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MINERAL COMPOSITION AND HEAVY METALS CONTENT OF SWEET CHESTNUT FRUITS IN FIVE NATURAL POPULATIONS FROM CENTRAL CROATIA

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Introduction

The sweet chestnut (*Castanea sativa* Mill.) is a noble hardwood providing multiple economic benefits (wood, fruit, honey, tannin, preservation of ecological and landscape values). It is distributed in the Mediterranean region, where chestnut forests spread over an area of 2,530,000 ha (Conedera *et al.* 2004). In Croatia, the sweet chestnut grows in the forests of the colline belt of the continental part, in Istria and on the islands of Krk and Cres. The largest part of the range of the sweet chestnut extends through central Croatia, from the Slovenian border to the border with Bosnia and Herzegovina (Medak *et al.* 2009).

Chestnut fruits are an important food in many European countries. In recent years, the consumers have been showing an increased interest in chestnut fruits because of their nutritional qualities and potential beneficial health effects (Barreira *et al.* 2012). Chestnuts are rich in carbohydrates and are a good source of essential fatty acids and minerals (Borges *et al.* 2008; Barreira *et al.* 2009; De Vasconcelos *et al.* 2010). Many studies about the nutritional composition of chestnut define them as fruit which, in contrast to the most other nuts, have high water content, contain small quantities of fat and oil and are cholesterol free (De La Montaña Míguez 2004; Borges *et al.* 2007, 2008; Peña-Méndez *et al.* 2008; Barreira *et al.* 2009, 2012). They also contain high quality protein and are gluten free. Except of that, they are good source of vitamin C, B1, B2, and folates, as well as appreciable levels of fibre (De Vasconcelos *et al.* 2010). Breisch (1995) indicated that the main macroelements are potassium, phosphorus, calcium and magnesium.

Material and Methods

Sweet chestnut fruits were collected during October 2007 in five natural populations in Croatia, as shown in Figure 1. Fruits were collected from five different trees per each

population. Directly after collection, the freshly picked fruits were peeled. The pericarp and seed coat were removed, after which only the edible part of the fruit remained, i.e. the kernel. The cleaned seeds were shredded using a shredding blender. From each tree, 100 g of shredded sample was placed in hermetically closed containers and stored in a freezer at -20 °C. Macro and micronutrients (K, Ca, Mg, Na, Fe and Zn) and toxic metals (Pb, Cd, Hg, As) were determined by atomic absorption spectrometry using a Varian SpectrAA 220 device.

For all of the studied variables, descriptive statistical parameters were calculated. Assumptions of normality were checked using the Shapiro-Wilk test, and the assumption of homogeneity of variance using Levene's test. Statistically significant differences between studied groups of trees were established using the analysis of variance (ANOVA). The differentiation among studied groups for variables (Hg, Cd) with a skewed and heteroscedastic distribution was verified using the Kruskal-Wallis ANOVA. The data used in the PC analysis were previously standardized using the z-score method. The specified statistical analyses were conducted using the STATISTICA 8.0 software.

