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SOIL AND WHEAT GRAIN SELENIUM CONTENT IN THE VICINITY OF KOPRIVNICA (CROATIA)

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This paper describes the findings of selenium measurement in three types of soil (semigley, pseudogley and hypogley) and in wheat grain cultivated in these types of soil in the vicinity of Koprivnica using hydrid atomic absorption spectrometry. The results showed relatively low selenium levels in all three types of soil (145-333 µg Se/kg dry weight) as well as the wheat grain (22-62 µg Se/kg dry weight). The analysis of covariance suggested that the concentration of selenium in soil, pH in KCl, and K_2O affect the concentration of selenium in wheat grain. After the analysis was repeated using only selenium in soil, pH in KCl, and K_2O as covariates, the selenium content in wheat grain was found to vary significantly with the type of soil. Although this study was performed in a small area in relation to the area of the country, it may be a good starting point for further investigation of selenium in Croatia.

KEY WORDS: *hypogley, pedology, plants, pseudogley, selenium, semigley, soil, wheat grain*

Selenium is present in soil in different forms which affect its bioavailability to plants. It is generally acknowledged that selenate (SeO_4^{2-}) is biologically more available than selenite (SeO_3^{2-}). In soils which tend toward alkaline selenates are more frequent, and vice versa, selenite is commoner in acid soils (1). Unlike selenate, selenite forms very stable insoluble compounds with iron [iron selenite $Fe_2(SeO_3)_3$ and basic iron selenite $Fe_2(OH)_4SeO_3$] (1). Sulphur competes with selenium and its presence in soil significantly decreases the selenium biovailability of selenium to plants. Apart from the soil composition, rainfall and temperature may contribute to the presence of selenium in soil. In cold and humid areas with acid soil the selenium content in vegetation will be significantly lower than in warm and drier area with more alkaline soil (2). As many factors affect selenium biovailability from soil to plant, parallel plant analysis is highly recommended (3).

Most soils contain 100-2000 µg Se/kg. Toxic seleniferous soils (1-2000 mg Se/kg) are found in the northern areas of the USA, in some areas of Canada, in the south of Africa, in Columbia, Venezuela and in China (the Hubel Province). Low selenium levels are found in New Zealand, some areas of Australia, then in Ireland, Scotland, Scandinavia, the Baltic countries, Poland, Slovakia, the Balkans, some areas of Greece and southwest and northeast regions of China (1, 4-8). The data on selenium content in Croatia are very scarce. Samples of typical pseudogleyic luvisols from Požeška kotlina (a basin in the east of the country) revealed low selenium levels from 20 to 48 µg/kg (9). In Podravina, the soil along the river Drava contains more selenium (50-280 µg/kg) (10). In the vicinity of a coal burning plant Plomin in Istria, the total selenium in soil was high, ranging from 600 to 22970 µg/kg. This reflected on the soil of the Risnjak National Park which had 530-1230 µg Se/kg (11).

The selenium content in plants is also influenced by a number of factors (12). The most important is the plant species. For example, *Astragalus rascemosus* accumulates high levels of selenium (14990 µg/kg). Generally, plants may be classified in three categories: primary selenium accumulators which often contain over 1000 mg Se/kg dry weight, secondary selenium accumulators which rarely contain more than a few hundred mg Se/kg dry weight and non-accumulators which include many weeds and most crop plants, grains and grasses which rarely contain more than 30 mg Se/kg dry weight (13).

The selenium content in wheat varies a lot. In 1936, *Robinson* (14) reported selenium concentrations of 100-1900 µg/kg in wheat samples gathered from all over the world. The highest levels of selenium in wheat were found in the USA (range 20-3120 µg Se/kg dry weight) (15). The lowest levels were found in some European countries (Finland, Sweden, Denmark, Germany, Great Britain) with means ranging from 8 to 30 µg Se/kg dry weight) (16, 17). Until now, Croatia has lacked any data on selenium content in wheat. The aim of this work was to determine the selenium content both in soil samples and in wheat grain collected in the vicinity of Koprivnica, a town in the north of the country (Podravina), and to analyse their relationship.

