (1996). Although ECM development is enhanced in proper wet condition (Kennedy, 2007) moisture trapped in clay particles is not easily accessible to ECM fungi. Moisture availability mostly depends on soil type not altitude. However, in research done by Chandrasekara et al. (2005) they mentioned the effect of altitude on increased amount of water at lower altitudes which results in higher water potential and enhanced spore germination as well as increased spore diversity and density in case of arbascular mycorrhizae.

Plant growth is a process dependant on the environmental conditions, life history of the species and exposure time to environmental variables (Aikman and Scaife, 1993). Tree species adapt to harsh environmental conditions generally through adjusting their metabolic processes, such as increasing their protein content (Chkhubianishvili et al., 2011). Results of this investigation indicated that increase in total protein content of tree leaves growing in upper altitudes of forests was because of colder temperatures. Enzymes, such as catalase and peroxidases are produced under stressful conditions and used as indicators of diurnal or local stresses within a plant community (Korori, 1999). Catalase is an enzyme important in breaking down hydrogen peroxide produced during metabolic activities of plant (Zolfaghari et al., 2010), especially in the roots where microbial interactions are frequent and diverse (Zare-Maivan, 2013). Peroxidases, on the other hand, are most active when hydrogen peroxide is at its lowest level in the cell (Korori, 1999). Results obtained in this investigation showed negative correlation between peroxidase and catalase in sampled trees (Table 4). However, activities of both enzymes have proved necessary for eliminating ROS generated during growth, particularly where contamination of heavy metals, such as Al and Fe is noticed. As ROS is scavenged (removed) by one enzyme, the activity of the other enzyme is lowered. Since, tree species at the same altitude displayed significantly different levels of enzyme actvity, generally peroxidase and total protein lowered with hike in altitude and catalase and MDA increased in contrast. MDA production in plant cells is species specific and produced when integrity of the cell membrane is damaged and peroxidation of membrane lipids has taken place. Similarly, flavonoid contents of cells also supported species specific habit of tree species as was evident in all species, i.e. each species responding differently to changes in altitude in different forests. Flavonoids are non-enzymatic secondary antioxidant metabolites produced by plant cells as an adaptive response to maintain cell biochemical integrity via scavenging ROSs under stressful environmental conditions.

Pigment content of leaves in plant species is also species specific and varies depending on the age of the leaf, position on the canopy, latitude and altitude, soil nutrient balance and season (Lin and Ehleringer, 1982; Vinod et al., 2012). *A.velutinum* grew better in sandy soils while *A.subcordata* dominanted in *clay soils*. Although, nutrient status of the soil was different within each soil type, cation competition between Ca and Mg and high concentration of Fe in soil, most likely, affected root performance and consequentially pigment content in leaves, particularly in upper altitudes where temperatures were low and UV radiation was strong (not measured in this investigation). UV radiation induces anthocyanin biosynthesis which mainly protects the DNA from damage (Fuglevand et al., 1996). In this research, Chl/ Car ratio reduced with increasing altitude perhaps because of photoprotection (Polle et al., 1999).

Reference

Abdel Latif, A. A., 2013. Growth and some physiological activities of pepper (*Capsicum*

annuum L.) in response to cadmium stress and mycorrhizal symbiosis. Journal of Agriculture, Science and Technology, 15: 1437-1448.

Adams, B., & Bioeng, B., 1995. Bioregenerating soils of problematic high altitude disturbed sites using specialty fertilizers, Proceeding of 19th Annual British Columbia Mine Reclamation Symposium in Dawson Creek, 37-44.

Aikman, D., and Scaife, A., 1993. Modelling plant growth under varying environmental conditions in a uniform canopy. Annals of Botany, 72: 485-492.

Ames, B., Shigenaga, M., and Hagen, T., 1993. Oxidants, antioxidants, and the degenerative diseases of ageing. Proceedings of the National Academy of Sciences, 90: 7915-7922.

Anderson, D., Prasad, K., and Stewart, R., 1995. Changes in isozyme profiles of catalase, peroxidase and glutathione reductase during acclimation to chilling in mesocotyls of maize seedlings. Plant Physiology, 109: 1247-1257.

Aprile, F., 2012. Evaluation of cation exchange capacity (CEC) in tropical soils using four different analytical methods. Journal of Agricultural Science, 4: 278-289.

Arrigoni, O., De Gara, L., Tommasi, F., and Liso, R., 1992. Changes in the ascorbate system during seed development of *Vicia faba* L. Plant Physiology, 99: 235-238.

Asada, K., 1994. Production and action of active oxygen species in photosynthetic tissues. In: C.Foyer and P. Mullineaux, eds., Photooxidative Stresses in Plants: Causes and Amelioration. 77-104.

