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FOLIAR RESORPTION OF SOME MACRO- (N, P, S) AND MICRONUTRIENTS (FE, ZN, CU, MN) IN *PTEROCARYA FRAXINIFOLIA* (POIRET) SPACH FORESTS IN TURKEY

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RÉSUMÉ.— *La résorption foliaire de quelques macro- (N, P, S) et micronutriments (Fe, Zn, Cu, Mn dans les forêts de* Pterocarya fraxinifolia *(Poiret) Spach en Turquie.— Pterocarya fraxinifolia* (Poiret) Spach a une distribution plutôt restreinte en Turquie dans les forêts marécageuses. La résorption foliaire de quelques macro- (N, P et S) et micronutriments (Fe, Mn, Zn, Cu) a été étudiée dans des populations de *P. fraxinifolia* du nord et du sud de la Turquie. Comparativement aux populations méridionales, les populations nordiques ont montré une résorption de l'azote (NRE) plus efficiente mais une résorption du phosphore (PRE) plus faible. Les populations tant nordiques que méridionales se sont montrées P-proficientes alors que seules les populations nordiques se sont révélées P-proficientes. Des valeurs négatives pour ZnRE et MnRE ont été trouvées dans les populations méridionales. SRE s'est révélée plus élevée que celle d'autres espèces décidues. Des ratios NRE/PRE > 1 ont été trouvés dans les populations nordiques mais < 1 dans les méridionales.

SUMMARY.— *Pterocarya fraxinifolia* (Poiret) Spach has a rather restricted distribution in Turkey and occurs in swamp forests. Foliar resorption of some macro- (N, P and S) and micronutrients (Fe, Mn, Zn, Cu) in northern and southern populations of *P. fraxinifolia* in Turkey was studied. In northern populations, comparatively to southern populations, nitrogen resorption efficiency (NRE) was higher, while phosphorus resorption efficieny (PRE) was lower. Both northern and southern populations were P-proficient, while only northern populations were N-proficient. Negative RE values were found with respect to Zn and MnRE in southern populations. SRE was found to be higher as compared to other deciduous species. It has been found that NRE/PRE ratios >1 in northern populations, while <1 in southern populations.

Plants have a high capacity to absorb mobile ions by diffusion, while they have a relatively lower capacity to absorb immobile ions like phosphate. For example, plants absorb any form of soluble nitrogen that is available in the soil, while they usually differ in their relative preference for different N forms like ammonium (NH_4^+) and nitrate ($NO3^-$). Leaves with high nutrient concentrations lose more nutrients than those with low tissue concentrations, in either gaseous form (NH_3) or as solutions (Aerts & Chapin, 2000). Foliar resorption enables a plant re-using the same nutrients (Busotti *et al.*, 2003). Resorption can be expressed as resorption efficiency (RE) and resorption proficiency (RP) respectively. RE is defined as the percentage of a nutrient recovered from a senescing leaf (Kilic *et al.*, 2010; Yilmaz *et al.*, 2014). RP is known as the amount of a particular nutrient that remains in fully senesced leaves and is not subject to temporal variation in nutrient concentration in green leaves or the timing of sampling (Killingbeck, 1996, 2004). There are several studies about foliar resorption of nutrients and the factors that influence foliar resorption (Aerts & Chapin, 2000; Busotti *et al.*, 2003; Kutbay & Ok, 2003; Covelo *et al.*, 2011; Kilic *et al.*, 2010, 2012). Most of these studies indicate that foliar resorption may be changed in different individuals of a plant population. It has been stated that northern and southern populations of a particular species may have different RE and RP with respect to foliar macro- and micronutrient concentrations (Oleksyn *et al.*, 2002, 2003). Vergutz *et al.* (2012) stated that mass loss correction factor (MLCF) should be used for the calculation of foliar resorption because foliar mass will be changed between green and senesced leaves (Reed *et al.*, 2012; Yilmaz *et al.*, 2014).

