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INTERACTIONS OF HUMIC ACIDS WITH METALS

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1. INTRODUCTION

Humic acids (HA) belong to the most valuable components of the soil environment, determining soil fertility. They reduce bulk density of soil, give optimal aggregate construction and improve air infiltration. They are also a valuable source of nutrients for plants and energy for soil microorganisms. HA have a high sorption capacity, which makes them capable of holding macro- and microelements in the surface layer of soil. Thus, important elements – plant nutrients, may be gradually sorbed and are not leached quickly from the soil.

In connection with numerous functions of HA in the soil environment, those compounds have been the object of studies for a long time. However, the state of knowledge about their functioning in the soil environment is still insufficient. Research difficulties are mainly due to the fact that HA are very complex substances. The particles are in the form of macromolecules without fixed and well-known molecular weight, with variable composition of carbon, oxygen, hydrogen, nitrogen and other elements. They are characterised by varying amounts of aliphatic and aromatic structures, as well as by a large variety of functional groups. For this reason, humic substances may exhibit significant variation in physicochemical properties, which may be explained by different mechanisms of interactions with substances contained in soil. In some cases, these mechanisms may have a negative impact on the environment.

The topic of interactions of HA with metal ions, despite numerous studies, is still poorly understood, and among researchers there are many divergent opinions about those processes. This problem is even more complicated because metals have different chemical nature as well as HA also differ in terms of origin, kind of soil or climatic conditions in which they were formed. As a result, it should be stressed that described topic is still actual, because it covers a large part of issues connected with environment protection and modern agriculture. Evidence for this statement could be, for example, the large-scale problem of accumulation of heavy metals and remediation of soils contaminated with these elements. Moreover, HA in soil environment undergo continual and dynamic changes, both humification and mineralisation. Such factors as heavy metals or pH changes may affect the equilibrium between the processes mentioned above and, in consequence, contribute to disadvantageous phenomena against nature.

Studies on interactions between HA and metals should be continuously extended and results obtained by the application of the newest instrumental techniques should supplement the current scientific knowledge. Understanding of interaction mechanisms, depending both on HA properties and chemical character of metal, may have a positive influence on the development of integrated, pro-ecological soil cultivation, based on modern technology as well as on minimisation of the use of toxic chemicals.

2. SOIL ORGANIC SUBSTANCES

2.1. Definition and classification

Soil organic matter is understood as the universe of chemical compounds containing carbon of organic origin. It also consists of still flora and fauna in various stages of decomposition. The edafon (living organisms) is generally not included in soil organic matter, despite the fact that the content of plant roots and microorganisms in the surface layers of soil is often 10-15% of the total weight of soil organic matter (Prusinkiewicz 1999). As a result of complete decomposition of organic residues, and through re-synthesis processes running with the participation of soil microorganisms, humus substances are formed. They are characterised by amorphous form, dark colour and colloidal nature. The complex mechanisms that occur during the formation of humus substances are called humification.

The complex nature and lack of a clear boundary between individual, formed chemical compounds make that definition and division of soil organic matter, as well as its various factions, including humus substances, not clear and various studies and publications provide slight differences in the names and classification of the fractions. The literature presents a common division of humus substances into non-specific and specific substances (Zbytniewski *et al.* 2003). The former group includes compounds of commonly well-known structure such as carbohydrates, fats, amino acids, lignins and tannins. Specific substances, which include humic substances, are the macromolecular compounds with complex, poorly-known structure. The most common criterion for their distribution is solubility in different liquids (Dobrzański and Zawadzki 1995, Kononowa 1968). Generally, three main fractions of specific substances are listed: fulvic acids, humic and humins (Hoffmann *et al.* 2010). The abovementioned classification of soil organic substances may be presented in the manner depicted at Figure 1.

Among the various fractions of soil organic matter, humic substances are still the least defined, and their division is conventional and based primarily on the methodology of isolation of individual compounds. An example could be one of the first classifications of humic substances proposed by Kononowa (Kononowa 1968) which divides them into the following fractions:

- Humic and ulmic acids: soluble in alkali,
- Apokrenic and krenic acids (fulvic acids): soluble in water, acids and alkali,
- Hymatomelanic acids: soluble in alcohol,
- Humins and ulmins: insoluble.

Oden's classification (Trojanowski 1973) takes into account other solvents:

- Fulvic acids: soluble in water, alkali, acids and alcohol,

- Humic acids: soluble in aqueous alkaline solutions, sodium oxalate and fluoride,
- Hymatomelanic acids: insoluble in acids, soluble in alkali and alcohol,
- Bitumins: insoluble in water solutions.