Asada, K., 1999. The water-water cycle in chloroplasts: scavenging of active oxygen and dissipation of excess photons. Annual Reviews of Plant Physiology and Plant Molecular Biology, 50: 601-639.

Baar, J., and ter Braak, C., 1996. Ectomycorrhizal sporocarp occurrence as affected by manipulation of litter and humus layers in Scots pine stands of different age. Applied Soil Ecology, 4: 61-73.

Baghvardani, M. and Zare-Maivan, H., 2000. Evaluating adsorption of heavy and radioactive metals in Endomycorrhizal spores. Plant Diseases Journal. 36 (1-2):1-14. (In Persian with English abstract).

Bahram, M., Polme, S., Koljalg, U., Zarre, S., and Tedersoo, L., 2012. Regional and local patterns of ectomycorrhizal fungal diversity and community structure along an altitudinal gradient in the Hyrcanian forests of northern Iran. *New Phytologist*, 193: 465–473.

Barr, P.M., 1930. The effect of soil moisture of the establishment spruce reproduction in British Columbia. Bulletin. Yale University, School of Forestry, 57 P.

Bolwell, G., Bindschedler, L., Blee, K., Butt, V., Davies, D., Gardner, S., et al., 2002. The apoplastic oxidative burst in response to biotic stress in plants: a three-component system. Journal of Experimental Botany, 53:1367-1376.

Bougher, N., and Malajczuk, N., 1990. Effects of high soil moisture on formation of ectomycorrhizas and growth of karri (*Eucalyptus diversicolor*) seedlngs inoculated with *Descolea maculata*, *Pisolithus tinctorius* and *Laccaria laccata*. New Phytologist, 114: 87-91.

Bradford, M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein binding. Analytical Biochemistry, 72: 248–254.

Chandrasekara, C. M., Madawala Weerasinghe, H. M., Gunatilleke, I. A., and Seneviratne, G., 2005. Spatial distribution of arbuscular mycorrhizas along an altitude and adaphic gradient in the forest dynamics plot at Sinharaja, Sri Lanka. Ceylon Journal of Science (Biological Sciences), 34: 47-67.

Chkhubianishvili, E., Kacharava, N., Badridze, G., Chanishvili, S., and Kurdadze, T., 2011. Activity of peroxidase, catalase and content of total proteins in leaves of some herbaceous plants of high mountains of the caucasus. Plant Physiology, 5: 96-100.

Colpaert, J. V., Wevers, J. H., Krznaric, E., and

Adriaensen, K., 2011. How metal-tolerant ecotypes of ectomycorrhizal fungi protect plants from heavy metal pollution. Annals of Forest Science, 68:17–24.

Ermakov, A., 1987. Metody biokhimicheskogo issledovaniya rasteniy. Leningrad, 41: 42 (in Russian).

Foyer, C., Descourvieres, P. & Kunert, K., 1994. Protection against oxygen radicals: An important defense mechanism studied in transgenic plants. Plant, Cell & Environment, 17: 507-523.

Fuglevand, G., Jackson, J., & Jenkins, G., 1996. UV-B, UV-A and blue light signal transduction pathways interact synergistically to regulate chalcone synthase gene expression in Arabidopsis. Plant Cell, 8: 2347-2357.

Gisbert, C., Ros, R., Deharo, A., Walker, D., Pilarbernal, M., Serrano, R., et al., 2003. A plant genetically modified that accumulates Pb is especially promising for phytoremediation. Biochemical and Biophysical Research Communications, 303: 440-445.

Gorfer, M., Persak, H., Berger, H., Brynda, S., Bandian, D., & Strauss, J., 2009. Identification of heavy metal regulated genes from the root associated ascomycete *Cadophora finlandica* using a genomic microarray. Mycological Research, 113:1377–1388.

Griffiths, R., Bradshaw, G., Marks, B., and Lienkaemper, G., 1996. Spatial distribution of ectomycorrhizal mats in coniferous forests of the Pacific Northwest, USA. Plant and Soil, 180: 147-158.

Harley, J., and Smith, S., 1983. Mycorrhizal Symbiosis. New York: Academic Press.

Harvey, A. E., Larsen, M. J., and Jurgensen, M. F., 1979. Comparative distribution of ectomycorrhizae in soils of three western montana forest habitat Types. Forest Science, 25: 350-358.

Heath, R and Packer, L., 1968. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Archives of Biochemistry and Biophysics, 196: 385-395.

Jackson, R., & Caldwell, M., 1993. The scale of nutrient heterogeneity around individual plants and its quantification with geostatistics. Journal of Ecology, 81: 683-692.