Euro-Siberian phytogeographical region in Turkey has peculiar characteristics. This region includes two different regions as Euxine and Colchic provinces and also includes different species belonging to different phytogeographical regions in addition to typical Euxine and Colchic species. Especially the eastern part of Euxine province more closely resembles the Hyrcanian province of North Iran and adjacent Talysh than any other part of the Euro-Siberian region. Two provinces are separated by the lower Aras valley and they are often treated as a single province as Hyrcano-Euxine province (http://www.paeon.de/h1/davis/16_phyto.html). *Pterocarya fraxinifolia* (Poiret) Spach is one of the most remarkable relict species in swamp forests in Turkey belonging to Hyrcano-Euxine phytogeographical region and this species was previously known only from the Caspian lowland forests. In the Hyrcanian area, *P. fraxinifolia* is a thermophilous tree growing mostly in swamp forests and valleys with running water at an altitude mostly below 1000 m (Sheykholislami & Ahmadi, 2009). *P. fraxinifolia* has a rather restricted distribution area in Turkey and it has a discontinuous distribution and occurs in northern and southern parts of Turkey. In studied localities *P. fraxinifolia* formed pure stands and outside the study area this species formed mixed stands with other species mainly *Alnus* L., *Populus* L., etc (Akhani & Salimian, 2003).

There were many studies on foliar macroelement especially nitrogen (N) and phosporus (P) resorption in temperate deciduous forests (Kilic *et al*., 2010; Covelo *et al*., 2011; Yilmaz *et al*,. 2014; Surmen *et al*., 2014). However, comparatively, studies regarding foliar resorption of micronutrients in temperate forests were scarce (Killingbeck, 1985; Oleksyn *et al*., 2003; Killingbeck, 2008; Housman *et al.*, 2012). In the present study, northern and southern populations of *P. fraxinifolia* in Turkey were compared with each other (i) to find whether foliar resorption of macro- and micronutrients patterns were differed or not with respect to resorption efficiency (RE) and resorption proficieny (RP); (ii) to find the differences among RE values with and without MLCF and (iii) to find which populations were proficient regarding macro- and micronutrients and uncover relationships among leaf macro- and micronutrient concentrations and foliar RE and RP of macro- and micronutrients.