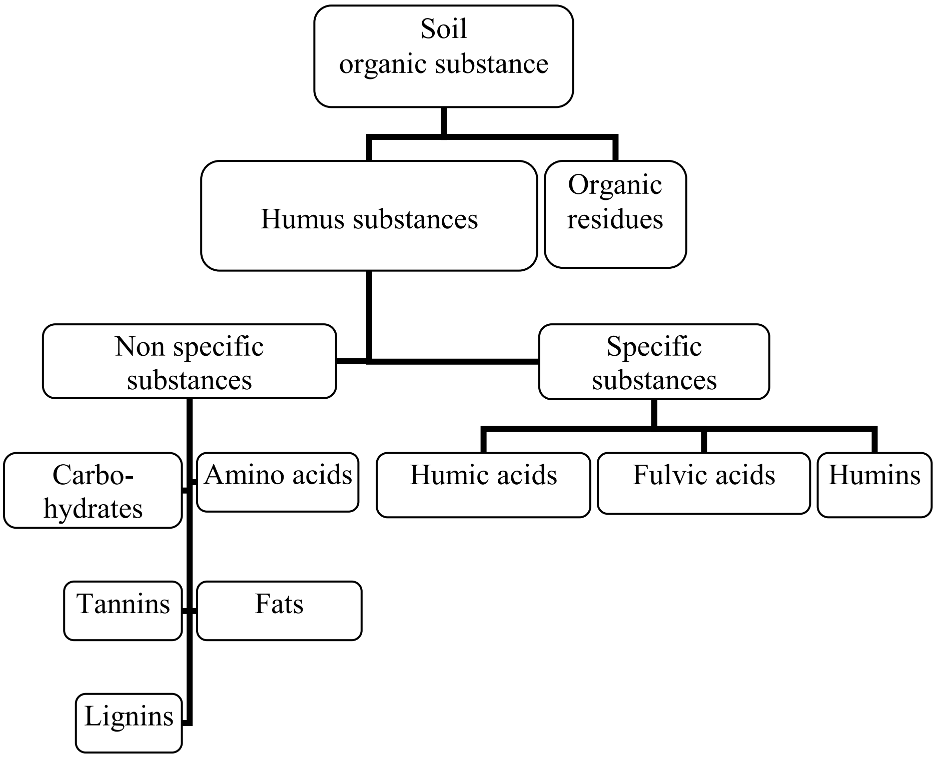


Fig. 1. Scheme of division of soil organic substance

Another less common criterion for the distribution of soil organic substance is also the dynamics of transformations of organic compounds in soil. According to McLauchlan and Hobbie (2004), soil organic substance is divided into stable and labile. The first group includes lignin and other substances that are highly degraded by soil microorganisms. The labile fraction includes:

- Microbial biomass (1-5% of organic matter)
- So-called light fraction (LF)
- Dissolved organic matter (DOM)

The light fraction includes remnants of fauna and flora being in various stages of decomposition. It may contain about 8% of total organic carbon, and 5% of total nitrogen (Gregorich *et al.* 2003). The water-soluble fraction is highly mobile and unstable as well as the most decomposed (Bolan *et al.* 2004). The above division is shown in Figure 2.

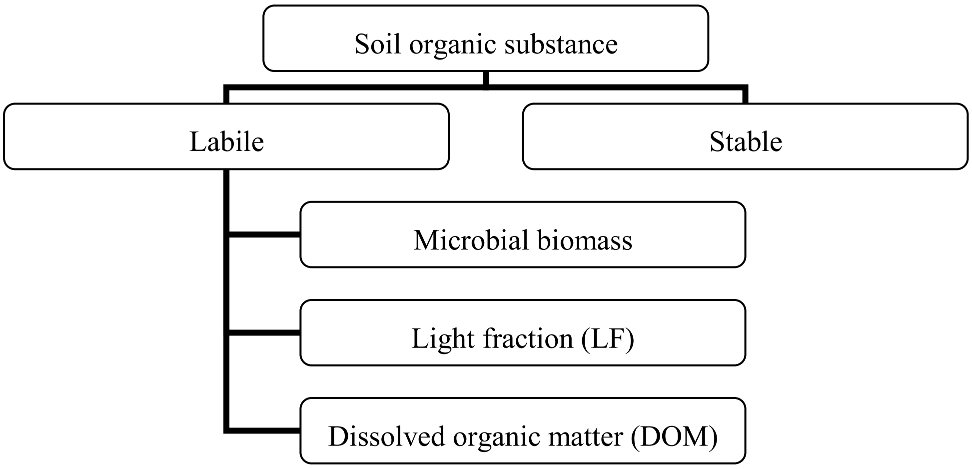


Fig. 2. Classification of soil organic substance according to McLauchlan and Hobbie (2004)

2.2. Genesis and transformations of soil organic substances

Soil organic substance is a product subject to continuous, dynamic changes. It is a result of two competitive processes: mineralisation and humification. The ratio of mineralization to humification equals app. 80% to 20%. These processes depend mainly on vegetation, hydrothermal conditions, activity of microorganisms and physicochemical soil properties (Badura 2006, Franzluebbers 2002, Sokołowska *et al.* 2004). The two basic, core and closely related processes: mineralisation and humification can be marked out on the way of formation of the individual fractions of soil organic substance.