Results and Discussion

The results of the descriptive statistical analysis are shown in Table 1. With regard to macronutrients, K (613.96 mg/100 g f.w.) was the most abundant, followed by Ca, 35.17 mg/100 g f.w.; Mg, 29.99 mg/100 g f.w.; and Na, 3.69 mg/100 g f.w. A similar pattern was observed by Bellini *et al.* (2007) and Poljak *et al.* (2016) for the Italian and Croatian chestnut cultivars. A smaller mass fraction of potassium and calcium was recorded in Spanish (Pereira-Lorenzo *et al.* 2006; Peña-Méndez *et al.* 2008) and Portuguese (Ferreira-Cardoso *et al.* 2005; Borges *et al.* 2008) chestnuts. Magnesium content on average is within the range reported by Pereira-Lorenzo *et al.* (2006) for Spanish chestnut cultivars. Smaller magnesium content on average is reported by Bellini *et al.* (2007) for the Italian chestnut cultivar 'Marrone del Mugello' and higher by Borges *et al.* (2008) and Ferreira-Cardoso *et al.* (2005) for Portuguese chestnut cultivars, and Peña-Méndez *et al.* (2008) for Spanish chestnut cultivars. The sodium content of the chestnuts from natural populations is within the range reported by Pereira-Lorenzo *et al.* (2006) and Peña-Méndez *et al.* (2008) for Spanish chestnut cultivars. Smaller sodium content on average is reported by Borges *et al.* (2008), Ferreira-Cardoso *et al.* (2005), and Bellini *et al.* (2007) for the Portuguese and Italian chestnut cultivars. The average mass fraction of zinc in the chestnut fruits from the natural populations is in line with the data published for sweet chestnut cultivars (Ferreira-Cardoso *et al.* 2005; Pereira-Lorenzo *et al.* 2006; Peña-Méndez *et al.* 2008; Bellini *et al.* 2007; Borges *et al.* 2008; Poljak *et al.* 2016). Iron content ranged between 1.29 mg/100 g f.w. in population Gvozd and 1.56 mg/100 g f.w. in population Ozalj, with 1.37 mg/100 g f.w. as a mean value. Smaller mass fractions of iron was reported by Peña-Méndez *et al.* (2008) and Pereira-Lorenzo *et al.* (2006) in Spanish chestnut cultivars, and greater by Borges *et al.* (2008) and Ferreira-Cardoso *et al.* (2005) in Portuguese chestnut cultivars. This study confirmed that chestnuts from natural populations are a good source of macro- and micronutrients. The content of detected toxic heavy metals was lower in all samples than the maximum allowed amounts for the sweet chestnut fruits, which indicates an unpolluted environment. The cadmium and mercury level in approximately 25 % of the samples was below the limit of detection (< 0.01 mg/kg). According to the results of the ANOVA (Table 1), the studied populations differed in the mass

fraction of potassium ($p < 0.01$) and magnesium ($p < 0.05$). The differentiation in the mass fraction of calcium, sodium, iron, zinc and toxic metals was not statistically significant. The PC analysis for the six studied macro and microelements established that the first two principal components account for 74.5 % of the total variability (Figures 2 and 3). The first principal component, which accounts for 54.0 % of variability, separates the populations with high potassium content, which is highly positively correlated with it, from the populations with high iron and zinc content, which is highly negatively correlated with the same principal component. The second principal component participates in the overall variance with 32.2 % and is in highly negative correlation with the content of magnesium. In other words, the bottom side of the diagram contains populations with a higher portion of the magnesium than those on the upper side. The differences found among the populations in mineral composition may reflect genetic factors and environmental conditions.

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Table 1. Descriptive statistical parameters, and level of significance.

Minerals and toxic metals	Population					P
	Ozalj	Moslavačka gora	Vojnić	Gvozd	Topusko	
K mg/100 g f.w.	602,58±36,67	579,18±20,54	632,07±23,74	629,95±10,05	626,02±21,01	< 0.01
Ca mg/100 g f.w.	37,04±5,68	39,21±3,74	32,42±5,15	34,67±4,73	32,49±2,63	ns
Mg mg/100 g f.w.	26,67±1,47	32,80±5,07	28,07±3,03	34,44±5,61	27,98±4,52	< 0.05
Na mg/100 g f.w.	4,70±2,80	3,92±1,60	3,96±1,99	3,90±2,37	1,98±0,35	ns
Fe mg/100 g f.w.	1,56±0,30	1,35±0,16	1,33±0,23	1,29±0,24	1,30±0,20	ns
Zn mg/100 g f.w.	0,87±0,24	0,69±0,12	0,67±0,21	0,59±0,08	0,69±0,19	ns
Pb mg/kg f.w.	0,048±0,016	0,044±0,032	0,053±0,029	0,039±0,016	0,037±0,009	ns
Cd mg/kg f.w.	0,018±0,006	0,014±0,004	0,019±0,003	0,017±0,008	0,022±0,008	ns
Hg mg/kg f.w.	0,013±0,005	0,034±0,018	0,040±0,008	0,010±0,000	0,016±0,005	ns
As mg/kg f.w.	0,034±0,005	0,034±0,009	0,032±0,015	0,018±0,008	0,032±0,004	ns