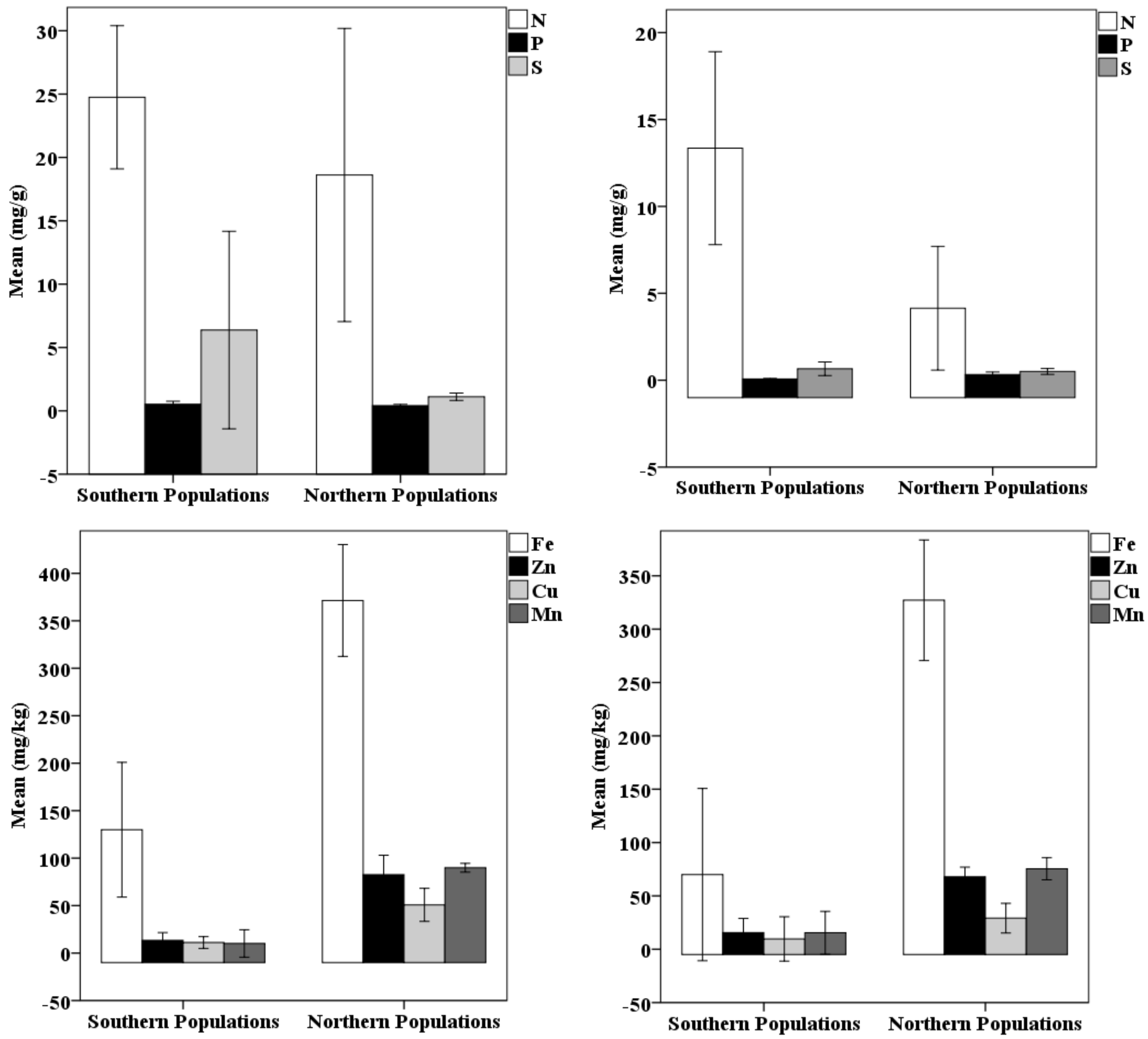
MATERIALS AND METHODS

STUDY AREA

P. fraxinifolia specimens were taken from northern (one station: Samsun: 41°18'N; 36°55'E) and southern parts (3 stations: Kilis, Hatay and Gaziantep: 36°52'N, 37°34'E; 36°49'N, 36°32'E; 36°56'N, 37°30'E) of Turkey. In northern part of the study area, mean annual temperature and mean annual precipitation respectively are 13.8 ºC and 895.2 mm, against 16.8ºC and 714.4 mm respectively in southern part. Northern part of the study area has an oceanic-type climate, whereas semi-arid Mediterranean climate is seen in southern part. Hydromorphic alluvial soils are widespread in the study area (Hüseyinova *et al*., 2013).

SAMPLING AND CHEMICAL ANALYSIS

Van Heerwaarden *et al*. (2003) suggested pre-selection of leaves in order to minimize the risk of comparing green and senescent leaves of different cohorts. Five individual plants were selected from each population. Seven fully expanded leaves per individual plant were marked by tying a small tag at their base and were pooled for analysis (Kilic *et al*., 2010; Zhang *et al*., 2015). Individuals were selected $\geq$ 2.5 m from the stems of neighbouring canopy trees to avoid potential microsite variation (Boerner & Koslowsky, 1989). Green leaves were sampled in the end of June. When a leaf or at least two-thirds of its area turned yellow or brown, it was considered senesced (Williams-Linera, 2000; Kilic *et al.*, 2012). Senescent leaves were sampled in December. *P. fraxinifolia* leaf samples were scanned and specific leaf area (SLA) was calculated by using Net Cad software (Anonymous, 1999).



(a) (b)

Figure 1.— Full leaf (a) and senescence (Resorption proficiency; RP) (b) micro and macroelements concentrations. Data are mean and ± 1 SD.