Mineralisation

Mineralisation of organic substance refers to the stage of decomposition of organic remnants, takes place in parallel with humification and occurs both in the humus and prehumus phase. The final products of mineralisation processes are simple inorganic compounds (Drobnik 2010). Mineralisation is determined by the presence of specialised soil microorganisms, pH, temperature, organic carbon content, soil type and moisture. This process may occur in two directions: in aerobic conditions (rotting) to form CO_2, H_2O, as well as Ca^{2+}, K^{+}, SO_4^{2-}, PO_4^{3-},

NO_3^- ions, and the other in anaerobic conditions (putrefaction), to form compounds such as CO_2, H_2O, H_2S, CH_4 (Prusinkiewicz 1999).

The rate of the mineralisation process depends on content of lignin, tannins and waxes that are difficult in decomposition. Sugars, starch and proteins are quickly degraded. The decomposition process takes place in the following phases:

- During the *initial phase*, processes of hydrolysis and oxidation of organic substance (directly after decay of living organisms) take place.
- In the *phase of mechanical distribution*, macro-and mesofauna plays the central role.
- In the *final stage* processes of microbial degradation dominate and lead to the formation of appropriate, simple mineral compounds.

Humification

In general, soils contain a large variety of organic materials that are grouped into humic and non-humic substances. The process of humification means the formation of humus from non-humic substances. Humus formation is called humification. According to some scientists, humification depends on biotic and abiotic factors (Kumada 1987). Recently, the humification process has been the subject of multiple speculations and studies. In natural systems, biomass consisting of still fauna and flora is converted into soil organic matter (humus) by degradation reactions catalysed by enzymes. Part of decomposed organic matter is oxidized and re-synthesised to compounds with altered physicochemical properties revealing macromolecular nature and high stability. This process is accompanied by condensation, addition and polymerisation reactions (Rosa *et al.* 2005, Wershaw 1994). On the basis of literature (Księżopolska 2009), a few directions of humification were emphasised. In Waksman' theory, humus compounds are derived from modified lignin particles. Polyphenols theory assumes conversion of compounds, such as lignin or cellulose molecules, to polyphenols, which in result of enzymes (phenol oxidase) action are oxidised to quinones. Reactions of quinones with organic nitrogen compounds lead to creation of humus compounds. The theory of carbohydrate-protein condensation assumes the presence of a polymerisation reaction of sugars and amino acids, which are metabolic products of soil microorganisms' activit. As a result of these processes, nitrogen compounds are created and, in the next step, they may combine with other molecules of carbohydrates and may be fragmented to form highly reactive compounds such as aldehydes and ketones. These, in turn, easily polymerise into complex, brown-colour compounds. The occurrence, intensity, and the order of the humification mechanisms depend largely on the type of soil and water-air relations that prevail in the soil (Stevenson 1994). The way of soil usage, climate, growth of plants and microorganisms activity have also a great influence on transformation processes of soil organic matter.

The measure of organic substance humification is the degree of humification which expresses the percentage of humic substances in the total mass of dead organic material (Prusinkiewicz 1999).

2.3. Importance of soil organic substances with special emphasis on humic acids

Humus compounds, including humic acids, perform a number of important functions in the soil environment. In broad terms, it can be concluded that HA, affecting the physical, chemical and biological properties of soil, help to improve plants growth.

Influence of humic acids on the physical properties of soil

Humic acids give a colour to soil, from brown to almost black, and therefore affect the rate of heating of soil surface. HA have structure-building influence. Thanks to the ability of adhesion of soil grains, they contribute to the creation of aggregate structure of soil (Piccolo 2002), which in turn improves the air-water relations, porosity, viscosity, and soil compaction. HA have large water capacity, and therefore their presence in the soil improves the retention capabilities. This is particularly important in the case of sandy soils, where the presence of humus, including HA, significantly improves the compactness of soil and can help maintain higher amount of water available for plants.

Influence of humic acids on chemical and physicochemical properties of soil

Humic acids have a large sorption capacity, in the range of 300-1400 meq $(100\ g)^{-1}$, which makes them play a key role in shaping the sorption capacity of soils (mineral parts have from 2 to 30 times lower sorption capacity). Particularly significant impact of humus substances on sorption capacity refers to the A-horizon of soil, where 30-90% of total sorption capacity originates from humus compounds. HA have also buffering properties. Due to this fact, they stabilise soil pH (Garcia-Gil *et al.* 2004, Pertusatti and Prado 2007). HA regulate redox processes, concentration of the soil solution, including for example concentrations of mineral components such as Ca^{2+}, Mg^{2+}, NH_4^+, Na^+, K^+ and H^+, and also play an important role in the economy of phosphorus and nitrogen.