Jourand, P., Ducousso, M., Loulergue-Majorel, C., Hannibal, L., Santoni, S., Prin, Y., et al., 2010. Ultramafic soils from New Caledonia structure Pisolithus albus in ecotype. FEMS Microbiology Ecology, 72:238–249.

Kabata-Peudias, A., & Pendias, H., 1992. Trace elements in soils and plants. Boca Ratón, Florida: CRC Press, 365 pp.

Kennedy, P. G., & Peay, K. G., 2007. Different soil moisture conditions change the outcome of the *ectomycorrhizal* symbiosis between Rhizopogon species and *Pinus muricata*. Plant Soil, 291:155–165.

Kialashaki, A., & Shabani, S., 2011. Differentiation ecosystem units of caspian forests in relation to physiographic factor and some of soil characteristics. Annals of Biological Research, 2 (3) :276-282.

Kim, C., Power, S., & Bell, J., 2003. Effects of cadmium on growth and glucose utilization of ectomycorrhizal fungi in vitro. Mycorrhiza, 4: 223-226.

Korori, S. A. A., Teimouri, M., Khoshnevis, M., et. al., 1999. Evaluation of pollution damage from the Persian Gulf war on mangrove and *Ziziphus* trees through study ofenzyme activity, cations and anions. Pazuhesh and Sazandegi . 43:102-107.

Krizek, D., Britz, S. J., & Mirecki, R., 1998. Inhibitory effects of ambient levels of solar UV-A and UV-B radiation on growth cv. new leaf fire lettuce. Physiologia Plantarum, 103:1-7.

Lichtenthaler, H., and Wellburn, A., 1983. Determination of total carotenoids and chlorophyll ‘a’ and ‘b’ of leaf extracts in different solvents. Biochemical Society Transactions, 11: 591-592.

Lin, Z., & Ehleringer, J., 1982. Effects of leaf age on photosynthesis and water use efficiency of Papaya. Photosynthetica, 16(4): 514-519.

Liu, N., Lin, Z., & Mo, H., 2012. Metal (Pb, Cd, and Cu)- induced reactive oxygen species accumulations in aerial root cells of the Chinese banyan (*Ficus microcarpa*). Ecotoxicology, 21:2004–2011.

Marvie Mohadjer, M., 2006. Silviculture. University of Tehran, Press, 387 pp.

Meharg, A., & Cairney, J., 2000. Co-evolution of mycorrhizal symbionts and their hosts to metal-contaminated environments. Advanced Ecology Research, 30:69–112.

Michalak, A., 2006. Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. Polish Journal of Environmental Studies, 15: 523-530.

Nagajyoti, P., Dinakar, N., Prasad, T., Suresh, C., and Damodharam, T., 2008. Heavy metal toxicity: Industrial effluent effect on groundnut (*Arachis hypogaea* L.) seedlings. Journal of Applied Sciences Research, 4 (1): 110-121.

Naqinezhad, A., Zare-maivan, H.and Gholizade, H., 2013. Understorey vegetation as an indicator of soil characteristics in the Hyrcanian area, Iran. Flora. *http://dx.doi.org/10.1016/g.flora.2012.1*

Naqinezhad, A., Zare-maivan, H. and Gholizade, H., 2015. A floristic survey of the Hyrcanian forests in northern Iran, using two lowland mountain transects. Journal of Forestry Research. 26 (1):187–199.

Olga, B., Eija, V. and Kurt, V., 2003. Antioxidants, oxidative damage and oxygen deprivation stress a review. Annals of Botany, 91: 179-194.

Polle, A. and Rennenberg, H., 1993. Significance of antioxidants in plant adaptation to environmental stress. In: Mansfield T, Fowden L, Stoddard F, eds. In Plant Adaptation to Environmental Stress. London: Chapman & Hall, 263-273.

Polle, A., Rennenberg, H., and Scholz, F. 1999. Antioxidative systems in spruce clones grown at high altitudes. Phyton (Austria), 39: 155-164.

Pringle, A., Bever, J. D., Gardes, M., Parrent, J. L., Rillig, M. C., and Klironomos, J. N., 2009. Mycorrhizal Symbioses and Plant Invasions. Annual Review of Ecology, Evolution, and Systematics, 40:699–715.

Rastgoo, L., and Alemzadeh, A., 2011. Biochemical responses of Gouan (Aeluropus littoralis) to heavy metals stress. Australian Journal of Crop Science, 5(4):375-383.

Read, D., and Armstrong, W., 1972. A relationship between oxygen transport and the formation of the ectotrophic mycorrhizal sheath in conifer seedlings. New Phytologist, 71: 49-53.