Leaf samples were dried at 70 °C until constant weight, and grounded, sieved to a mesh and digested in a mixture of concentrated nitric and perchloric acids. Nitrogen was determined by the micro Kjeldahl method with a Kjeltec Auto 1030 Analyser (Tecator, Sweden) after digesting the samples in concentrated H_2SO_4 with a selenium catalyst. P was determined with stannous chloride method by using a Jenway spectrophotometer (Allen *et al*., 1986; Kutbay & Ok, 2003) filtered through a Whatman filter paper No.42. Sulphur concentrations were determined by using turbidimetric calcium-sulphate method

(Bayrakli, 1987; Hüseyinova *et al*., 2009) Micronutrient (Fe, Zn, Cu and Mn) concentrations were determined by a Perkin Elmer 2280 atomic absorption spectrophotometer, using an air /acetylene flame after digesting HNO_3 and $HClO_4$ (Allen *et al.*, 1986).

Macro- and micronutrient resorption efficiency (NRE, PRE, FeRE, ZnRE, CuRE, MnRE, and SRE) (%) was calculated as the percentage of macro- and micronutrients recovered from senescing leaves and calculated by RE = (macro- or micronutrient in mature green – macro- or micronutrient in senescent) / macro- or micronutrient in mature green × 100 %, where: macro- or micronutrient in mature green = macro- or micronutrient in mature green leaves, macro- or micronutrient in senescent = macro- or micronutrient in senescent leaves (Killingbeck, 1985; Kilic *et al*., 2010). MLCF is defined as the ratio of dry mass of senesced leaves to the dry mass of green leaves (Yilmaz *et al.*, 2014). Foliar resorption was also calculated by accounting mass loss correction factor (MLCF) by the help of following equation:

Foliar resorption = (1-nutrients in senescent leaves/nutrients in green leaves x MLCF) x 100

Nutrient resorption proficiency (RP) is simply the amount of a nutrient that remains in fully senesced leaves (Killingbeck, 1996; Kilic *et al*., 2012).

