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MINERAL ELEMENT UPTAKE STATUS OF ENDEMIC *ISOETES ANATOLICA* PRADA & ROLLERI POPULATIONS FROM BOLU-TURKEY

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Abstract

Isoetes genus is commonly known as the "quillworts" and considered to be "fern allies". There are about 200-250 species, with a cosmopolitan distribution but often scarce to rare. *Isoetes* genus members often grow in extremely sensitive aquatic environments such as temporary ponds, streams and lakes. They are therefore good indicators of environmental quality. *Isoetes anatolica* Prada & Rolleri is an endemic plant grows on calcareous sediment/soil on the edges of seasonal ponds located in a mountainous area near the southern coast of the Black Sea at 1400 m above sea level at Bolu, Turkey. In this study, mineral element uptake statuses of *I. anatolica* populations were studied on the background of plant-sediment/soil-water interactions. The study materials were collected from the place where this narrow endemic species only lives in the world (Abant Region, Bolu/Turkey) by using standard methods and plant and sediment/soil mineral element measurements (Al, B, Ca, Cu, Fe, K, Mg, Mn, Na, Ni and Zn) were done. ICP-OES was employed for the measurements during the study. Interrelations between mineral element contents in the sediment/soil, water and plant were discussed. The data revealed that *I. anatolica* is capable of accumulating considerable amounts of certain mineral elements (B, Ca, Mn and Na).

Introduction

Genus *Isoetes* is known as a cosmopolitan heterosporous lycopsids (Pteridophyta), comprising about 200-250 species and is found mainly in the tropics (Taylor & Hickey, 1992; Hickey, 1997). The distinctive features are: submerged or emergent aquatic, sometimes terrestrial in moist places, perennial herbs with 2-5 lobed, corm-like rootstocks, bearing a rosette of leaves, and dichotomous roots from between the lobes. Leaves terete or flat and linear, often lacking stomata, with 4 longitudinal air canals and a single central vascular strand, expanded basally, glabrous, a small triangular ligule on the adaxial surface above the sporangium. Sporangia very large, borne adaxially in depressions at the base of the sporophylls, containing either large, trilete megaspores, or minute, monolete microspores, sometimes partially or entirely covered by a membranous vellum, extending down from the apex of the sporangium (Fuchs, 1962; Jermy, 1965; Croft, 1980; 1985).

It is indicated that there are of 11 species in the Mediterranean region of which 8 are endemic (Greuter *et al*., 1984). *Isoetes anatolica* Prada & Rolleri was first discovered on the edges of seasonal ponds located in a mountainous area near the southern coast of the Black Sea at 1400m above sea level at Bolu (Fig. 1), Turkey (Prada & Rolleri, 2005). A search for additional populations of *I. anatolica* in similar habitats around Abant Lake was produced no result (Bolin *et al*., 2008). It grows in calcareous sediment/soil (Prada & Rolleri, 2005). The data revealed that the diploid chromosome number is 2n = 22 for *I. anatolica*, a first count for this species (Bolin *et al*., 2008). *I. anatolica* is a robust and has well-formed megaspores and microspores (Prada & Rolleri, 2005). Since *Isoetes* genus members often grow in extremely sensitive aquatic environments such as temporary ponds, streams and lakes, they are good indicators of environmental quality (Al Arid *et al*., 2011; Bolin *et al*., 2011). Despite it being a well-known flora, little is known about genus *Isoetes*.

The present work focuses on mineral element uptake statuses of *I. anatolica* Prada & Rolleri populations on the background of plant-sediment/soil-water interactions, whose characteristics clearly differ from the other *Isoetes* species.

Materials and Methods

Surface water and sediment/soil samples were collected at each location in 2012. Samples of water were taken from 5 different points at each sampling site. The pH of water was determined at each before the water was sampled. Sediment/soil sampling (about 500 g for each) was conducted by using a stainless steel shovel from a depth of about 10cm at 5 different points for each sampling site. A bulk was formed from the samples at each sampling site and a representative sample was obtained from that bulk. The sediment/soil sample collections were carried out by hand using disposable gloves to prevent contamination at each sampling site and following the collection, the samples were put into white transparent labeled polythene bags.

Plant parts were isolated and oven-dried at 80°C for 24 h, milled in micro-hammer cutter and fed through a 1.5 mm sieve. Samples were weighed as 0.3g and transferred into Teflon vessels and then 8ml 65% HNO_3 was added (Aksoy *et al*., 2012; Yasar *et al*., 2012).

