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INFLUENCE OF HUMUS ACIDS ON MOBILITY AND BIOLOGICAL AVAILABILITY OF IRON, ZINC AND COPPER

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Soil organic matter is known as an important condition for the mobility of trace elements in soils, their geochemical migration and availability to plants. However, various components of soil organic matter have different effect on these processes due to their significant differences in structure and properties. **Aim**. To establish the role of humic and fulvic acids in the process of formation of microelement mobility in soils and their accumulation in plants. **Methods.** A model experiment with sand culture was used to investigate the release of trace elements from preparations of humic and fulvic acids and their uptake by oat plants. **Results**. It was found that among biologically needed elements humic acids are enriched with iron, fulvic acids – with zinc, and copper distribution between these two groups of substances may be characterized as even. These elements have unequal binding power with components of soil organic matter, as evidenced by their release into the cultivation medium and accumulation in plants. In the composition of fulvic acids zink has the most mobility – up to 95 % of this element is in the form, accessible for plants; the lowest mobility was demonstrated by copper in the composition with humic acids, for which no significant changes in the concentration of mobile forms in the substrate and in the introduction to the test culture were registered. Despite significantly higher iron content in humic acids, the application of fulvic acids in the cultivation medium provides a greater increase in the concentration of mobile forms of this element. **Conclusions.** The results confirm the important role of organic substances of fulvic nature in the formation of zinc and iron mobility in the soil and their accumulation in plants.

Key words: soil, trace elements, humic acids, fulvic acids, mobility, accessibility.

INTRODUCTION

The most severe shortage of trace elements in the mineral nutrition of cultivated crops is primarily related to the properties of soils, affecting the accessibility of these trace elements to plants, rather than to their low concentration in soil. The increase in the concentration of protons and in the soil humidity as well as the decrease in the oxidation-reduction potential (ORP) are referred to as the factors, enhancing the mobility of most trace elements. On the contrary, the factors, inhibiting the mobility of trace elements, include carbonate content of soils, increase in ORP and the increase in the concentration of OH^- and PO_4^-. The organic matter of soils has ambiguous effect on the performance of the cations of trace elements – the increase in its content enhances the availability of Zn and Mn and inhibits the mobility of Cu [1]. However, it is known that this property of soil may act both as a regulator of the mobility of trace elements and their source for plants and soil biota. In particular, many trace elements are remarkable for the accumulation of different types of soils in the organic matter, for instance, for Mo, Ni, Cu, V, Co, Zn, Pb the enrichment factor is in the range from 10 to 1,000 [2]. Still some components of soil organic matter have a different capacity of binding trace elements – their majority is concentrated in fulvic acids (FA). This is a regular phenomenon, taking into consideration high dispersibility, hydrophilicity and higher reaction capacity of FA which are more saturated with functional groups compared to humic acids (HA). Thus, compared to HA, FA have a higher capacity of interacting with trace elements.

Notable ways of interacting with trace elements are ion exchange, chelation and adsorption. One kilo of humic acid is known to adsorb up to 200 g of metals and fulvic acids have even higher base exchange capacity. The carboxyl and phenolic groups are the most active in the processes of fixing metals, but due to the ion exchange heavy metals may leave the composition of these compounds rather easily. A separate kind of organic and mineral derivatives is complex salts. In this case the cations of trace elements displace hydrogen ions and penetrate the inner sphere. Here the chelate structures may be formed due to the interaction of metal with two groups of $COOH^-$ or with $COOH^-$ and OH^- groups. This is notable for Zn, Cu and Pb that easily form covalent bonds due to high electronegativity which was proven by infrared spectroscopy [4].

One of the main mechanisms, conditioning the accessibility of trace elements for living organisms, is the formation of chelates, and in this process FA are the main chelating agent. Contrary to single salts, the chelate compounds of trace elements maintain their increased mobility in a wide range of pH values, while the concentration of ionic forms of trace elements decreased by tens of times. This phenomenon is of utmost importance to soil-forming processes and to the input of nutrients to plant roots. The trace elements in the composition of FA or more low molecular organic compounds of soils are more susceptible to microbiological destruction and, as a result, more available for plant roots than the ones, accumulated in HA [5].