TABLE I

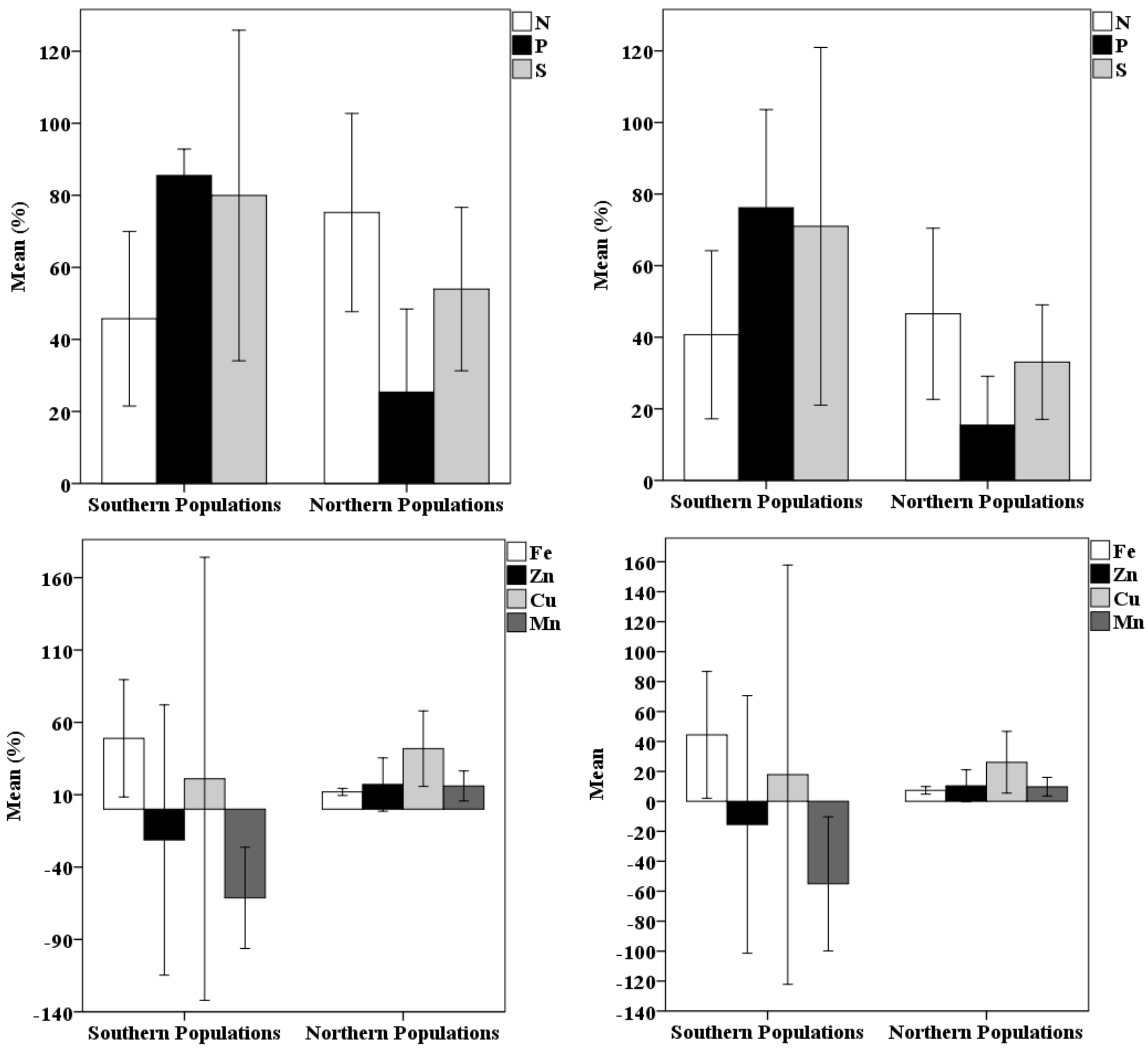
Full leaf and senescence macroelements (mg/g) and microelements (mg/kg) concentrations and their comparisons between populations

Dependent Variable	Locality	Mean	Std. Deviation	N	Type III Sum of Squares	df	Mean Square	F	Sig.
				Full leaf					
N	Southern Populations	24.74	2.82	15	141.09	1	141.09	10.34	**0.01***
	Northern Populations	18.61	5.78	5					
P	Southern Populations	0.51	0.12	15	0.04	1	0.04	3.50	0.07
	Northern Populations	0.41	0.04	5					
S	Southern Populations	6.37	3.89	15	103.91	1	103.91	8.79	**0.01***
	Northern Populations	1.11	0.14	5					
Fe	Southern Populations	129.91	35.48	15	218583.27	1	218583.27	186.45	**0.01***
	Northern Populations	371.34	29.46	5					
Zn	Southern Populations	13.40	4.07	15	18005.87	1	18005.87	499.63	**0.01***
	Northern Populations	82.70	10.19	5					
Cu	Southern Populations	11.10	3.09	15	5891.29	1	5891.29	242.46	**0.01***
	Northern Populations	50.74	8.70	5					
Mn	Southern Populations	10.12	7.20	15	22.22	1	22.22	50.92	**0.01***
	Northern Populations	89.88	2.32	5					
				Senescence					
Dependent Variable	Locality	Mean	Std. Deviation	N	Type III Sum of Squares	df	Mean Square	F	Sig.
N	Southern Populations	13.35	2.77	15	317.84	1	317.84	47.50	**0.01***
	Northern Populations	4.14	1.77	5					
P	Southern Populations	0.07	0.01	15	0.23	1	0.23	168.29	**0.01***
	Northern Populations	0.32	0.07	5					
S	Southern Populations	0.65	0.19	15	0.08	1	0.08	2.77	0.11
	Northern Populations	0.50	0.08	5					
Fe	Southern Populations	15.62	6.62	15	247874.39	1	247874.39	171.63	**0.01***
	Northern Populations	67.94	4.48	5					
Zn	Southern Populations	9.66	10.42	15	10263.26	1	10263.26	265.61	**0.01***
	Northern Populations	29.10	6.95	5					
Cu	Southern Populations	15.42	10.03	15	0.25	1	0.25	6.09	0.02
	Northern Populations	75.46	5.19	5					
Mn	Southern Populations	15.42	10.03	15	13518.00	1	13518.00	160.35	**0.01***
	Northern Populations	75.46	5.19	5					