Influence of humic acids on the biological properties of soil

Humic acids influence the activity of micro flora and micro fauna of soil. Macro-and micro-organisms obtain from organic substances energy and mineral nutrients necessary for their life. Therefore, soil rich in humus has higher biological activity. HA have also a positive effect on many chemical and biochemical processes through the regulation of oxidation-reduction mechanisms.

Influence of humic acids on plant growth

Humus compounds, including humic acids, are a source of nutrients for plants. They are subject to mineralisation, and then gradually release trace elements necessary for proper growth and development of plants (Arancón *et al.* 2006). HA are an important source of nitrogen, phosphorus and carbon. This fraction may indirectly protect some plants from disease. Phytosanitary action is caused in this case by strong proliferation of saprophytic microorganisms in the environment of humus compounds which are antagonists of phytopathogens (Kowaliński 1993, Stevenson 1994). When examining the humic substances, the presence has also also found of growth substances such as vitamins, auxins, other organic acids and substances with the nature of antibiotics which at low concentration have a stimulating effect, while at high concentration have inhibiting influence on growth and development of plants. Results of studies also indicate the possibility of binding, by humus compounds, herbicides and other chemical compounds used for plant protection (Stevenson 1994). Inactivation of herbicides is related to their adsorption, as well as to acceleration of their decomposition by providing microorganisms with high-energy compounds (Kowaliński 1993).

Potential negative effects of humic acids for the environment

High concentrations of humic acids as well as other fractions of humus substances in water may cause undesirable odour and colour of water. Chelating action of HA may also contribute to the accumulation of toxic substances: heavy metals, phosphorus and ammonia compounds, adsorption of pesticides, phthalates, and polycyclic aromatic hydrocarbons (PAHs) (Kaleta 2004). In the process of water disinfection using chlorination, HA can form trihalomethanes compounds (THMs) and halogen acids which may have carcinogenic effects on living organisms (Galland and von Gunten 2002). Therefore, numerous studies are conducted on effective removal of HA. In this case various methods have been examined: capability of flotation and impact of various factors on HA removal from water – e.g. pH, concentration and type of flocculants (Zouboulis *et al.* 2003).

3. STRUCTURE AND PROPERTIES OF HUMUS COMPOUNDS WITH REFERENCE TO THEIR REACTIVITY IN SOIL ENVIRONMENT

Humus is one of the most chemically and physically active components of soils, but the structure of humus compounds (HS) is still not well examined. From the chemical point of view, HS are heterogeneous and polydisperse macromolecules with structure of different flexibility depending on units building the particle. HS are composed of various lengths of aliphatic chains, functional groups, aromatic rings and different kind of "bridges" linking given units (Schnitzer and Khan 1972). They are classified as macro ions because they have a polyelectrolyte character and therefore carry electrostatic charge in aqueous solution. Amounts and ratios of abovementioned structures determine the degree of sphericity or linearity as well as the properties of humus compounds.

Various functional groups, shape and size of particles, determine reactivity of humus compounds and classification to a given group of humus substances which are commonly known as: fulvic acids, humic acids and humins. In this classification (Kononowa 1966), HA are soluble in strong base but precipitate at pH lower than 2; fulvic acids are soluble in any pH; humins are insoluble in both basic and acidic solutions. This classification is only contractual limitation between the three groups of compounds, however it is still commonly used in analyses. Figure 3 presents a more extensive classification of humus substances based on their solubility in different media.

<table>
<tr><td colspan="6">Specific humus substances</td></tr>
<tr><td colspan="2">Fulvic acids</td><td colspan="3">Humic acids</td><td rowspan="2">Humins</td></tr>
<tr><td>Krenic acids</td><td>Apokrenic acids</td><td>Hymatomelanic acids</td><td>Brown humic acids</td><td>Grey humic acids</td></tr>
<tr><td colspan="3">Soluble in acetyl bromide</td><td colspan="3">Insoluble in acetyl bromide</td></tr>
<tr><td colspan="5">Soluble in NaOH</td><td>Insoluble in NaOH</td></tr>
<tr><td colspan="2">Lack of precipitate under acids influence</td><td colspan="3">Precipitate under acids influence</td><td>–</td></tr>
<tr><td colspan="2">Soluble in water and alcohol</td><td>Soluble in alcohol</td><td colspan="2">Soluble in alkali solutions</td><td>Insoluble in alkali solutions</td></tr>
</table>

Fig. 3. Classification of humus substances on the basis of their solubility in different media

3.1. Fulvic acids

Fulvic acids (FA) belong to the fraction of soil humus compounds with overall solubility in the whole pH range**.** The colour of fulvic acids varies from light yellow to yellow-brown.

