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DETERMINATION OF ELEMENTS BY ATOMIC ABSORPTION SPECTROMETRY AND ENERGY DISPERSIVE X-RAY SPECTROSCOPY IN HUMIC ACIDS AND SOIL SAMPLES

OZNACZANIE PIERWIASTKÓW ZA POMOCĄ ATOMOWEJ SPEKTROMETRII ABSORPCYJNEJ I SPEKTROMETRII RENTGENOWSKIEJ ENERGII ROZPROSZONEJ W PRÓBKACH KWASÓW HUMINOWYCH ORAZ GLEBY

Abstract: Determination of various elements in humic acids and soil samples is important with regard to their biological availability. This work was focused on the identification of trace elements presented in soil samples and humic acids preparates. The object of our study was the samples of grassland soil classified according to FAO as Eutric Cambisol (locality Vatín, Czech-Moravian Upland). Labile and total several element contents in the studied samples were determined. The labile forms were measured in the 0.01 M $CaCl_2$ extract by using high resolution continuum source atomic absorption spectrometer. Total element contents were determined after extraction in *aqua regia*. Basic soil properties were measured by commonly used methods - soil reaction and conductivity by potentiometric method, texture by pipette method, CEC according to Kappen method and C_{ox} by titrimetric oxidation. Soil *humic acids* (HA) were isolated according to standard IHSS method. Elemental composition of HA was determined by CHSN/O analyzer. Energy dispersive X-ray spectroscopy allowed us to detect elements *in situ* in HA molecule and following elements were found: Fe, Cu, Zn, Ti, Ca, K, S, P, Si and Br. The presence of mentioned elements in humic acids preparates was compared with the determined amounts of labile forms and total contents in the soil samples.

Keywords: humic acids, labile forms, atomic absorption spectrometry, energy dispersive X-ray spectroscopy

Quality and content of soil organic matter, together with processes of mineralization and microbial activity are the main factors affecting the health/quality of agricultural soils. Many authors dealt with evaluation of selected soil parameters influencing mobile and potential mobile heavy metals content showed that humic substances quality strongly affected metals mobility [1-3]. Most of metals in plants originate in uptake by roots from soils. Metals of anthropogenic origin are considered as more available from soils than those from parent rock. The way of uptake influences metal distribution in plant. Some metals are accumulated in roots, which is again the case especially for Pb, while others are well mobile in plants [4]. Humic acids (HA), the main component of humic substances are considered to be very active in interaction with metals and their sorption properties are given by their structure and chemical properties [5-7]. HA consist mainly of hydroxyphenols, hydroxybenzoic acids, and others aromatic structures with linked peptides, amino compounds, and fatty acids as pointed by Hayes and Malcolm [8]. Frequently is given their active interaction with copper, zinc, lead and cadmium. The highest stability constant for copper(II) binding was determined. Stability constant values were decreasing in order: $Cu^{2+} > Ba^{2+} > Pb^{2+} > Cd^{2+} > Ca^{2+}$ [9].

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Our work represents a part of research carried out in Bohemian-Moravian Upland and dealing with total and water-soluble levels of some micronutrients in soils of permanent grassland. The aim of this study was to identify total and labile trace elements presented in the soil, isolate humic acids and give their chemical characterization. We try to characterize portion of selected elements strongly bound into humic acids molecule and show distinct differences for the various metals.

Materials and methods

The monitoring of total and labile elements content in grassland was carried out at locality Vatín (Mendel University Enterprise) - Bohemian-Moravian Upland (altitude 530 m a. s. l., yearly average of air temperature 6.9°C, yearly sum of precipitation 620 mm). Soil type was classified according to FAO as Eutric Cambisol. Grassland was sown by clover-grass mixture (*Sanguisorba-Festucetum comutatae* association). Part of the re-established area was left for spontaneous grassing. Two modes of management were used - mulching once a year and mulching 3 times a year. The experimental variant was established in four replicates, the size of individual plots was 12 m x 10 m. Soil was sampled in 20 cm upper layer twice a year (in spring and autumn).

Soil analysis

Basic soil properties were determined by commonly used methods. Soil reaction was determined by the potentiometric method in distilled water and in 1 M KCl solution. Particle size analysis was determined by the pipette method. The soil was extracted using the Kappen method for *cation exchange capacity* (CEC) determination. Total organic carbon content (C_{ox}) was determined by wet digestion according to Nelson and Sommers [10]. Fractional composition of humic substances was determined according to Kononova and Beltchikova [11]. *Humic substances* (HS) quality was assessed by humic acids (HA)/*fulvic acids* (FA) ratio, humification degree and by *colour indexes* (Q4/6). The labile forms of elements were measured in the 0.01 M $CaCl_2$ extract by high resolution continuum source atomic absorption spectrometry (ContrAA 700, Analytik Jena). Total element contents were determined after extraction in *aqua regia* after microwave extraction (Milestone Ethos 1 microwave system) by *inductively coupled plasma mass spectrometry* (Agilent 7500 ce ICP-MS spectrometer).