**P<0.01*

STATISTICAL ANALYSIS

Statistical analysis was performed by using a SPSS (21.0 version) software (IBM Corp., 2012). Data were analysed for normality by using Shapiro-Wilk test, and square, ln (square) and invers transformation of the data was used for normal distribution before performing the one-way MANOVA tests. One-way MANOVA was performed to show the differences between populations with respect to macro- and microelement concentrations. Mann-Whitney U test and student's t test were used to show the differences between populations with respect to RE. Northern and southern populations were assigned as independent variable, while macro- and microelements concentrations and RE were selected as the dependent variables. The Pearson's and Concordance correlation tests were used to fit between the calculation of macro- and microelement RE with and without MLCF correction.



(a) **(b)**

Figure 2.— The macro- and microelements resorption efficiency without MLCF (a) and with MLCF correction (b).
Data are mean and ± 1 SD.

TABLE II

The macroelements resorption efficiency (%) with and without MLCF correction and their comparisons between populations

Without MLCF correction							
Dependent Variable	Locality	N	Median	Range	Mean	SD	Sig.
N	Southern Populations	15	45.43	53.83	45.72	12.11	**0.01***
	Northern Populations	5	75.35	34.93	75.21	13.75	
P	Southern Populations	15	84.77	12.03	85.48	3.67	**0.01***
	Northern Populations	5	23.02	30.24	25.38	11.53	
S	Southern Populations	15	91.01	76.04	79.93	22.93	**0.01***
	Northern Populations	5	57.04	30.41	53.96	11.33	
With MLCF correction							
Dependent Variable	Locality	N	Median	Range	Mean	SD	Sig.
N	Southern Populations	15	42.12	42.83	40.69	11.75	0.34
	Northern Populations	5	45.01	31.10	46.55	11.95	
P	Southern Populations	15	76.23	49.29	76.20	13.70	**0.01***
	Northern Populations	5	12.73	17.51	15.45	6.82	
S	Southern Populations	15	71.55	110.67	70.99	24.99	**0.01***
	Northern Populations	5	32.91	22.48	33.07	7.99	

**P<0.01*

TABLE III

The microelements resorption efficiency (%) with and without MLCF correction and their comparisons between populations.

Without MLCF correction							
Dependent Variable	Locality	N	Median	Range	Mean	SD	Sig.
Fe	Southern Populations	15	52.19	56.12	48.98	20.30	**0.01***
	Northern Populations	5	11.74	30.07	11.95	1.23	
Zn	Southern Populations	15	-32.46	166.63	-21.24	46.70	**0.01***
	Northern Populations	5	15.80	24.38	17.06	9.21	
Cu	Southern Populations	15	68.98	174.33	21.03	76.55	0.34
	Northern Populations	5	38.92	34.13	41.86	13.03	
Mn	Southern Populations	15	-58.48	60.98	-61.26	17.51	**0.01***
	Northern Populations	5	16.96	13.61	16.04	5.21	
With MLCF correction							
Dependent Variable	Locality	N	Median	Range	Mean	SD	Sig.
Fe	Southern Populations	15	41.19	65.16	44.40	21.17	**0.01***
	Northern Populations	5	7.01	3.48	7.35	1.28	
Zn	Southern Populations	15	-24.78	162.33	-15.46	43.02	0.03
	Northern Populations	5	9.99	14.54	10.34	5.35	
Cu	Southern Populations	15	54.94	192.63	17.83	69.99	0.30
	Northern Populations	5	24.60	27.11	26.08	10.31	
Mn	Southern Populations	15	-55.01	96.53	-55.08	22.39	**0.01***
	Northern Populations	5	10.11	7.80	9.76	3.11	