MATERIAL AND METHODS

The wheat (Sana) was cultivated in three types of soil (semigley, pseudogley, and hypogley) in three localities near Koprivnica with intensive agricultural production. All three regions are far from urban, industrial and transport sources of environmental contamination. Each field (24-40 ha) was divided into 15 tables. From each table 15 soil samples (0-25 cm below the surface) were taken, totalling 225 samples. All 15 samples from one table were mixed together to achieve 15 average samples (1500-2000 g) per table (totalling 45 samples for all three types of soil). The samples were air-dried and ground using agate mortar. They were sieved twice to pass through 1.2 and 0.8 mm holes. The residue was ground again in mortar and sieved. The powdered soil samples were stored in polyethylene bags until analysis. Meanwhile, pedologic characteristics (pH, nitrogen, potassium, phosphorous and humus content) were determined in native soil samples.

The wheat grain samples were obtained from the same tables as the soil samples. The wheat grain sampling followed the same procedure as the soil sampling; 15 wheat samples were collected from each table (totalling 225 samples) and mixed to create 15 average wheat samples per soil (45 samples for all three types of soil). The grain was removed from the husks manually, stored in polyethylene bags, and frozen at -18 °C until analysis. Before the analysis, the samples were dried at 70°C for 60 min and milled into powder. The sample moisture was controlled by desiccation at 130°C until they reached constant weight (18).

Soil samples were decomposed with hydrochloric and nitric acid using the Digestion System 6 (Tecator, Sweden) (19). About 1.5 g of each dried and powdered sample and 14.0 ml of an acid mixture of 10.5 ml HCl and 3.5 ml HNO_3 was added into a wet-digestion Teflon tube. The tubes were stored at room temperature overnight, and the following day the mixture was heated for five hours (at 50 °C for 30 min, at 80 °C for 90 min and at 120 °C for 180 min) in the digester. After cooling, the solution was quantitatively transferred with deionised water to a 20 ml flask.

Wheat grain samples were decomposed with nitric acid and hydrogen peroxide in the MDS-2000 Microwave Sample Preparation System-CEM (19). About 0.5 g of each dried and powdered sample was added into a Teflon tube, mixed with 5 ml of concentrated nitric acid, and heated in the microwave oven in five stages (the power in all five stages was 90%, pressure 20, 40, 80, 135, 135 psi, respectively, running time 15, 10, 10, 10, 20 min, respectively). After the fourth stage, 1 ml hydrogen peroxide (30%) was added. After cooling, the solution was guantitatively transferred with deionised water to a 10 ml flask.

Selenium was analysed using the Mercury/ Hydride System (MHS) connected with Perkin-Elmer 3110 atomic absorption spectrometer equipped with electrodeless discharge lamp for selenium, power supply and a strip chart recorder.

Selenate was reduced to selenite with 5 M hydrochloric acid at 60 °C for 30 min (20) and selenite transformed into hydrogen selenide in MHS using 3% sodium tetrahydro-borate in 1% sodium hydroxide solution. Argon was used as the purge gas, and the tubes were heated with the acetylene mixture to 900 °C. The 196.0 nm line with a spectral line bandwidth of 2 nm was used for all measurements. Calibration was performed using the method of standard additions. All steps of the analysis were carried out in triplicate.

The method was validated using reference soil materials and whey powder (wheat reference material was not available) for wheat grain. The soil reference materials included ISE sediment sample 976 from the Netherlands (3320±460 μg Se/kg) (Sample A) and two US samples GXR-2 (570±171 μg Se/kg) (Sample B) and GXR-6 (1010±170 μg Se/kg) (Sample C). The mean results obtained by ten analyses for each sample are as follows: 3061±270 μg Se/kg for Sample A, 505±63 μg Se/kg for Sample B, and 922±59 μg Se/kg for Sample C.

For wheat grain we used IAEA-155 Whey Powder (mean: 64; range: 51-77 μg Se/kg) from the International Atomic Energy Agency (Austria) (Sample D). The mean result obtained by ten analyses of the sample was 55±3 μg Se/kg. The relative standard deviation of the method for soil selenium was 9.09%, and it was determined by ten analyses of a sample containing 220 μg Se/kg. The relative standard deviation for wheat grain was 5.25%, and it was determined by ten analyses of a sample containing 50 μg Se/kg.