Humic acids isolation

The isolation of soil HA was made according to the standard international method (IHSS). Hundred grams of air-dried soil sample was sieved at the mesh size of 1 mm, washed with 10% HCl and stirred for 1÷2 h (decalcination process). After the negative reaction for CO_2 (detected visually) was achieved, the soil rest was washed with 0.05 M HCl. After the negative reaction for Ca^{2+} (detected by ammonium oxalate) was obtained, the soil rest was washed with distilled water. After the negative reaction for Cl^- (detected by $AgNO_3$), was obtained, the soil rest was shaken in 0.1 M NaOH for 7÷8 h. We allowed it to precipitate overnight and centrifuge for 15 min at 5000 rpm. The elution with 0.1 M NaOH and centrifugation was carried out twice and the supernatants were combined. The dark-brown solution of HS was precipitated with concentrated HCl added to pH = 1.

The coagulated HA were decanted, washed several times, extensively purified with 0.5% mixture of HCl + HF, dialysed against distilled water until chloride-free, and finally freeze-dried [8]. Humic acids elemental analysis was made by the standard method of Carlo Erba and elementary CHNS/O analyser - Thermo Finnigan was used. Results of elemental analysis were compared with IHSS Elliot HA standard 1S102H, (IHSS, 2010). Energy dispersive X-ray spectrofluorimeter XEPOS allowed us to detect elements *in situ* in HA molecule.

Results and discussion

Eutric Cambisol under permanent grassland had acid soil reaction, soil was loamy sands textured, with medium CEC, and low C_{ox} content. Quality of HS given by colour index (Q4/6) was less than 1. Humification degree was low (about 20%). Average amount of HS, HA and FA was 7.6, 3.6 and 4 mg/kg, respectively. Isolated HA contain in their molecule 32.73% C, 46.48% H, 2.52% N, and 18.27% O (in atomic %). ^{13}C NMR study showed that isolated HA contain more aliphatic and less aromatic part in their molecule [12].
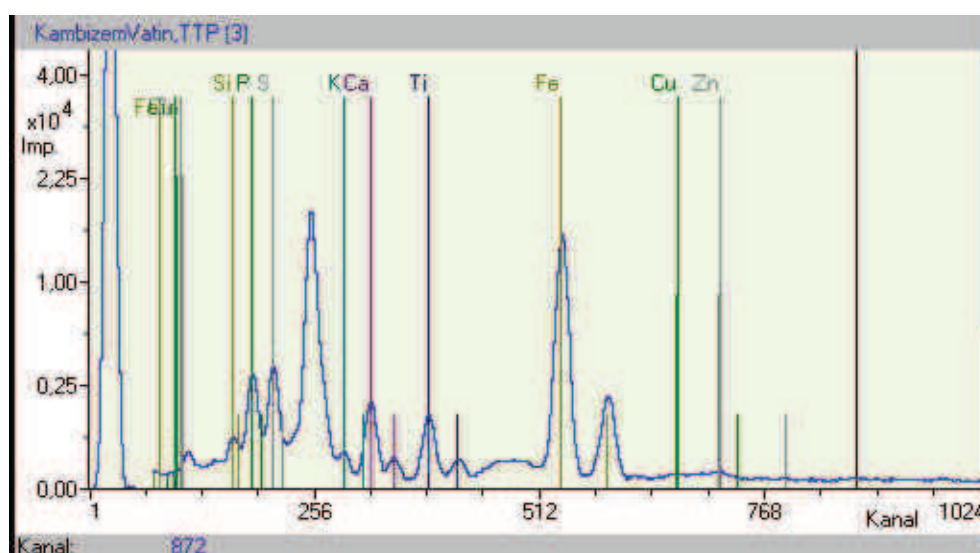

Fig. 1. EDX-ray spectra of humic acids isolateds from Eutric Cambisol (Vatín)

The amount of the most of elements was decreasing in time. The significant decrement was observed in the case of Ca, Cd and Mn (54.0, 40.9 and 34.8%, respectively). Results of total content of selected elements determined in *aqua regia* showed the highest content of Fe in both soil and HA samples. Higher content of K, Fe, V, Cu, Cr and Mo was determined in HA sample to compare with soil. Measured values are typical for this soil type [13]. Limits given for hazardous elements in soils were not exceeded [4, 14, 15]. The correlation between labile form and total element contents was not significant (p < 0.05). The most significant dependence was observed in the case of Co (r = 0.921). Within labile

forms measured in the 0.01 M $CaCl_2$ extract the highest content of Fe and K was determined in both HA and soils samples. HA contain hundred times more Fe, twice more Cu and Co to compare with soil sample. High adsorption of Cu and Co by HA form is also confirmed by literature data [9]. Higher values to compare with background content were found. Also Pb and Zn are usually highly adsorbed by HA, but our measurements not confirmed it. We can conclude that this area is not polluted by atmospheric deposition of heavy metals, because all of determined hazardous elements were in the range of background content. Results of EDX-ray spectroscopy confirmed the presence of Fe, Cu, Zn, Ti, Ca, K, S, P, Si and Br in HA molecule (Fig. 1).

Conclusions

The most important conclusion was that all of determined hazardous elements were in the range of background content. Both mineral soil samples and isolated HA contained the highest amount of *iron* and *potassium*. HA samples contained twice higher amount of *copper* and *cobalt* to compare with soil samples. Statistically significant dependence for *cobalt* (r = 0.921) was found.

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