The role of HA in the fixation of trace elements is revealed not only in the background soils. In case of contamination these substances serve as a natural barrier to fix the excessive amount of heavy metals. The prevailing significance of fulvic compounds is noted in this process as well – the share of Zn, Pb and Cd in the composition of fulvic acids of alluvial soils with technogenic pollution in Czech Republic is 95.7, 82.0 and 98.4 % respectively. The significance of this ecologic factor is also evidenced in the close interrelation of the total content of metals and their concentration in fulvic acids. The correlation coefficient for Zn, Pb and Cd is 0.928, 0.855 and 0.873, respectively, $p = 0.001$ [6].

However, the complex compounds in the biosphere are not mere regulators of the input of chemical elements to plants – FA are the main complexone of natural surface waters and soil solutions. Therefore, the organometallic complexes are the main form of migration of trace elements and heavy metals in soils which is an integral part of geochemical circulation of these elements [7]. For instance, the fulvates of manganese, aluminum, cobalt, copper and other elements are usually soluble. In the anaerobic acid medium these compounds of metals are so mobile that they are capable of penetrating ground waters and permanent streams in large amounts which is primarily notable for rivers of the boreal belt. It is known that 50–70 % of organic matter content in the Dnieper's water is composed of the compounds of Fe, Mn, Ni, Cu with humus acids. The compounds of fulvic and oxy-acids and cations of metals migrate in the geochemical stream and get accumulated in depressions. The soils of swamps, flood plains and river deltas are usually enriched with these components. On the contrary, humic acids precipitate iron, manganese, copper, zinc, nickel and cobalt. This results in the formation of insoluble precipitates of humates which are both salt compounds and the products of mutual coagulation and adsorption [2]. The interaction of heavy metals and HA depends on the availability of alkaline-earth metals. For instance, in presence of Ca and Mn ions the interaction of Cu with the organic matter decreases 5-fold. However, the mechanism of interaction between the cations of heavy metals and humates and fulvates does not have any considerable differences from their binding to HA and FA, where metal displaces hydrogen from the phenolic group [8]:

$$Cu^{2+} + H\text{-}FA\text{-}COOCa^+ \leftrightarrow Cu\text{-}FA^+\text{-}COOCa^+\ H^+.$$

MATERIALS AND METHODS

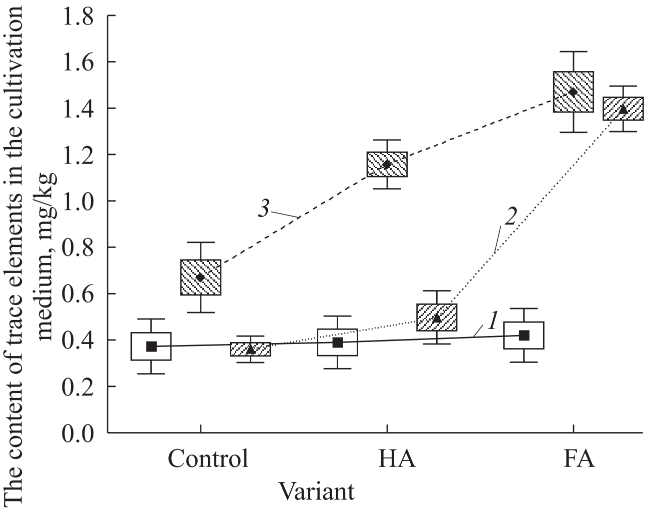
We have determined reliable increase in the mobility of zinc, manganese and iron along with the increase in the share of fulvic acids in the organic matter [9, 10]. However, in natural conditions the availability of trace elements in soils is affected by a considerable number of factors, the main among them being the granulometric composition of soils, humus content, pH of the medium, the presence of $CaCO_3$ (especially in the active form) and a number of other abovementioned soil characteristics. In order to bring out the effect of merely humus acids we have conducted a model experiment with sandy culture and Mitscherlich's nutrient mixture (without Fe addition), the composition of which and the conditions of preparing sand for the experiment are presented in fine detail in [11]. The experiment was conducted in three variants with four repeats – with the introduction of preparations of humic and fulvic acids and control, respectively. The preparations were obtained by six isolations of the organic matter from the patch of soil using 0.1 m NaOH after its decalcination with 0.05 n H_2SO_4. The fractions of HA and FA were separated by the acidification of the obtained and refiltered solution to pH of 1.5–2 with 10 % H_2SO_4. After the corresponding purification and drying-up according to [12] the preparations of HA and FA were analyzed for the content of trace elements (Table 1).