The pedologic analysis of soil pH in H_2O, pH in KCl, N_2(%), K_2O (mg/100g), P_2O_5 (mg/100g) and humus (%) was performed in the laboratory of the Development of Technology and Control department within the food processing company Podravka, Croatia. Soil pH in H_2O and KCl was measured using the potentiometric method with glass electrode (21). The nitrogen was analysed using the Kjeldahl's method (22), potassium using the flame atomic absorption spectrometry (23), phosphorus using the spectrophotometric method (24), and humus using the Tjurin' s method (21).

The results in soil and wheat grain were expressed in μg Se/kg dry weight. As the Kolmogorov-Smirnov goodness of fit test (25) indicated that selenium in wheat grain was not normally distributed, we used the logarithmic scale for this variable. Correlations between selenium in soil, selenium in wheat grain, and other pedologic characteristics were considered significant at the level $P<0.10$. The analysis of covariance was used to test the difference in wheat grain selenium between grains taken from three types of soil with different selenium concentrations as well as to test all pedologic variables (26). The homogeneity of variance was tested using the Leven test (27, 28). Pairwise differences in selenium concentration in wheat grain between the soil types were analysed using the Tukey's HSD test (29). All analyses were performed on a PC computer using Statistica® version 5.0.

RESULTS

Table 1 shows selenium concentrations in soil and wheat grain as well as the pedologic characteristics of the three types of sampled soil. Judging from the range of selenium in all three types of soil (145-333 μg Se/kg) and from the range of wheat grain selenium (22-62 μg Se/kg),

Table 1 *Selenium concentrations in soil and in wheat grain with basic pedology of three types of soil (Mean±SD)*

Parameter	Type of soil		
	Semigley	Pseudogley	Hypogley
Selenium in soil (μg/kg)	219±44.11	257±51.53	210±34.57
Selenium in wheat grain (μg/kg)	30±4.54	43±8.09	33±10.38
pH in H_2O	7.82±0.11	6.65±0.65	6.49±1.08
pH in KCl	7.07±0.16	5.38±0.68	5.67±0.69
N_2 (%)	0.14±0.01	0.30±0.11	0.17±0.02
K_2O (mg/100g)	11.19±1.64	10.41±1.03	13.95±1.49
P_2O_5 (mg/100g)	28.20±2.40	15.92±1.75	14.42±13.59
Humus (%)	1.31±0.15	4.41±2.01	4.42±0.47

the selenium content in this area of the country is relatively low. Pseudogley contained more selenium than the two other types of soil. The same was found in wheat grain grown in pseudogley. This type of soil had the highest content of nitrogen, but the lowest content of potassium and the lowest pH in KCl. Other pedologic measurements (pH in H_2O, phosphorous and humus content) were similar to those of hypogley.

Selenium in wheat grain showed a strong positive correlation with selenium in all three types of soil (Figures 1-3). In relation to the pedologic measurements, selenium in wheat grain showed significant positive correlation with pH in KCl for all three types of soil (Figures 4-6), positive correlation with K_2O for pseudogley and hypogley, significant negative correlation with K_2O for semigley (Figure 7), non-significant positive correlation with K_2O for pseudogley

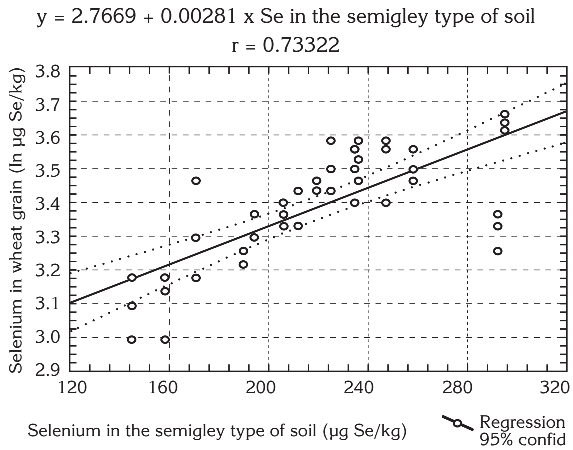

Figure 1 *The relationship between Se in semigley (µg Se/kg) and Se in wheat grain (ln µg Se/kg) (N=45) P<0.001*

