

Chemical Composition of Seeds of Two Endemic *Lathyrus* L. Species from Turkey

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Abstract

Genus *Lathyrus* L. belonging to Fabaceae (Leguminosae) family consisted of 77 taxa at the species, subspecies, and variety level at Turkey and 25 of that were known as endemic. *Lathyrus egirdiricus* H.Genc & A.Sahin and *Lathyrus tefennicus* H. Genç & A. Şahin were endemic species and described at the date of 2008 and 2011, respectively. The seeds of *Lathyrus tefennicus* and *Lathyrus egirdiricus* were collected in Tefenni of Burdur province and Eğirdir of Isparta province in May of 2016 at Turkey. The concentration of Cd, Cr, Ni, Cu, Mn, Zn, Fe, Ca, Mg, Na and K in the *Lathyrus tefennicus* and *Lathyrus egirdiricus* seeds were determined by Atomic Absorption Spectrometry. A microwave oven was used for the sample digestion. The accuracy of proposed method was confirmed by the analysis of the certified reference material (EnviroMAT Contaminated Soil). All results were given at 95% of confidence level. The presence of different concentrations of elements in the seeds of both species strengthens the belief that this data can be used in plant taxonomy.

Keywords: Chemical composition, Elements, *Lathyrus egirdiricus*, *Lathyrus tefennicus*, Turkey

1. Introduction

Lathyrus L. belongs to the tribe Vicieae within the Fabaceae (Leguminosae). It contains more than 200 taxa and has an almost worldwide distribution (Allkin et al., 1986). The main centers of diversity of *Lathyrus* were the Mediterranean region, Asia Minor and North America, as well as South America and East Africa (Klamt & Schifino-Wittmann, 2000). Tutin et al. (1968) reported that 54 species of *Flora Europaea* were known. Davis (1970) stated that the genus was represented as 67 taxa at the species, subspecies, and variety level in *Flora of Turkey*. However, the number of taxa at Turkey was increased to 77 and 25 of that were known as endemic (Davis et al., 1988; Güner et al., 2000; Genç & Şahin, 2008; Genç, 2009; Genç & Şahin, 2011; Güneş & Çırpıcı, 2012; Güneş, 2014). *Lathyrus* L. was important owing to economically and nutritionally. In general, *L. sativus* L., *L. hirsutus* L., *L. cicera* L., *L. ochrus* (L.) DC. and *L. sylvestris* L. were cultivated for forage and human food. These species were highly nutritious and favorite food for domestic animals (Yamamoto et al., 1984). It was stated that *Lathyrus sativus*, *L. cicera*, *L. ochrus* and *L. clymenum* L. were cultivated at Turkey (Genç & Şahin, 2001).

If the atomic number and mass of elements were bigger than 20 and 5 g/cm³, respectively, they were called as heavy metals (Lajayer et al., 2017). The metal pollutants in the environment caused hazard effect for all living organisms. In last decades, different industrial activities such as battery and paint manufacture, fertilizer usage and wastes have caused to metal contaminations in the soil, water and air (Amari et al., 2017) causing the accumulation of them in the plants (Filipiak-Szok et al., 2015).

The heavy metals were transferred from the soil to plant and following accumulated in the seeds, cereals, fruits, roots, stalks and leaves. To investigate the physiological activity of the plant, the concentrations of Cr, Mn, Mo, Zn, Fe, Co, Cu, Al, Ni should be determined (Filipiak-Szok et al., 2015; Heredia et al., 2016). When the plant included excessive or low amount of these metals, it caused to a negative effect for the plant health (Heredia et al., 2016). Because some metals such as Pb, Ni, and Co hinder to ecesis as well as make prohibition effects on the plants (Mohamed et al., 2017), determination of metals in the plants were important.

There were some analytical methods such as flame atomic absorption spectrometry (FAAS), graphite furnace atomic absorption spectrometry (GFAAS), inductively coupled plasma optical emission spectrometry (ICPOES), and inductively coupled plasma mass spectrometry (ICPMS) were used for the determination of metals in any samples (Santos Júnior et al., 2017). Among them, FAAS was frequently used because of its some advantages such as more speed, lower cost and higher accessibility (Elik et al., 2017).