In relation to other humus substances, FA are characterised by lower molecular weight in comparison to HA. This parameter may vary from 300 to 6000 (Trojanowski 1973). Schnitzer studies have revealed that the average molecular weight of fulvic acids equals 670 (Schnitzer and Khan 1972). FA have also smaller degree of polymerisation, less organic carbon and nitrogen content, higher content of oxygen and high acidity, and consequently better solubility in relation to humic acids. FA are also more heterogenic in relation to HA, however, they consist of simpler units of lower molecular mass.

The equivalence point of these compounds is included in pH between 8 and 9. It is a result of the presence of surface functional groups which mainly include carboxylic, carbonyl and hydroxyl groups. Larger amount of carboxylic groups in relation to HA makes fulvic acids more chemically reactive, and makes them have extremely high cation exchange capacity, from 520 to 1120 cmol (H^+) kg^{-1}.

FA form readily soluble compounds with metal ions**,** which may contribute to increased mobility of toxic metals in the environment, as well as cause excessive leaching of valuable micronutrients from the soil.

Sometimes distribution of FA on krenic and apokrenic acids is also encountered. The first fraction has a lighter tint and may change into the second fraction (apokrenic acids) after oxidation.

3.2. Humic acids

The reactivity of humic acids has a direct relationship with its structure. It results from the presence of various chemical groups and units, which is dependent on the nature and degree of transformation of HA. Principal and the most important differences in HA structure which may affect reactivity result from aromatic components condensation, amount of aliphatic structures and presence of different functional groups. However, the structure of HA, despite numerous studies, is still not completely understood. Difficulties in defining the construction of HA are mainly connected with continuous, dynamic changes of these compounds. The structure, molecular weight and the functional groups of HA differ from each other depending on the compounds' origin and age (Siéliéchi *et al.* 2008, Tipping 2002). This is mainly due to constantly ongoing process of humification of organic matter and changes of forming HA.

The structure of HA include, among others: aromatic rings (hydroxyphenols derivatives), heterocycled and condensed rings with heteroatoms such as nitrogen, sulphur or oxygen, side aliphatic chains and a lot of functional groups like: carboxylic, phenolic, alcoholic, amino, amide, metoxylic, quinonic and ketonic. General structure of HA proposed by Stevenson (Stevenson 1994) is presented in Figure 4.

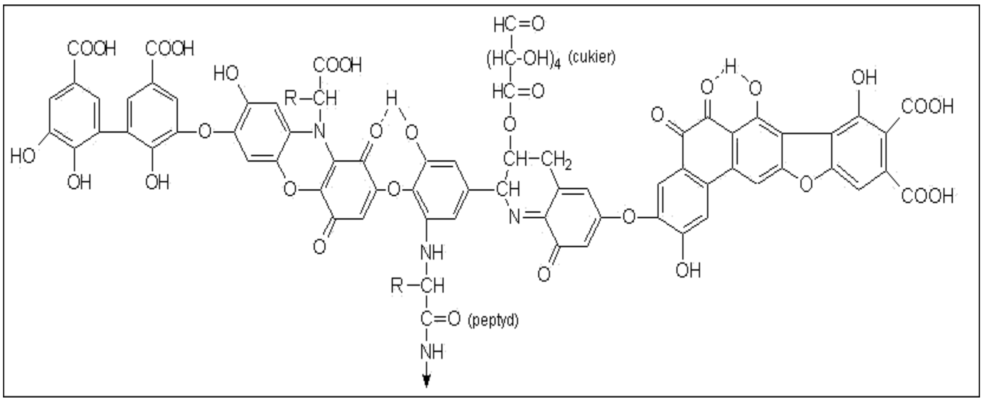


Fig. 4. Hypothetical structure of humic acids (Stevenson 1994)

Aliphatic chains are more specific to molecules of low molecular weight. In HA with high molecular weights, the main units of the structure are aromatic rings. Schulten *et al.* (1991) argues that the basis for the flexible structure of HA are alkyl benzene rings, as well as phenantrene and naphthalene units. The presence of bridges linking aromatic rings of humic acids causes that these substances are not compact but have a porous structure. These features mainly determine the water absorption capacity and sorption properties.

Humic acids have an acidic character and the nature of negative colloids. According to Schnitzer and Khan (1972) and Swift (1989), the surface of HA is highly charged and possesses 3-4 charged sites per 1000 Daltons, which makes HA one of the most charged substances among naturally occurring polyelectrolytes. This fact is related to the presence of surface functional groups in the particle structure, mainly carboxylic, phenolic hydroxyl, methoxy, carbonyl and quinone, which are dissociated to various degrees depending on the environment (Plaza *et al.* 2005a, Shen 1999, Sokołowska *et al.* 2009b). Typical functional groups present in humic acid structures are presented in Figure 5.