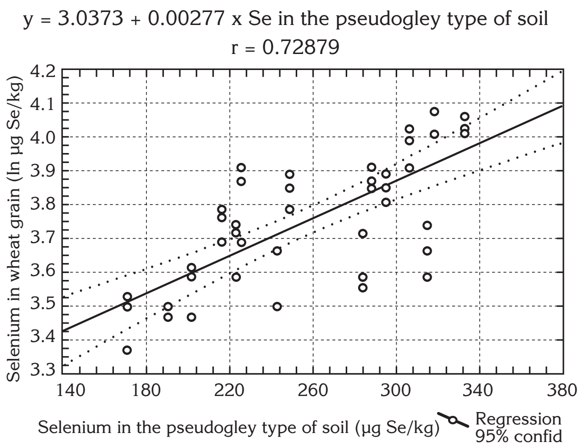

Figure 2 *The relationship between Se in pseudogley (µg Se/kg) and Se in wheat grain (ln µg Se/kg) (N=45) P<0.001*

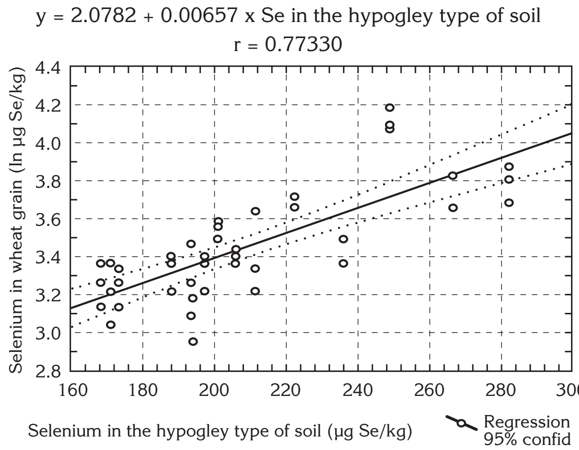

Figure 3 *The relationship between Se in hypogley (µg Se/kg) and Se in wheat grain (ln µg Se/kg) (N=45) P<0.001*

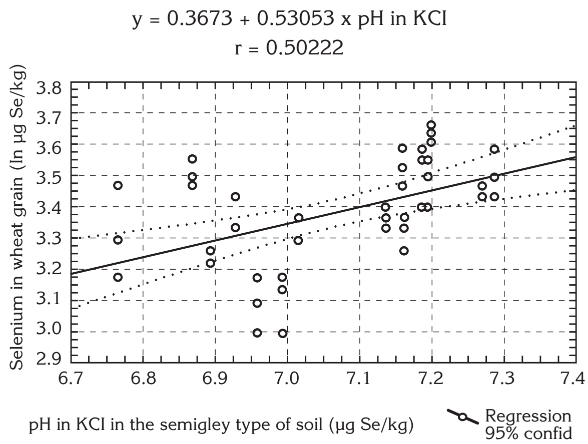

Figure 4 *The relationship between pH in KCl in semigley and Se in wheat grain (ln µg Se/kg) (N=45) P<0.001*

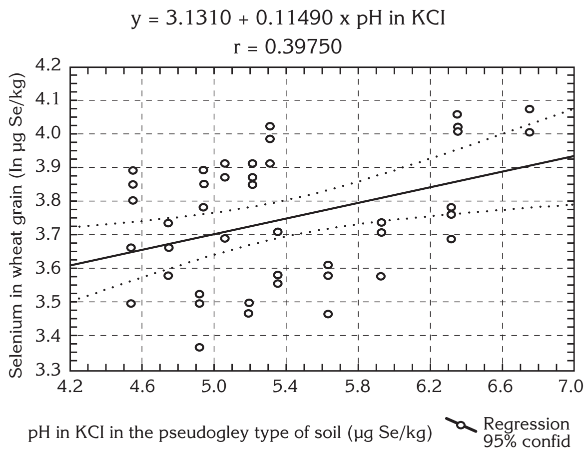

Figure 5 *The relationship between pH in KCl in pseudogley and Se in wheat grain (ln µg Se/kg) (N=45) P<0.01*