f.w. – fresh weight

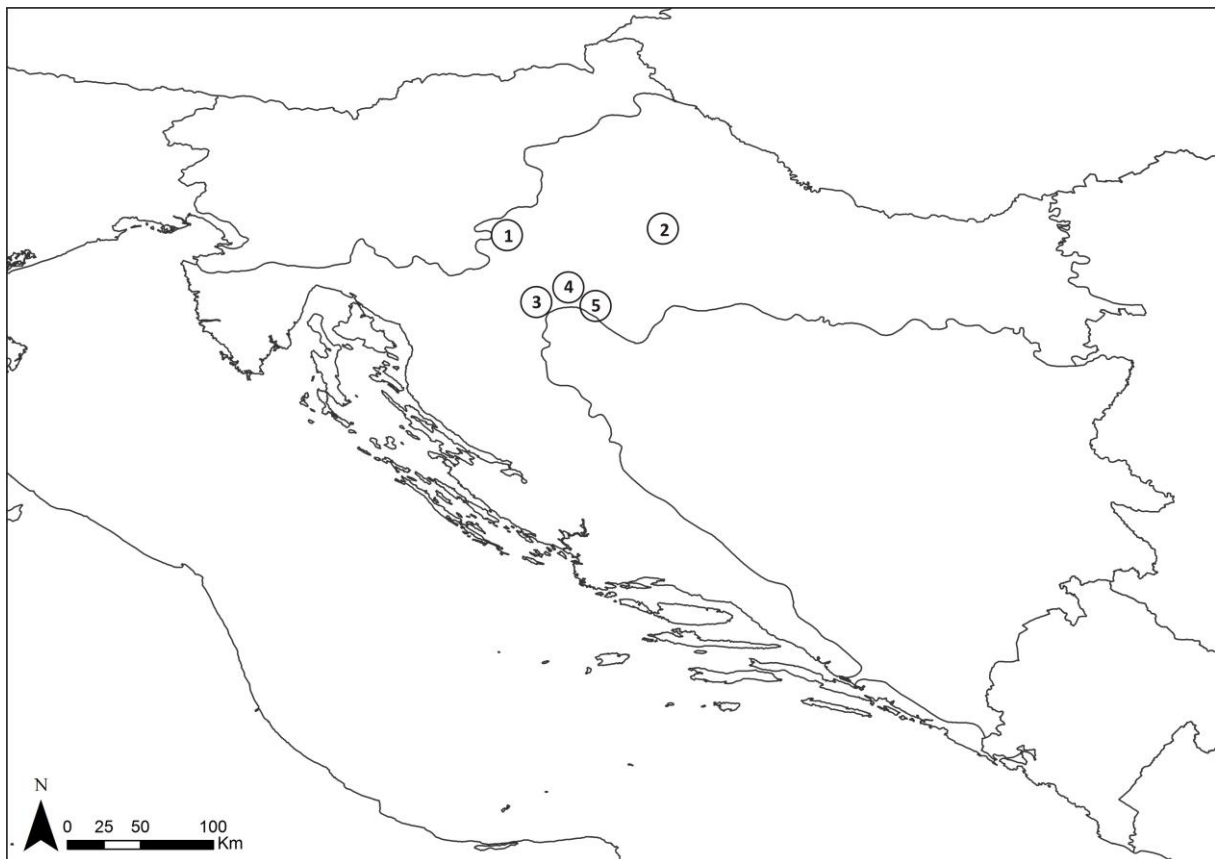


Figure 1. Sampled populations: (1) Ozalj; (2) Moslavačka gora; (3) Vojnić; (4) Gvozd; (5) Topusko.