The data presented testify that out of biologically required elements the humic acids are enriched with iron and the fulvic acids – with zinc. The distribution of manganese and copper may be characterized as even. The preparations were introduced in the amount of 1.2 g/vessel (0.4 %) which corresponds to the natural content of humic and fulvic acids in soils with sandy texture. The oat plants (*Avena sativa L.*) were cultivated until the tillering stage. The changes in the concentration of trace elements in the substrate and their accumulation in plants were determined. We studied the changes in the content of trace elements, the concentration of which in the composition of humus acids was found to be the highest, namely – Fe, Zn, and Cu, for more accurate definition of their balance using atomic absorption analysis. The content of mobile forms of trace elements was determined using 1 N of ammonium-acetate buffer solution (pH 4.8), the soil:extractant ratio was 1:5. The concentration of trace elements in the preparations of humus acids and herbage of a test-crop was determined after annealing at 550 °C and the dissolution of the ash obtained in 10 % solution of HCl.

The statistical processing of the data obtained was conducted using Dospehov and Statistica 10 programs.

RESULTS AND DISCUSSION

Humic and fulvic acids have ambiguous effect on the mobility of trace elements in substrates. It was determined that the content of iron in the cultivation medium after the cultivation of the test-crop in the control variant was 0.67 mg/kg. The introduction of preparations of humus acids to the cultivation medium increases this index up to 1.15 mg/kg for humic acids and up to 1.47 mg/kg for fulvic acids at the value of LSD_{05} which corresponds to 0.25 mg/kg (Figure). However, the data of the analysis of HA and FA preparations testify that 1 kg of the mixture was introduced 25.94 and 5.42 mg of iron respectively which evidences to high accessibility of this element in the composition of fulvic acids, regardless of a considerably smaller total content. The form, accessible for plants, was noted for 14.8 % Fe from the composition of exogenous FA, and only 1.9 % – from the composition of HA preparation.



The impact of humus acids on the content of trace elements in the cultivation medium: *1* – copper; *2* – zinc; *3* – iron

The efficiency of Fe in the complex with FA was also determined [14]. Fulvate iron does not concede the EDTA–Fe and citrate–Fe complexes in the stability constants; its introduction to the nutrient solution in conditions of the sandy crop resulted in more active input of this metal into the plants of sunflower and beans [13].

The effect of zinc in the composition of humus acids was proven to be somewhat different. Contrary to Fe, zinc was mostly present in fulvic acids – 273.5 mg/kg of the preparation against 57.6 mg/kg in humic acids which turned out to be the additional factor of the release of this trace element from the preparations. The highest content of mobile zinc compounds was observed in the variant with FA introduction – 1.40 mg/kg of the substrate with the control value of 0.36 mg/kg. The reliability of the difference was proven by the results of the dispersion analysis − LSD_{05}

Table 1. The content of trace elements in the preparations of humic and fulvic acids from typical chernozem

Preparation	Content of trace elements, mg/kg of preparation								
	Fe	Mn	Cu	Zn	Co	Ni	Cr	Cd	Pb
HA	6485.8	33.8	257.4	57.6	13.4	15.37	22.2	0.72	37.0
FA	1354.9	33.1	255.0	273.5	3.7	4.99	20.8	1.12	26.5
LSD_{05}	411.7	5.07	9.12	22.21	4.62	4.36	5.17	0.33	15.59