**P < 0.01*

RESULTS

Macroelement concentrations (N, P, S) were found to be higher in southern populations, while microelement concentrations (Fe, Zn, Cu, Mn) were found to be higher in northern populations. Northern populations were N, P and S-proficient because lower concentrations were found in senescent leaves. However, southern populations were Fe, Zn, Cu and Mn-proficient (Fig 1).

Significant differences were found between northern and southern populations with respect to macro and micro-element concentrations except for P concentrations in full leaves. However, no significant differences were found between northern and southern populations with respect to S and Cu concentrations in senescent leaves (Tab. I).

It has been found that Mn and Zn were accreted in southern populations, while they were resorbed in northern populations. In addition to this, RE was found to be lower with MLCF correction and significant differences were found between northern and southern populations except for NRE with MLCF correction (Fig 2; Tab. II).

ZnRE was not significantly changed between northern and southern populations without MLCF correction. However, NRE and CuRE were not significantly changed with MLCF correction (Tab. III).

The calculation of macro- and micolements RE with or without MLCF correction were supported each other by statistical models (Tab. IV).

TABLE IV

The results of Pearson's and Concordance correlation tests between the calculation of macro- and microelements resorption efficiency with and without MLCF correction

Elements	N	Concordance correlation coefficient	95% Confidence Interval	r	P
N	20	0.52	0.25-0.71	0.72	**0.01***
P	20	0.86	0.71-0.94	0.92	**0.01***
S	20	0.77	0.56-0.89	0.87	**0.01***
Fe	20	0.94	0.86-0.97	0.95	**0.01***
Zn	20	0.97	0.94-0.98	0.98	**0.01***
Cu	20	0.97	0.93-0.98	0.98	**0.01***
Mn	20	0.93	0.85-0.97	0.94	**0.01***

**P<0.01*

DISCUSSION

According to Killingbeck (1996) foliar resorption is highly proficient in plants if nitrogen and phosphorus concentrations below 7 $mg.g^{-1}$ and 0.5 $mg.g^{-1}$of dry matter respectively in their senescent leaves (Kilic *et al.*, 2010). According to threshold values both northern and southern populations were P-proficient, while only northern populations were N-proficient. It has been found that northern populations were N-, and S- proficient, while southern populations were P, Fe, Zn, Cu, and Mn-proficient because their concentrations were lower in senescent leaves although there is no threshold values for micronutrients.

NRE was found to be higher in northern populations as compared to southern populations, while the opposite trend was found with respect to PRE in the present study. Salehi *et al.* (2013) studied NRE and PRE in some poplar species in Hyrcano-Euxine phytogeographical region. Northern and southern populations of *P. fraxinifolia* had high NRE than that of poplar species, while northern populations had similar PRE. However, southern populations had rather high PRE than poplar species. It has been emphasized that foliar nutrient concentrations and foliar resorption vary largely among individuals of the same species and between different species (Salehi *et al*., 2013). S concentrations in senescent leaves were found to be low and SRE was found to be high. Liu *et al.* (2014) reported that S is only significantly resorbed in deciduous tree species among functional groups and found SRE was 12.5% in deciduous trees. Bilgin *et al.* (2015) also found low S concentrations in leaves of *Vaccinium* species and reported that S is weakly resorbed in deciduous trees. However, SRE was much higher in southern populations than the reported values and SRE was found to be about three-fold higher even in northern populations with MLCF correction (33.06 %). Nutrient concentrations in plants vary depending on the ability to transfer nutrients from xylem or phloem by plants and it has been reported that S is known to be highly mobile nutrient in phloem (Mailard *et al.*, 2015).