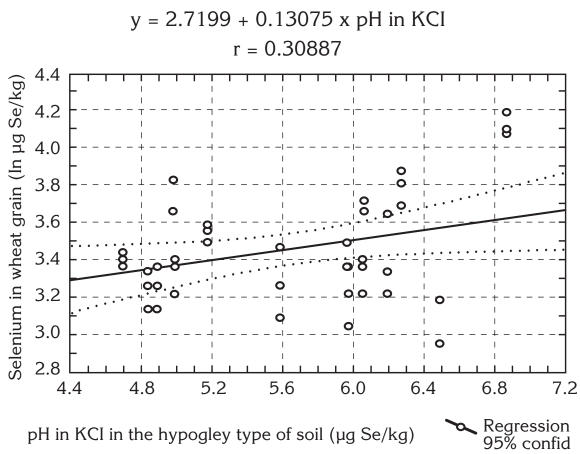

Figure 6 T*he relationship between pH in KCl in hypogley and Se in wheat grain (ln µg Se/kg) (N=45) P<0.05*

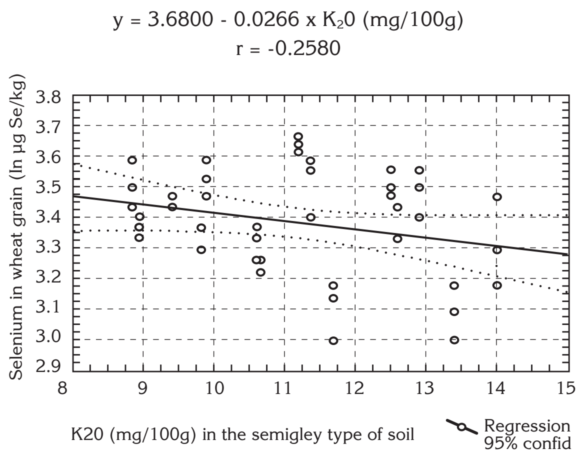

Figure 7 *The relationship between K_2O (mg/100g) in semigley and Se in wheat grain (ln µg Se/kg) (N=45) P<0.05*

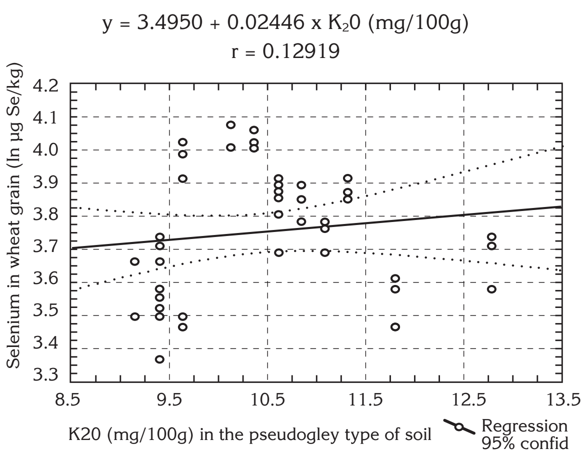

Figure 8 *The relationship between K_2O (mg/100g) in pseudogley and Se in wheat grain (ln µg Se/kg) (N=45) N.S.*

(Figure 8), and significant positive correlation with K_2O in hypogley (Figure 9).

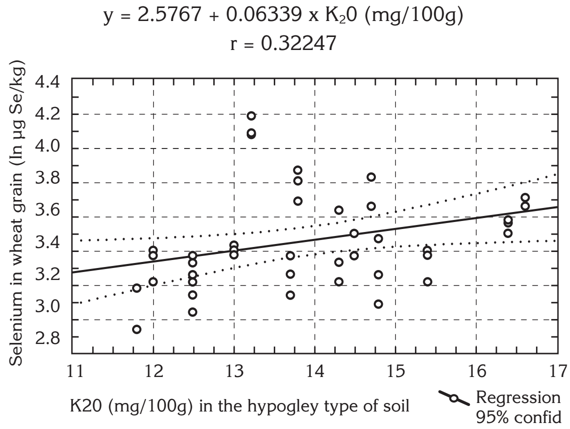

Figure 9 *The relationship between K_2O (mg/100g) in hypogley and Se in wheat grain (ln µg Se/kg) (N=45) P<0.05*

Variables pH in H_2O and pH in KCl strongly correlated (r=0.849; P<0.001). Only pH in KCl was included in the model to avoid multicollinearity of predictors.