The sediment/soil samples were also oven-dried at 80°C for 24h and fed through a 2mm sieve. They were weighed as 0.3g and transferred into Teflon vessels and then 5ml 65% HNO_3, 3 ml 37% HCl and 2ml 48% HF were added. Water samples were filtered and then 0.5ml was taken from each sample and finally 8ml 65% HNO_3 (Merck) was added. All samples were mineralized in microwave oven (Berghof-MWS2) as follows: in 145°C for 5 min, in 165°C for 5 min and in 175°C for 20 min. After cooling, the samples were filtered by Whattman filters, and made up to 50 ml with ultra-pure water in volumetric flasks and then stored in falcon tubes. Standard solutions were prepared by using multi element stock solutions-1000 ppm (Merck) and mineral element (Al, B, Ca, Cu, Fe, K, Mg, Mn, Na, Ni and Zn) measurements were done by Inductively Coupled Plasma Optical Emission Spectroscopy (PerkinElmer-Optima 7000 DV).

Results and Discussion

The plant, soil and water samples were collected from the place situated in Southern coast of Black Sea of Turkey at an altitude of 1400 m above sea level at Abant-Bolu (Fig. 2) by using standard methods.

In the region, a transition climate between the Mediterranean with a very cold, less rainy winter and oceanic is seen (Turker & Guner, 2003; Cobanoglu & Akdemir, 2004). Due to geographical conditions, totally closed to the dry southern wind and opens to the northern winds, high degree humidity is occurred in the region (Anon., 2011). According to the meteorological data obtained between 1930 and 2001 (71 years) in Bolu, the mean annual temperature is 10.3°C. Abant Lake is 600m higher than Bolu. Therefore, the mean annual temperature of Abant is expected to be 7.3°C (Mater & Sunay, 1985). The mean annual precipitation in Bolu is 534.4mm. of this precipitation, 32% is in winter, 29% in spring, 21% in autumn and 18% in summer. According to the elevation factor, the mean annual precipitation of Abant is expected to be between 800 and 900mm (Mater & Sunay, 1985). The lake region is famous for its scenic beauty and is recognized as Natural Park surrounded by a rich flora and fauna. The lake is an important geographical feature in the park and considered to have unique species richness as a result of its long geological history (Celekli *et al*., 2007).

Abant Lake and its vicinity were formed as a consequence of various events in the Paleozoic times. The sandy, clayey and calcareous soils were created by dissolving of bedrock formed from schist and serpentine (Mater & Sunay, 1985). Table 1 showed that the water in which *I. anatolica* grows has pH values of 6.12-6.47. The average pH value is 6.23 ± 0.17. In regards to pH, *I. anatolica* grows in slightly acidic sediment/soil and water. Soil mineral element measurements (Al, B, Ca, Cu, Fe, K, Mg, Mn, Na, Ni and Zn) were done. The data revealed that the concentrations of macro-elements in the plant, sediment/soil and water (mg/kg), respectively, were 3651.2, 1068.707 and 192.872 for Ca, 3195.2, 4557.355 and 247.064 for K, 1388.36, 2471.634 and 113.416 for Mg. The concentrations of micro-elements in the plant, sediment/soil and water (mg/kg) were 508.96, 4914.148 and 51.64 for Al, 7.95, 5.913 and 0.023 for B, 4.99, 10.041 and 0.245 for Cu, 431.0, 3032.572 and 43.192 for Fe, 186.672, 95.583 and 0.801 for Mn, 3.03, 9.743 and 0.015 for Ni, 208.176, 110.518 and 10.001 for Na and 27.008, 35.664 and 2.21 for Zn, respectively.

An attempt was made to explain interrelations between mineral element contents in sediment/soil, water and the plant. According to the literature, the normal limits of Al, B, Cu, Fe, Mn, Ni and Zn in plants are in range of 7-104, 10-100, 5-30, 2-250, 30-300, 0.1-5, 25-150 mg/kg dw and between or over 400, 50-200, 20-100, 400-1000, 300-500, 10-100, 100-400 mg/kg dw are accepted as toxic levels, respectively (Kabata-Pendias & Pendias, 2001; Kabata-Pendias & Mukherjee, 2007). According to these values, the B, Cu, Ni and Zn concentrations in this study were within normal limits in the plant. But for Al, Fe and Mn, the normal limits were exceeded. Especially, Al was in the range of toxic level.