NRE/PRE ratios were found >1 in northern populations, while <1 in southern populations. Reed *et al.* (2012) stated that if NRE/PRE ratios were < 1 more P was consistently resorbed relative to N. It has been found that PRE was higher as compared to NRE in southern populations. Several authors implied that P compounds in a leaf are more readily resorbed as compared to N compounds therefore PRE is more significant with respect to nutrient use efficiency than NRE (Aerts & Chapin, 2000; Salazar *et al*., 2011; Yilmaz *et al*., 2014). However, the opposite trend was found in northern populations and NRE was higher than PRE. Reed *et al.* (2012) and Brant & Chen (2015) reported that NRE was increased while PRE was decreased with increasing latitude and tree species in northern regions tend to be more N-limited mainly due to N losses regarding leaching and denitrification. High RE also indicated low litter quality and low mineralization rates. The swamp forests in northern part of Turkey exhibit low rates of soil organic matter decomposition due to high precipitation (Horuz *et al*., 2014).

Foliar Zn and Cu concentrations of *P. fraxinifolia* in the present study were similar to those of *Fraxinus excelsior* L. populations in Europe. Foliar Fe concentrations were similar to those of *Fagus sylvatica* L. However, micronutrient especially foliar Mn concentrations were lower than some European tree species quoted by Hagen-Thorn *et al*. (2006).

Negative Zn and MnRE values were found in southern populations. This means Zn and Mn are accreted in southern populations. Housman *et al.* (2012) and Medina *et al.* (2015) also found that Mn and Cu were accreted and they stated that the accretion of foliar micronutrients is probably due to lower nutrient mobility or lack of a driving force for their nutrient conservation and that resorbed micronutrients are more limiting or they may be close to the resorption of macronutrients. Mn was classified as a low mobility micronutrient in phloem (Maillard *et al.*, 2015). Zn and Mn were more limiting in northern populations of *P. fraxinifolia* because they were resorbed, while they were accreted in southern populations. The differences between northern and southern populations may likely be due to differences in the uptake process of micronutrients among different individuals (Oleksyn *et al.*, 2002, 2003).

N, Zn, Cu and MnRE were found to be higher in northern populations, while P, S and FeRE were found to be higher in southern populations. This may be interpreted on the basis that northern populations of *P. fraxinifolia* have higher N, Zn, Cu and Mn requirements, while southern populations have high P, S and Fe requirements (Yan *et al.*, 2015). If a particular nutrient is limiting, plant resorbs more that particular nutrient (Brant & Chen, 2015). This would explain that NRE, MnRE, ZnRE and CuRE were found to be higher in northern populations whereas PRE, FeRE, and SRE were higher in southern populations.

Foliar RE should be corrected due to leaf mass loss during leaf senescence and MLCF correction should be applied (Vergutz *et al*., 2012; Surmen *et al*., 2014). There were significant differences among foliar RE values when MLCF was used or not. Additionally, NRE was found to be lower for woody deciduous species at global scale. However, PRE was higher than global estimates for PRE when MLCF was not used, while very close to global PRE values when MLCF was used (Brant & Chen, 2015). We also found that the calculations of macro- and microelements RE with or without MLCF correction were each supported by statistical models.

In summary, we found that northern populations were N-proficient, while southern populations were P-proficient. It has been found that micronutrients (Zn and Mn) were more limiting in northern than southern populations because they were resorbed in northern populations but accreted in southern populations. SRE was found to be higher than in other deciduous species. We agree with Liu *et al*. (2014) who stated that nutrient resorption patterns strongly depend on a particular nutrient and plant species.

González (2012) stated that the ecological integrity of floodplain forests may be protected by effective restoration and management measures. Patterns of foliar resorption and of macro- and microelement use may help to achieve these goals and our results may serve to assess nutrient using patterns of swamp forest species. Such studies may also contribute to specify threshold-values for resorption proficiency of micronutrients.

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