In this study, determinations of 11 different metals such as Cd, Cr, Ni, Cu, Mn, Zn, Fe, Ca, Mg, Na and K in the both *Lathyrus tefennicus* and *Lathyrus egirdiricus* seeds were performed by FAAS method. The microwave method was used to digest seeds because of its rapid and repeatable properties (Tüzen, 2003). *Lathyrus tefennicus* an endemic species was found in Tefenni of Burdur province at Turkey and grewed in *Pinus* L. forests. The flowering and fruiting time was between May and July. The colour and size of seeds were varied from dark brown to dark green with the rough-surfaced and $2.9\text{--}3.8 \times 4.8\text{--}6$ mm with a $1.5\text{--}3.2$ mm long hilum, respectively (Genç & Şahin, 2011). Furthermore, *Lathyrus egirdiricus* an endemic species was found about 3 km far from Eğirdir of Isparta province at Turkey. The colour of its flower was light purple and bloom in between May and July. However, the colour of seed was varied from light brown to dark brown with rough-surfaced. The size of seed is 6–8 per legume, 2×4 mm, hilum 0.5–1 mm (Genç & Şahin, 2008). The accuracy of the method was performed by using certified reference material (EnviroMAT Contaminated Soil) at 95% confidence level with good agreement. It was thought that the data obtained in this study could be used for plant taxonomy based on chemotaxonomics.

2. Material and Methods

2.1 Material

The seeds of *Lathyrus tefennicus* and *Lathyrus egirdiricus* were collected from natural habitat in Tefenni (Burdur) and Eğirdir (Isparta) in May 2016.

2.2 Reagents and apparatus

All reagents were of analytical reagent grade. The stock solutions (1000 mg/L) of Cd, Cr, Ni, Cu, Mn, Zn, Fe, Ca, Mg, Na and K were prepared from $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (Aldrich), $\text{Cr}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ (Merck), $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (Merck), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (Sigma-Aldrich), $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ (Sigma-Aldrich), ZnCl_2 (Merck), $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (Merck), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (Sigma-Aldrich) $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (Riedel-de Haën), NaCl (Sigma-Aldrich) and KNO_3 (Merck), respectively. 18.2 MΩ.cm deionized water (PURIS, Expe-up) was used for all

experimental studies. The concentrated HNO_3 , HCl , and HF solutions used for the digestion step were supplied from Merck, Riedel de Haën and Merck, respectively. A flame atomic absorption spectrometry (ATI Unicam 939 model) was used for determination of all metals. The operating conditions in determination step were shown in Table 1.

The microwave digestion system (CEM, Mars 6 240/50 model) was used to digest both the seeds and certified reference material (EnviroMAT Contaminated Soil). The digestion conditions for the seeds and certified reference material were given in Table 2.

2.3 Digestion procedure

The seeds were first washed with distilled water and then dried at the room temperature. Afterwards, they were homogenized using a porcelain agate and stored in polyethylene bottles until experiment. During the digestion, 1.0 g of seed samples and 0.2 g of the certified reference material (EnviroMAT Contaminated Soil) were introduced into a Teflon vessel. Then, 3 mL of concentrated HNO_3 , 3 mL of concentrated H_2O_2 , 1 mL of concentrated HClO_4 and 3 mL of deionized water were added on all samples, respectively. After digestion procedure given in Table 2, the samples were transferred into polyethylene bottles and kept in the refrigerator until analysis.

3. Result and Discussion

3.1 Concentrations of metals in the seeds

The concentrations of some metals such as Cd, Cr, Ni, Cu, Mn, Zn, Fe, Ca, Mg, Na and K were determined using by FAAS after digestion procedure. The standard solutions were prepared between 0.2 and 10 mg/L depending on metals and the absorbance values of the metals were measured in both standard and sample solutions using the calibration method. The linear equations of calibration graphs for all metals were given in Table 3. The concentrations of metals in the *Lathyrus tefennicus* and *Lathyrus egirdiricus* were calculated by using these linear equations depending on metals.

Cd in plants decreased the plant cultivation and microorganism variety (Sobariu et al., 2017). The Cr(III) was an essential metal and played important roles in the biological systems. However, the Cr(VI) was toxic for plants, animals and human beings. In general, Cr was transferred from the soil to plant (Sobariu et al., 2017). Ni was a serious pollutant due to burn of coal and petroleum and using of excessive fertilizer. The Ni concentration in the plant was increased with increasing Ni concentration in the soil. Its concentration in the plants was affected by the climate and soil pH. The excessive amount of Ni slowed down the plant growth (Kabala & Singh, 2001). Cu was an essential metal for all creatures. Cu was attended in some important biological mechanisms such as carbohydrate and lipid metabolism. However, the excessive amount of Cu could cause to harmful effects on the blood and kidney (Xiang et al., 2010). Mn was another essential metal and played an important role in the growth of plants. Because of this, the Mn salt was added to the soil (Lemos et al., 2009). Zn was toxic for plant growth when it was taken at higher than essential amount. In addition to this, the adsorption of Cd by the plant was diminished in the soil because of Zn (Pehlivan et al., 2008). Fe was essential for all creatures and it had significant functions for some