The data suggest that the plant accumulates excessive Al in the plant. Much of Al enters the cell is resulted in compartmentalization of Al through action of antiporters found on the vacuolar membrane. Hence, the toxic effects of Al accumulation are reduced by its sequestration into the vacuole (Apse *et al*., 1999; Gaxiola *et al*., 1999). Also, our results showed that considerable amounts of B, Ca, K, Mg, Mn, Na and Zn were accumulated by the plant although some of them were within the limits (Table 2). For example, B and Mn levels in the whole plant were much higher than the sediment/soil and water showing a high degree accumulation although they were not in the range of toxic levels. Soil solution pH is the major factor affecting the availability of micro-elements among the different factors. Among the effects of pH, the important one is mainly on the solubility of nutrients and their ionic forms (Ronen, 2007). Some nutrients might become unavailable while others might reach high concentrations leading to deficiency or toxicity, respectively, at different levels of pH. At high basic pH, availability problems occur for the most micro elements. The pH range within 6.0 to 6.5 is preferable by most plants for growing optimum. This will assure high availability of most demanded nutrients for plants (Ronen, 2007). Harris (1937) noted that there is a relationship between soil pH and K fixation and K fixation occurs in such soil pH ranging from 5.3 to 8.5. In acidic soils, the K fixation rate is reduced as a result of competition between K and Fe, Al and H ions; however, in alkaline soils, by contrast, K fixation rate is increased (Sezen, 1975; 1991). Our results were consistent with this fact. Table 2 showed that K level was much higher than Al and Fe levels in the plant with respect to pH value of the sediment. Individual of soil characteristics has the effects on the plant elements but also joint impacts are taken into account because of interactions between them. In a previous study, an attempt was made to determine relationships between the soil characteristics of soils and the mineral element content of the *C. tinctoria* and *R. tinctorum* plants. In *R. tinctorum* positive correlations between soil pH and plant potassium and soil potassium and plant calcium were obtained. However, negative correlations exist between soil organic matter and plant sodium and soil organic matter and plant manganese content in *C. tinctoria*. In that study, the data implied that the soils of these plants appear to be poor in nutrients (Baslar & Mert, 1999; Dogan, 2001; Baslar *et al.*, 2003). In another study in the same area were reported three negative linear correlations were observed between plant calcium and soil pH, plant nitrogen and soil calcium carbonate, plant potassium and soil calcium carbonate (Dogan *et al*., 2003). In our study, there was a positive correlation between soil K and plant Ca. And also, plant Na and Mn levels were high in comparison to the sediment/soil and water. It implies that the sediment/soil is poor in nutrients.

Fig. 1. Satellite view showing the study area (location of Abant Lake region, Bolu-Turkey)

Fig. 2. The photos showing A. *Isoetes anatolica* Prada and Rolleri and B. its natural habitat

Availability of micro-elements depends on some interactions occurring in the soil and the plant tissues. Some interactions cause interference on availability or damage in the plant tissues due to excessive concentrations. Our data revealed that quite high uptake rate for Zn was a consequence of low N and P levels or in other word; the sediment/soil was insufficient for organic materials. It means there were no significant antagonistic interactions between P-Zn and N-Zn (Camp & Fudge, 1945; Ozanne 1955; Thorne, 1957; Stuckenholtz *et al.*, 1966). Also, B accumulation was very high in comparison to the sediment/soil and water in the plant in our study. Higher Ca concentration can be employed to correct boron toxicity. A requirement for normal growth in plant is a certain balance between the uptake of Ca and B (Jones & Scarseth, 1944). Our results showed consistency with this fact. Accelerated Zn or Mn uptake causes a marked reduction in Fe concentration in plants (Epstein & Stout, 1951; Ronen, 2007). This was also observed in our work. Their adaptations and abilities of biotic relationships in plants for surviving in physical environment are fundamental. *I. anatolica* lives only in that small region reflecting its unique adaptation ability, specific for that area.

Table 1. pH values of water samples (WS) in habitat of *Isoetes anatolica* Prada & Rolleri.

WS 1	WS 2	WS 3	WS 4	WS 5	Average
6.12	6.33	6.13	6.09	6.47	6.23 ± 0.17

Table 2. Mineral element (Al, B, Ca, Cu, Fe, K, Mg, Mn, Na, Ni, and Zn) concentrations of sediment/soil, water and samples of *Isoetes anatolica* Prada & Rolleri.

Elements	Plant (mg/kg)	Sediment/soil (mg/kg)	Water (mg/L)
Al	508.960 ± 22.721	4914.148 ± 163.759	51.640 ± 3.800
B	7.950 ± 0.289	5.913 ± 0.247	0.023 ± 0.001
Ca	3651.200 ± 160.453	1068.707 ± 34.217	192.872 ± 5.607
Cu	4.990 ± 0.341	10.041 ± 0.287	0.245 ± 0.018
Fe	431.00 ± 15.28	3032.572 ± 168.415	43.192 ± 1.58
K	3195.200 ± 94.309	4557.355 ± 162.054	247.064 ± 9.575
Mn	186.672 ± 6.428	95.583 ± 2.942	0.801 ± 0.053
Mg	1388.360 ± 52.581	2471.634 ± 93.932	113.416 ± 5.212
Na	208.176 ± 6.015	110.518 ± 3.943	10.001 ± 0.761
Ni	3.03 ± 0.154	9.743 ± 0.165	0.015 ± 0.002
Zn	27.008 ± 0.181	35.664 ± 0.109	2.21 ± 0.073

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