The carboxyl groups are strongly acidic and they have dissociation constants in the range of 10^{-2}-10^{-5}, while OH groups have a dissociation constant in the range of 10^{-9}-10^{-11}. One of the methods of studying the electrochemical properties of HA is potentiometric titration. Figure 6 presents titration curves of HA solutions (Boguta and Sokołowska 2012). Humic acids have been isolated from peaty-muck soils with different degree of secondary transformation. The results show that when using this method it is possible to observe even small differences in negative charge of HA resulting from varied content of functional groups. The amount of consumed titrant (NaOH) is proportional to the amount of negative surface charge at given pH.

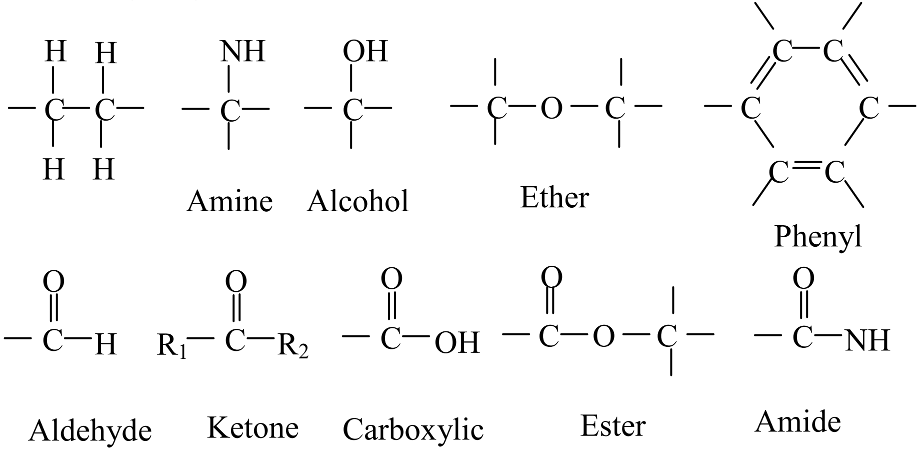


Fig. 5. Typical functional groups contained in humic acids

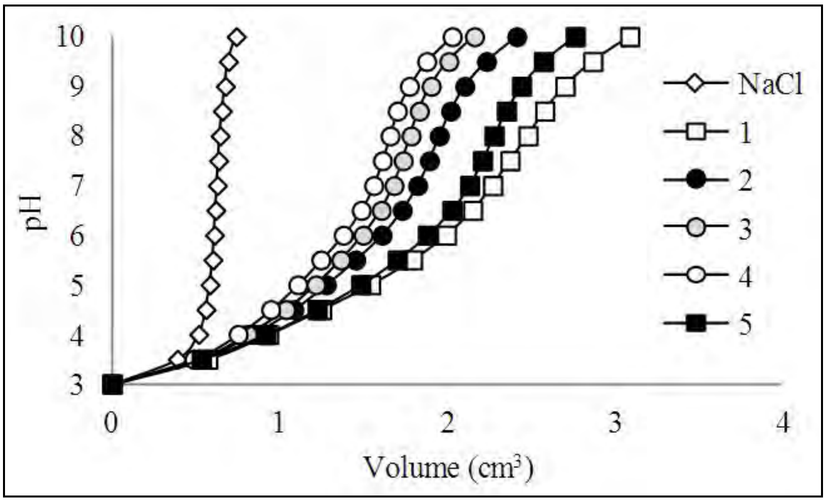


Fig. 6. Potentiometric titration curves of HAs. The samples abbreviated "NaCl" denote the reference solution of 1M NaCl. The samples abbreviated 1-5 refer to the number of studied HAs (Boguta and Sokołowska 2012)

The content of the functional groups determines the equivalence point, which for the HA is at pH 8-9 (Kononowa 1968), as well as properties such as hydrophilicity, acidity, ion exchange capacity, sorptivity, and abilities for creating organo-mineral connections (Flis-Bujak and Żukowska 2001). Studies have also demonstrated significance of aliphatic groups to interact with other substances of soil. According to Kopinke *et al.* (2001) and Salloum *et al.* (2002), processes of adsorption of hydrophobic impurities run better on HA containing larger amounts of aliphatic moieties than the aromatic and polar groups. The presence of acidic functional groups, especially carboxylic and phenolic on the surface of humic acid, makes these substances have a significant buffering capacity in soil (Boguta and Sokołowska 2012, Sokołowska *et al.* 2011). Sokołowska and Boguta in their research revealed that the buffer abilities of HA mainly originated from functional groups which dissociated at low pH, probably carboxylic groups. A second place of buffering was localised above pH 8 and probably came from phenolic groups dissociation. An example of the distribution of buffer capacity for HA with different humification degree, carboxylic and phenolic groups as well as varied elemental content is presented in Figure 7.