processes such as DNA synthesis, oxygen and electron transport. The high amount of Fe could cause the tissue damage (Maiga et al., 2005). Ca was an essential metal for plant growth and development. Additionally, Ca played the crucial role in regulation of membrane permeability, stabilization of tissues and quality of plants (Tuna & Özer, 2005). Mg was accepted as the iron of the plant world. Similar to the Fe-hemoglobin relationship in humans, Mg entered the chlorophyll structure in plants (Solak Görmüş & Ergene, 2004). Na played an important role to adjust the blood volume, pressure, the osmotic balance and the pH value in the body. However, higher amounts of Na may cause to hypertension, stroke, stomach cancer, osteoporosis and kidney diseases (Hwang et al., 2017). K played some important roles such as regulate of plant water potential, form and transport of proteins, carbohydrates, fats and convert of amino acids into proteins. K was the most important element affecting the quality parameters of the plant such as color, odor, aroma, durability, hardness, resistance against diseases and storage period (Kacar & Katkat, 1998).

The concentration of metals in both *Lathyrus tefennicus* and *Lathyrus egirdiricus* and other plants in the literature were compared and the results were given in Table 4.

3.2 Method validation

The accuracy of the proposed method developed was checked by applying certified reference material (EnviroMAT Contaminated Soil) for Cu determination. The Cu amount was found as 174 mg/kg in the certified reference material and there was a good agreement between found and certified values at 95% confidence level with -8.9% relative error.

4. Conclusion

To the best of our knowledge, this was the first study to determine metal composition of new *Lathyrus tefennicus* and *Lathyrus egirdiricus* seeds. The amounts of Cd, Cr, Ni, Cu, Mn, Zn, Fe, Ca, Mg, Na and K in the seeds were determined using by FAAS after digestion step with microwave oven. Significant toxic metals such as Cd, Cr and Ni were not found in seeds. Cu were determined as 4.71 ± 0.09 mg/kg and 3.74 ± 0.09 mg/kg in *Lathyrus tefennicus* and *Lathyrus egirdiricus*, respectively. Mn values were found as 9.65 ± 0.40 mg/kg in *Lathyrus tefennicus* and 11.0 ± 0.28 mg/kg in *Lathyrus egirdiricus*. Zn were found as 25.0 ± 0.32 mg/kg and 42.0 ± 1.3 mg/kg in *Lathyrus tefennicus* and *Lathyrus egirdiricus*, respectively. The amounts of Fe were found as 66.0 ± 3.0 mg/kg and 51.0 ± 3.0 mg/kg in *Lathyrus tefennicus* and *Lathyrus egirdiricus* seeds, respectively. The amounts of Ca were identified as 760 ± 62.0 mg/kg and 1343 ± 98.0 mg/kg in *Lathyrus tefennicus* and *Lathyrus egirdiricus* seeds, respectively. The Mg amounts were determined as 1374 ± 38.0 mg/kg and 1026 ± 82.0 mg/kg in *Lathyrus tefennicus* and *Lathyrus egirdiricus* seeds, respectively. The concentrations of Na were found as 6650 ± 59.0 mg/kg and 4967 ± 251 mg/kg in *Lathyrus tefennicus* and *Lathyrus egirdiricus* seeds, respectively. The amounts of K in *Lathyrus tefennicus* and *Lathyrus egirdiricus* seeds were found as 5306 ± 202 mg/kg and 5919 ± 210 mg/kg, respectively. Furthermore, the accuracy of method was confirmed by the analysis of certified reference material (EnviroMAT Contaminated Soil). According to results, it was decided that 2 different endemic *Lathyrus* taxa were also different for the chemical compositions based on t-test calculations at 95% confidence level. Additionally, the presence of elements at different

concentrations in the both endemic *Lathyrus* species could be used in the plant taxonomy. We thought that these results could provide a basis for studies on taxa.

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Table 1. The operating conditions of FAAS for metals

Metals	Wavelength (nm)	Slit width (nm)	Lamb current (mA)
Cd	228.8	0.5	8.0
Cr	357.9	0.5	12.0
Ni	232	0.2	15.0
Cu	324.8	0.5	5.0
Mn	279.5	0.2	12.0
Zn	213.9	0.5	10.0
Fe	248.3	0.2	15.0
Ca	422.7	0.5	6.0
Mg	285.2	0.5	4.0
Na	589	0.2	8.0
K	766.5	0.5	8.0

Table 2. Microwave digestion operating conditions for seeds

Steps	Time (min)
Heating process	15
Thawing process (200 °C)	15
Cooling process	15

Table 3. The linear equations for calibration graphs

Metals	Linear Equation	R²
Cd	$y = 0.078x + 0.004$	0.999
Cr	$y = 0.016x + 0.000$	1.000
Ni	$y = 0.021x + 0.001$	0.999
Cu	$y = 0.054x - 0.002$	0.999
Mn	$y = 0.065x + 0.001$	0.999
Zn	$y = 0.099x - 0.002$	0.999
Fe	$y = 0.017x - 0.006$	0.996
Ca	$y = 0.033x - 0.002$	0.997
Mg	$y = 0.047x + 0.009$	0.993
Na	$y = 11.80x + 6.482$	0.996
K	$y = 4.375x + 2.038$	0.998