corresponds to 0.19 mg/kg. The surplus of accessible Zn is 1.04 mg/kg with the introduction of 1.09 mg Zn in the composition of fulvic acid preparation, thus the accessibility of this trace element in the composition of the preparation of fulvic acids is 95 %. After the introduction of HA preparation the content of mobile forms of zinc was considerably smaller – 0.50 mg/kg, so the surplus is 0.14 mg/kg of the substrate which may be considered as a tendency only. If the introduction of Zn per 1 kg of the medium is 0.23 mg/kg in this case, the accessibility of zinc in the composition of humic acids is rather high – almost 61 %. The data obtained testify to high mobility of zinc, connected to humus acids. The result demonstrates that the presence of FA in soils is a considerable factor of the mobility of this trace element. The introduction of 0.4 % of the preparation of fulvic acids transfers soil to another category of provision regarding the content of mobile Zn – over 1 mg/kg according to commonly accepted gradations [1].

Quite different results were obtained for copper. Regardless of the high amount of the latter in both preparations of humus acids there were no registered reliable changes in mobile forms of this element. The content of mobile copper is 0.37 mg/kg in the control variant and 0.39 and 0.42 mg/kg – in variants with the introduction of the preparations of humic and fulvic acids respectively at the value of the lowest significant difference of 0.24 mg/kg of soil.

The data obtained brings one to the conclusion that the behavior of trace elements, located in humic acids, depends both on the nature of humus substances and on the properties of metals proper. Iron is primarily located in humic substances, but it is much more accessible in the composition of fulvic acids. The highest mobilization indices are attributed to zinc, which is notable for its accumulation in the fulvate humus. Copper is evenly distributed between the investigated components of the organic matter, but this metal has closest relations with humus acids, which is testified to by the absence of reliable changes in the concentration of its mobile forms.

Table 2. The accumulation of trace elements in the herbage of oats

Variant	Content of trace elements in plants, mg/kg of dry weight		
	Fe	Zn	Cu
Control	93.1	41.0	11.7
HA 0.4 %	109.6	40.6	10.5
FA 0.4 %	115.5	62.3	21.0
LSD_{05}	9.79	17.05	7.06

Still the most objective index of mobility and biological accessibility of nutrition elements is their accumulation in plants. The data on the effect of humus acids on the mobility of Fe, Zn, and Cu in soil are partially confirmed by their accumulation in test-crops (Table 2). The content of iron in test-crops in the control variant is 93.1 mg/kg of dry mass. The introduction of the preparations of humus acids to the cultivation medium increases the accumulation of Fe in plants up to 109.6 mg/kg for humic and up to 115.5 mg/kg – for fulvic substances. The enhanced uptake of iron by plants in these two variants compared to the control is statistically proven – LSD_{05} is 9.79 mg/kg.

The data on the accumulation of zinc in plants provide substantial evidence to the effect of humus substances on the provision of accessible forms of trace elements for soils. The content of Zn in oat plants in the control variant is 41.0 mg/kg, calculated per dry weight. The introduction of the preparation of humic acids to the cultivation medium does not induce stable changes in the indices of translocation of this trace element – 40.6 mg/kg. However, the application of FA preparation increases zinc accumulation by oat plants more than 1.5-fold – up to 62.3 mg/kg which proves the relevance of the availability of fulvic humus in the processes of forming the mobility of this trace element in soils.

The use of preparations of HA and FA has a different effect on the input of copper into plants. The introduction of humic acids to the cultivation medium does not induce stable changes in Cu accumulation in the herbage of oats – 10.5 mg/kg with the control value of 11.7 and LSD_{05} which equals 7.06 mg/kg. However, the introduction of 0.4 % of fulvic acids ensures almost twice enhanced introduction of copper into plants compared to the control which is crucially different from their effect on the copper release in the cultivation medium. This effect may be explained by higher susceptibility of plants to the consumption of trace elements i.e. bio-testing using plants is more sensitive to the changes in the mobility of Cu compared to the use of extract of ammonium-acetate buffer solution with pH 4.8.

The data obtained testify that the presence of even small amounts of humus substances, of fulvic origin in particular, in the cultivation medium results in the enhanced accumulation of Fe, Zn, and Cu in plants.