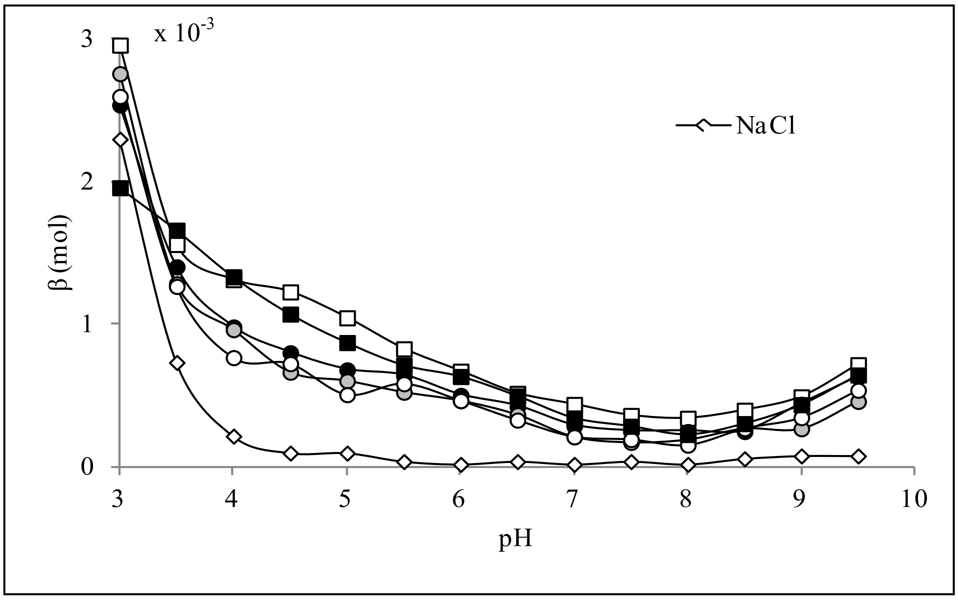


Fig. 7. Distribution of buffer capacity vs. pH for different humic acids. Symbols of humic acids refer to the same samples as in figure 6 (Boguta and Sokołowska 2012)

The results achieved allowed the researchers to prove that the differences observed on the β curves at low pH could be associated with the degree of secondary transformation of organic matter. β decreased (at low pH) with increasing degree of secondary transformation, which was confirmed by the high correlation coefficient. This also provided evidence of great diversity within amounts of surface carboxylic groups which dissociated at low pH range.

The functional groups of HA have a significant (but lower than FA) sorption capacity. Their total cation exchange capacity is equal to 487-870 cmol (+) kg^{-1}, whereas for FA this feature is equal to 900-1400 cmol(+) kg^{-1} (Zawadzki 1999). Comparison of the cation exchange capacity as well as other parameters of HA with the other soil components is presented in Table 1 (Zbytniewski *et al.* 2003).

Table 1. Comparison of selected properties of humic acids with other components of soil (Zbytniewski *et al.* 2003)

Properties	Montmorillonite	Illite	Kaolinite	Hydrated oxides of Fe and Al	Humic acids	Fulvic acids
Type of layer structure	2:1	2:1	1:1	–	–	–
surface charge	0.25-0.6	0.6-0.9	1.0	–	–	–
CEC (cmol kg^{-1})	80-120	20-50	1-10	4	485-870	900-1400
S ($\cdot 10^3$ m^2 kg^{-1})	600-800	70-120	10-20	–	800-900	
pH dependence	weak	medium	strong	strong	Strong	

CEC – cation exchange capacity, S – specific surface area.

The functional groups and the structural elements persenting the properties of chromophore (e.g. carboxyl, hydroxyl, and condensed benzene rings, chinoic systems formed by oxidation of hydroxyl groups in ortho- or para-positions) are also (next to pH and redox potential) responsible for HA colour. The colour of HA increases with increasing polymerisation, molecular weight and carbon content. Light, in particular UV radiation, changes HA structure, probably by disrupting quinone bonds.

According to Steelink, molecular weight of humic acid varies from 2000 to 50000 Da (Steelink 2002). However, even within these ranges, HA structure may differ significantly. Particles of smaller mass are more soluble in water than larger ones. Diameter of HA ranges between 8 and 10 nm, but in natural environment aggregated particles may reach dimensions of up to 100 nm.

The shape of the humic acid molecules undergoes dynamic changes, mainly due to the high sensitivity to pH conditions. Studies have revealed that at low pH HA has spherical coil form and at high pH the structure becomes more linear due to repulsion of charges of individual structure elements (Gosh and Schnitzer 1980, Myneni *et al*. 1999). Some researchers claim that HA have supermolecular structure, i.e. they are presented in the form of molecular aggregates with no macromolecular nature and interconnected by weak attractive forces (Piccolo *et al.* 2001, Simpson *et al.* 2001, Wershaw 1999). This theory of the structure confirms also the reduction of the size of the particles with decreasing pH due to destabilisation of supermolecular structure. High pH favours the maintenance of associated structure (Pédrot *et al*. 2010). The supermolecular structure theory is relatively young, but it explains in a good manner some phenomena such as e.g. the impact of HA on xenobiotics (Pacheco 2003).