Table 4. Concentration of metals in *Lathyrus tefennicus* and *Lathyrus egirdiricus*, $\bar{x} \pm \frac{ts}{\sqrt{N}}$

and other plants in the literature (N:11; N.D.*: Not detected)

Metals (mg/kg)	Found in <i>Lathyrus tefennicus</i>	Found in <i>Lathyrus egirdiricus</i>	Literature
Cd	N.D.*	N.D.	0.17±0.01mg/kg (in smaller coix seeds) (Zhang et al., 2017)
Cr	N.D.	N.D.	0.025±2.56 and 0.038±9.63 µg/g (in <i>Medicago sativa</i> L. seeds) (Liu et al., 2014) 0.18±0.01 mg/kg (in smaller coix seeds) and 0.02±0.00 mg/kg (in bigger coix seeds) (Zhang et al., 2017)
Ni	N.D.	N.D.	29.69±1.20 and 43.88±1.83 µg/g (in <i>Medicago sativa</i> L. seeds) (Liu et al., 2014) 0.79±0.03 mg/kg (in smaller coix seeds) and 0.16±0.01 mg/kg (in bigger coix seed) (Zhang et al., 2017)
Cu	4.7±0.1	3.7±0.1	3.8±0.3 and 5.0±0.8 µg/g (in maize seeds) (Heredia et al., 2016) 2.88±0.14 mg/kg (in smaller coix seeds) and 1.65±0.05 mg/kg (in bigger coix seed) (Zhang et al., 2017) 21.44±0.11 and 102.24±1.15 µg/g (in <i>Medicago sativa</i> L. seeds) (Liu et al., 2014)
Mn	9.7±0.4	11.0±0.3	18.2±1.3 and 52.8±18.5 µg/g (in maize seeds) (Heredia et al., 2016) 22.70±0.87 mg/kg (in smaller coix seeds) and 19.66±0.75 mg/kg (in bigger coix seed) (Zhang et al., 2017) 19.68±1.33 and 28.34±0.80 µg/g (in <i>Medicago sativa</i> L. seeds) (Liu et al., 2014)
Zn	25.0±0.3	42.0±1.3	5.5±0.7 and 17.4±7.9 µg/g (in maize seeds) (Heredia et al., 2016) 22.72±1.01 mg/kg in smaller coix seeds) and 23.88±1.02 mg/kg (in bigger coix seed) (Zhang et al., 2017) 27.44±0.72 and 57.01±0.67 µg/g (in <i>Medicago sativa</i> L. seeds) (Liu et al., 2014)
Fe	66.0±3.0	51.0±3.0	13.1±1.7 and 25.9±7.9 µg/g (in maize seeds) (Heredia et al., 2016) 34.86±0.87 mg/kg in smaller coix seeds) and 47.21±0.74 mg/kg (in bigger coix seed)

(Zhang et al., 2017)			
			125.94±0.36 and 528.81±2.13 µg/g (in <i>Medicago sativa</i> L. seeds) (Liu et al., 2014)
			14.0±0.9 and 84.9±10.7 µg/g (in maize seeds) (Heredia et al., 2016)
			92.54±3.61 mg/kg in smaller coix seeds) and 96.74±3.12 mg/kg (in bigger coix seed) (Zhang et al., 2017)
Ca	760.0±62.0	1343.0±98.0	2298.75±0.39 and 3611.25±0.68 µg/g (in <i>Medicago sativa</i> L. seeds) (Liu et al., 2014)
			5.61±2.62 µg/g (in maize seeds) (Heredia et al., 2016)
			1687.46±34.77 mg/kg in smaller coix seeds) and 1624.31±62.32 mg/kg (in bigger coix seed) (Zhang et al., 2017)
Mg	1374.0±38.0	1026.0±82.0	1886.25±0.58 and 2148.75±1.41 µg/g (in <i>Medicago sativa</i> L. seeds) (Liu et al., 2014)
			437±29 and 993±99 µg/g (in maize seeds) (Heredia et al., 2016)
			37.63±8.53 µg/g (in cotton seed), 25.44±7.66 µg/g (in sunflower seed), 23.70±7.60 µg/g (in tung seed), 36.40±3.97 µg/g (in soybean seed), 175±20 µg/g (in curcas bean seed), 86±5 µg/g (in fodder turnip seed) and 32.93±8.08 µg/g (in castor bean seed) (Chaves et al., 2010).
Na	6650.0±59.0	4967.0±251.0	
			6653.13±0.58 and 7358.75±0.12 µg/g (in <i>Medicago sativa</i> L. seeds) (Liu et al., 2014)
K	5306.0±202.0	5919.0±210.0	

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