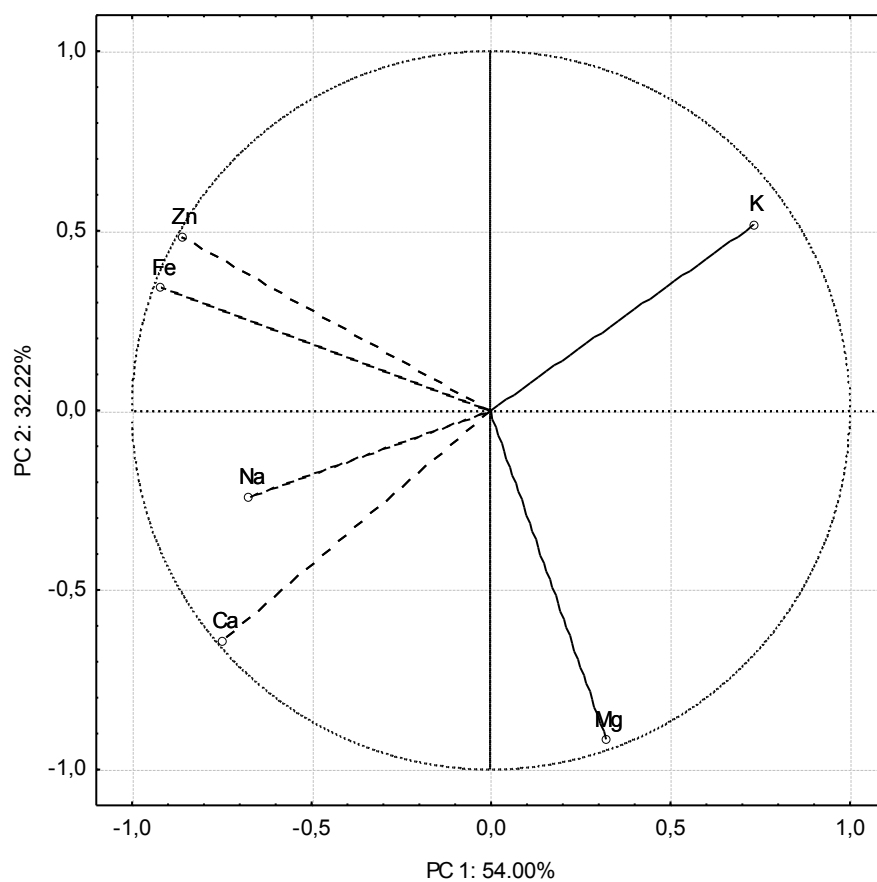


Figure 2. Score plot of the variables projected on the space of PC1 vs. PC2.

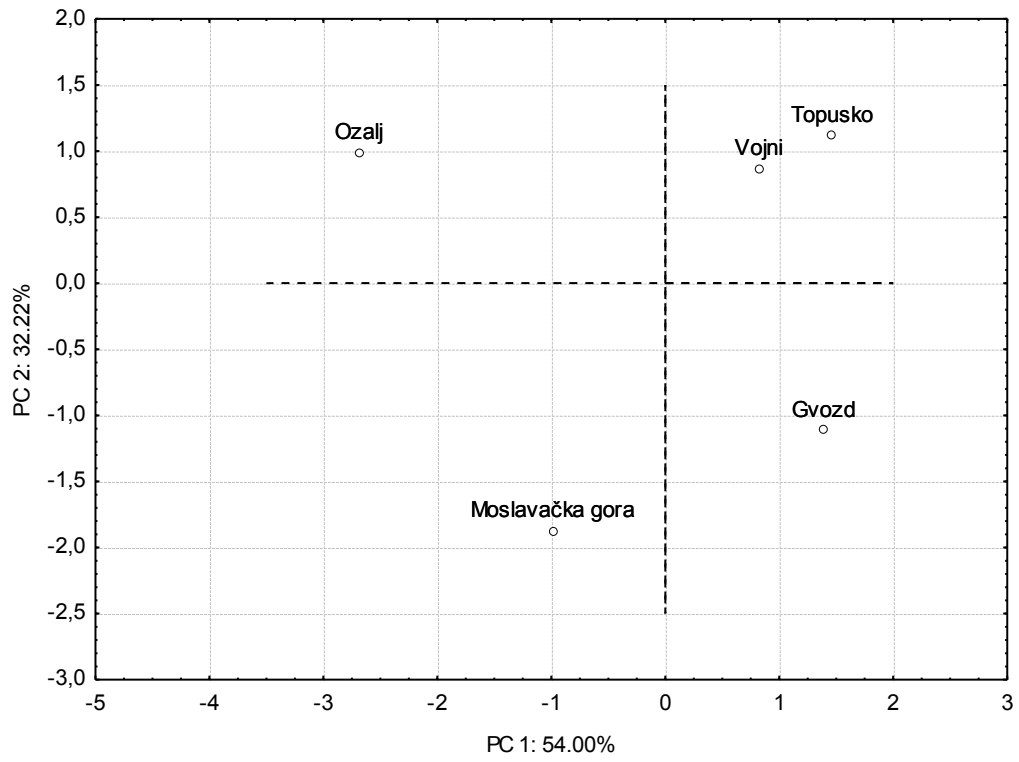


Figure 3. Score plot of the chestnut populations projected on the space of PC1 vs. PC2.