Rivera-Becerril, F., Calantzis, C., Turnau, K., Caussanel, J., Belimov, A., Gianinazzi, S., et al., 2002. Cadmium accumulation and buffering of cadmium induced tress by arbuscular mycorrhiza in three *Pisum sativum* L. Genotypes. Journal of Experimental Botany, 53: 1177–1185.

Schutzendubel, A. and Polle, A., 2002. Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. Journal of Experimental Botany, 53: 1351-1365.

Semane, B., Dupae, J., Cuypers, A., Noben, J., Tuomainen, M., Tervahauta, A., et al. (2010). Leaf proteome responses of Arabidopsis thaliana exposed to mild cadmium stress. Plant Physiology, 167: 247-254.

Silva, I., Ferrufino, A., Sanzonowicz, C., Smyth, T., Israel, D., and Júnior, T., 2005. Interactions between magnesium, calcium, and aluminum on soybean root elongation. Revista Brasileira de Ciência do Solo, 29: 747-754.

Slankis, V., 1974. Soil factors influencing formation of mycorrhizae. Annual Review of Phytopathology, 12:437-457.

Smirnof, N., 1998. Plant resistance to environmental stress. Current Opinion in Biotechnology, 9: 214-219.

Stankevičienė, D., and Pečiulytė, D., 2004. Functioning of ectomycorrhizae and soil microfungi in deciduous forests situated along a pollution gradient next to a fertilizer Factory. Polish Journal of environmental studies, 13: 715-721.

Stockwell, W., Kramm, G., Scheel, H., Mohnen, V., and Seiler, W., 1997. Ozone formation, destruction and exposure in Europe and in the United States. - In: Sandermann, H., Wellburn, A.R. & Heath, R.L. (Eds.), Forest decline and ozone: a comparison of controlled chamber and field experiments. In Ecological Studies Series. Springer Verlag, Berlin, 127: 1-38.

Teichmann, T., 2001. The biology of wood formation: scientific challenges and biotechnological perspectives. Recent Research Developments in Plant Physiology, 2: 269-284.

Umadevi, M. and Avudainayagam, S., 2013. Effect of cadmium and chromium on fast growing pulp wood tree species. International Journal of Biosciences, 3: 92-104.

Vafadar, M. and Zare-maivan, H., 2006. Studying adsorption of heavy metals by herbaceous plants of Ramsar forest, Iran. Journal Agricultural and Natural Resources. 13(4):142-150.

Vilkka, L., Aula, L., and Nuotreva, P., 1990. Comparison of the levels of some metals in roots and needles of *Pinus sylvestris* in urban and rural environment at two times in the growing season. Annales Botanici Fennici, 27: 53-57.

Vinod, K., Awasthi, G., and Chauhan, P., 2012. Cu and Zn tolerance and responses of the Biochemical and Physiochemical system of Wheat. Journal of Stress Physiology & Biochemistry, 8 (3): 203-213.

Vinton, M., and Burke, I., 1995. Interactions between individual plant species and soil nutrient status in short grass steppe. Ecology, 76: 1116-1133.

Vogt, K. A., Edmonds, R. E., and Gr i ~ rC, .. C. 1981. Seasonal changes in biomass and vertical distribution of mycorrhizal and fibrous-textured conifer fine roots in 23- and 180-year-old subalpine *Abies arnabulis* stands. Canadian Journal of Forest Research, 11: 223-229.

Wagner, G., 1979. Content and vacuole/extra vacuole distribution of neutral sugars free amino acids, and anthocyanins in protoplast. Plant Physiology, 64: 88-93.

Zare-Maivan, H., 2013. Mycorrhizae adsorb and bioaccumulate heavy and radioactive metals. Pp. 269-281. In: Mohamadi Goltapeh, A. Varma and (eds.). Fungi as Bioremediators. Springer. London.

Zare-Maivan, H. H. Khajehzadeh, F. Ghanati and M. Sharifi., 2014. Changes of enzyme activity and production of metabolites in *Artemisia aucheri* in different altitudes and its relationship to adaptation. J. Chemical Health Risks. 4:57-66.

Zare-maivan, H., H. Khajehzadeh, F. Ghanati and M. Sharifi., 2015. Effect of mycorrhiza in adaptation of Artemisia aucheri to altitude changes. Resarch Journal of Biological Sciences. 10(4-5):56-61.

Zolfaghari, R., Hosseini, S. M. and Korori, S. A., 2010. Relationship between peroxidase and catalase with metabolism and environmental factors in Beech (*Fagus orientalis* Lipsky) in three different altitudes. International Journal Environmental Sciences. 1: 243-252.