CONCLUSIONS

The results obtained allow the conclusion that humic acids are a relevant factor of the mobility of trace elements in soils and their accumulation by plants, but it depends both on the nature of humic substances and the properties of metals. The predominant role of fulvic acids in the processes of the formation of mobility of trace elements, the introduction of the preparation of which leads to the increase in the level of provisions for soils in terms of mobile forms of iron and, especially, zinc, has been proven.

Вплив гумусових кислот на рухливість та біологічну доступність заліза, цинку і міді

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Відомо, що органічна речовина ґрунтів є важливим чинником рухливості мікроелементів у ґрунтах, їхньої геохімічної міграції та доступності рослинам. Проте різні компоненти органічної речовини ґрунтів неоднаково впливають на зазначені процеси внаслідок значних відмінностей у побудові та властивостях. **Мета.** Встановлення ролі гумінових та фульвокислот у процесах формування рухливості мікроелементів у ґрунтах та їхнє накопичення у рослинах. **Методи.** За умов модельного досліду з піщаною культурою вивчали вивільнення мікроелементів з препаратів гумінових та фульвокислот та їхнє поглинання рослинами вівса. **Результати.** Встановлено, що з-поміж біологічно необхідних елементів гумінові кислоти збагачені залізом, а фульвокислоти – цинком, розподіл міді можна охарактеризувати як рівномірний. Перелічені елементи мають неоднакову силу зв'язку з компонентами органічної речовини ґрунтів, про що свідчить їхнє вивільнення в середовищі росту та накопичення в рослинах. Найбільша рухливість притаманна цинку у складі фульвокислот – до 95 % цього елемента знаходиться у доступній рослинам формі; найменша – міді у складі гумінових кислот, для якої не зафіксовано достовірних змін у концентрації рухливих форм у субстраті та в надходженні до тест-культури. Незважаючи на значно вищий вміст заліза в гумінових кислотах, додавання препарату фульвокислот у середовище росту забезпечує більший приріст концентрації рухливих форм даного елемента. **Висновки.** Отримані результати свідчать про переважну роль фульватних органічних сполук у формуванні рухливості цинку і заліза в ґрунтах та їхньому накопиченні в рослинах.

Ключові слова: ґрунт, мікроелементи, гумінові кислоти, фульвокислоти, рухливість, доступність.

Влияние гумусовых кислот на подвижность и биологическую доступность железа, цинка и меди

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Известно, что органическое вещество почвы является важным условием подвижности микроэлементов в почвах, их геохимической миграции и доступности растениям. Однако разные компоненты органического вещества почвы неодинаково влияют на указанные процессы, что обусловлено значительными отличиями в строении и свойствах. **Цель.** Установление роли гуминовых и фульвокислот в процессах формирования подвижности микроэлементов в почвах и их накопление в растениях. **Методы.** В условиях модельного эксперимента с песчаной культурой изучали высвобождение микроэлементов из препаратов гуминовых и фульвокислот и их поглощение растениями овса. **Результаты.** Установлено, что среди биологически необходимых элементов гуминовые кислоты обогащены железом, фульвокислоты – цинком, а распределение меди между указанными группами веществ можно охарактеризовать как равномерное. Данные элементы имеют неодинаковую силу связи з компонентами органического вещества почвы, о чем свидетельствует их высвобождение в среде роста и накопление в растениях. Наибольшая подвижность присуща цинку в составе фульвокислот – до 95 % данного элемента находится в доступной растениям форме; наименьшая – меди в составе гуминовых кислот, для которой не зафиксировано достоверных изменений в концентрации подвижных форм в субстрате и в поступлении в тест-культуру. Несмотря на значительно более высокое содержание железа в гуминовых кислотах, внесение препарата фульвокислот в среду роста обеспечивает больший прирост концентрации подвижных форм данного элемента. **Выводы.** Полученные результаты подтверждают важную роль органических веществ фульватной природы в формировании подвижности цинка и железа в почвах и в их накоплении в растениях.

Ключевые слова: почва, микроэлементы, гуминовые кислоты, фульвокислоты, подвижность, доступность.

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