The Leven test of homogeneity of variance revealed heterogeneity of variances for selenium in wheat grain in various soil types. After logarithmic transformation both non-normality and heterogeneity of variances disappeared.

The results of the analysis of covariance for selenium concentration in wheat grain (ln µg Se/kg) showed significant differences among the soil types even after accounting for all available soil characteristics (Table 2).

Table 2 *Analysis of covariance for selenium concentration in wheat grain (ln µg Se/kg)) with soil type as the main effect and all available soil characteristics as covariates*

Effect	Degrees of freedom	F	P
Soil type	2; 34	5.92	0.0062
Soil characteristics	8; 34	8.09	<0.0001

Table 3 shows the results of the within-soil-type regression of selenium concentration in wheat grain with all pedologic measurements as covariates. Since the sample is relatively small (N=45) the criterion of significance was P<0.10, and selenium concentration in soil, pH in KCl and K_2O were considered as significant predictors of selenium concentration in wheat grain.

Only the significant predictors were retained as covariates in the final model of analysis of

Table 3 *Analysis of covariance. Within soil type regression of selenium concentration in wheat grain (ln μg Se/kg) by all available soil characteristics*

Variable	Regression coefficient	Standard errors	P
Selenium in soil (μg Se/kg)	0.0034	0.0007	<0.0001
pH in H_2O	-0.0937	0.0556	0.1012
pH in KCl	0.1904	0.0683	0.0087
N_2 (%)	-0.1331	0.3957	0.7385
K_2O (mg/100g)	0.0306	0.0153	0.0545
P_2O_5 (mg/100g)	0.0010	0.0033	0.7541
Humus (%)	0.0273	0.0203	0.1873

covariance (see Table 4). Soil types differed significantly in regard to selenium concentration in corresponding wheat grain even after accounting for these covariates.

Table 4 *The final analysis of the covariance model for selenium concentration in wheat grain (ln μg Se/kg) with soil type as the main effect and the selected soil characteristics as covariates*

Effect	Degrees of freedom	F	P
Soil type	2; 39	9.26	0.0005
Soil characteristics	3; 39	19.27	< 0.0001

Table 5 shows the results of the within-soil-type regression of selenium concentration in wheat grain with selected pedologic measurements as covariates. Associations between the selenium concentration in wheat grain and all covariates were positive and statistically significant.

The parallelism test showed that the relationship between selenium content in wheat grain and selenium in soil, pH in KCl and K_2O did not significantly vary between the three types of soil. The slopes of the regression lines for each predictor did not differ significantly between the soil types, although the amount of selenium in wheat grain was different in each type of soil.

DISCUSSION

The selenium content in soil and in wheat grain in the vicinity of Koprivnica is relatively low. Its levels in soil are comparable to those found in the vicinity of the river Drava (10), and are higher than those found in Požeška kotlina (9). Although the results in this study refer to a small area, they are not far from the selenium findings in the soil of the low-selenium Keshan disease area in China (mean: 112 μg Se/kg; range: 59-190 μg Se/kg) (30). Low selenium status has also been reported in the neighbouring Hungary (31) and Yugoslavia (32) with geochemical and climate conditions similar to those of the studied area.

As there were no data for the selenium content in wheat grain in Croatia before this study, the most similar results were found in Great Britain (33) and Germany (16) where its levels averaged 30 μg Se/kg. In Hungary the wheat grain level varied from 5 to 235 μg Se/kg (34) and in Yugoslavia from 4 to 66 μg Se/kg (35). The lowest average level of selenium in wheat grain of 5 μg Se/kg was found in the Keshan disease area (Heilongjiang province in the northeast corner of China) (36).

It is also important to stress that selenium from wheat grain is highly available to the organism. *Levander and co-workers* found that selenium-rich wheat flour (200 μg per day for 11 weeks)

Table 5 *Analysis of covariance. Within soil type regression of selenium concentration in wheat grain (ln μg Se/kg) by selected soil characteristics*

Variable	Regression coefficient	Standard errors	P
Selenium in soil (μg Se/kg)	0.0034	0.0005	<0.0001
pH in KCl	0.0710	0.0392	0.0781
K_2O (mg/100g)	0.0267	0.0153	0.0887

steadily increased plasma selenium level and doubled the selenium-dependent enzyme glutathione peroxidase activity of platelets in middle-aged men with low selenium status (37).

The selenium content in wheat grain showed a good positive correlation with the selenium content in all three types of soil. The comparison between the transformed values of selenium in wheat grain (ln µg Se/kg) and its concentration in all three types of soil shows a significant correlation ($r=0.743$; $P<0.001$). The same is true for separate correlations for each type of soil (Figures 1-3). Additional statistical analysis revealed that pH in KCl ($P<0.0087$) and the K_2O content ($P<0.0545$) in soil are also important variables which significantly affect selenium levels in wheat grain. When only these three variables were included in the repeated analysis of covariance, the results showed that the type of soil was an important variable affecting the selenium content in wheat grain.

Biovailability of soil selenium to plants depends on many factors. This study was restricted to the selenium concentration in soil and wheat grain and the most important pedologic factors. We measured total selenium without specifying its chemical form. Having in mind that selenate is biologically more available than selenite, it is possible that their unequal distribution in the three types of soil we sampled could account for the differences in selenium wheat grain content according to the type of soil. In nature, the oxidation of selenium oxide (SeO) to selenite and selenate in soil is largely biotic and occurs at a relatively slow rate (38). The microbial volatilisation of selenium as dimethylselenide and dimethyldiselenide from soil into the atmosphere could also affect the distribution of selenium forms (39). Our study did not include soil measurements of sulphur which competes with selenium and could be yet another confounding factor relevant to the selenium differences in soil.

The low selenium content in soil and wheat grain in this part of Croatia calls for further investigation. Ideally, it would be best to make a monoelemental geochemical map of the country which would include the findings of selenium in wheat and other cereals cultivated in specific areas. This map could provide the scientific basis for the supplementation of the agricultural soil with selenium. The final effect would be an increase in selenium intake through food in humans and animals.

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Sažetak

KONCENTRACIJE SELENIJA U TLU I PŠENICI U OKOLICI KOPRIVNICE

U tri tipa tla (semiglej, pseudoglej i hipoglej) i u zrnu pšenice koja je uzgojena na tim tipovima tla u okolici Koprivnice ispitana je koncentracija selenija. Za analizu je primijenjena metoda hidridne tehnike atomske apsorpcijske spektrometrije. U tlu su određene i osnovne pedološke karakteristike (pH, sadržaj dušika, kalija, fosfora i humusa). U sva tri tipa tla i u zrnu pšenice uzgojene na tim tipovima tla rezultati su pokazali relativno niske razine selenija (raspon za tlo:145-333 µg Se/kg suhe tvari; raspon za zrno pšenice: 22-62 µg/kg suhe tvari). Tlo tipa pseudoglej i zrno pšenice uzgojene na tom tipu tla sadržavali su više selenija nego drugi tipovi tla. Između selenija u zrnu pšenice i selenija u tlu te pH u KCl za sva tri tipa tla utvrđena je dobra pozitivna korelacija, s K_2O pozitivna korelacija za tip tla pseudoglej i hipoglej, a negativna za tip tla semiglej. Druge korelacije nisu bile značajne. Analizom kovarijance utvrđeno je da koncentracija selenija u zemlji, pH u KCl i K_2O određuju koncentraciju selenija u zrnu pšenice. Nakon ponovljene analize kovarijance, kada su u modelu kao kovarijati zadržani samo selenij u zemlji, pH u KCl i K_2O sadržaj selenija se značajno mijenjao u odnosu na tip tla. To upućuje na to da druge karakteristike (možda kemijski oblik selenija i/ili prisustnost sumpora) mogu utjecati na koncentraciju selenija u zrnu pšenice. Iako je ovo ispitivanje obavljeno na maloj površini u odnosu na veličinu zemlje, ono može biti dobra znanstvena osnova za daljnja ispitivanja stanja selenija u Hrvatskoj.

KLJUČNE RIJEČI: *biljke, hipoglej, pedološke karakteristike tla, pseudoglej, selenij, semiglej, tlo, zrno pšenice*

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