HA particle size also decreases along with increasing electrolyte concentration in the solution, which results from weakening of electrostatic interaction of negatively charged structural elements of HA. At low ionic strength, electrostatic interactions have a greater impact, providing “extension” structure (Avena *et al.* 1999). Humic acids have fractal structure strictly dependent on pH. In consequence, this structure may be expressed via value as fractal dimension. Studies of specific surface area measured by adsorption of water vapour have revealed that heterogeneity and thus the fractal dimension of different soil formations has been enhanced by addition of humic acids (Sokołowska *et al.* 2009a).

With respect to elemental composition in humic acid molecules, dominating elements are carbon, oxygen, hydrogen, nitrogen atoms, and to a lesser extent - sulphur, phosphorus and metals. Research of Kononowa (1968) provides the following contents of individual elements:

C: 52-62%, H: 3-5.5%, N: 3.5-5%, O: 30-33%

These quantities depend widely on the source and maturity of humic compounds. Oxygen content of humic acids and hydrogen is smaller than in FA and carbon, and nitrogen content is greater (Stevenson 1994). The amount of oxygen in the structure of humic acids decreases with increasing molecular weight.

Among humic acids more specific fractions may be distinguished (Zawadzki 1999):

- Hymatomelanic acids: simplified humic acid fraction soluble in alcohol. They are probably the initial products of humic acid biosynthesis. This fraction has relatively low molecular weight from 250 to app. 500.
- Brown humic acids: they are poorer in nitrogen and more sensitive to the influence of electrolytes than hymatomelanic acids. They also have higher molecular weight and lower acidity.
- Grey humic acids: They are rich in nitrogen and highly sensitive to coagulating influence of electrolyte. They have high molecular weight and weakly acidic nature.

3.3. Humins

Humins are the fraction of specific humus substances which is the least understood and studied. The main problem is the isolation and purification of humins fraction. This fraction cannot be isolated by alkaline extractants. This group of compounds is weakly chemically active and truly they do not take part in significant number of soil processes. The lack of solubility in alkaline solutions is explained by strong binding to the mineral part of soil (Zawadzki 1999). Comparison of the most important chemical properties of humins, humic and fulvic acids is presented in Figure 8.

Humic substances				
Fulvic Acids		Humic acids		Humins
Light yellow	Brown yellow	Dark brown	Grey black	Black
→		Intensity of colour		→
→		Degree of polymerisation		→
→		Molecular weight		→
→		Carbon content		→
←		Oxygen content		←
←		Exchange acidity		←
←		Solubility		←

Fig. 8. Chart of changes in chemical properties of humic substances according to Stevenson (1994)

Soil is a source of many elements, especially metals. Their distribution in soil layers is varied and significantly depends on processes running in soil environment, such as:

- Dissolution,
- Occlusion,
- Sorption,
- Diffusion,
- Oxidation,
- Binding by organic matter,
- Creation of complex compounds.

4.1. Sources of metals in soil

A large part of metals present in soil originate from natural sources. However, very often, despite their high total content in the form of ores and minerals, metals are not available for plants under natural conditions. The most common minerals containing heavy metals are (Kociołek-Balawejder and Stanisławska 2012):

- cinnabar HgS,
- litharge PbO,
- lead galena PbS,
- cerussite $PbCO_3$,
- anglesite $PbSO_4$,
- monteponite CdO,
- greenockite CdS,
- arsenopyrite $FeAsS$,
- lollingite $FeAs_2$,
- cobaltite $CoAsS$,
- nickeline $NiAs$.

The circulation of metals in areas not covered by human activity may be considered as closed. Mineral components sorbed by plants are returned to soil as biomass and are subject to decomposition into simpler components, and afterwards they can be again sorbed by plants.

A separate group of metals are elements implemented artificially into the environment, either as by-products of industry or for intentional purpose as fertilizers and pesticides. Nutrient deficit that becomes especially visible at agricultural fields, where annual harvests result in gradual soil degradation, causes that supplementation of certain micronutrients becomes necessary. On the other hand, from the chemical point of view a significant part of microelements being simultaneously heavy metals are not only absorbed by plants, but also accumulated in form of weakly soluble compounds in soil. It is generally accepted that heavy metals are elements with density exceeding 4.5 g cm^{-3}. This group includes: zinc, manganese, lead, mercury, copper, nickel, strontium, barium, cadmium, cobalt, molybdenum, arsenic and aluminium (Baranowska-Morek 2003). Excess of metals introduced to soil as macronutrients is not significantly dangerous, however accumulation of heavy metals often playing role of micronutrients as well as lack of effective methods of soil remediation become a big challenge for contemporary ecology.

In broad terms, metal emission is mainly connected with various industry verticals, communication and transportation. Among industrial areas, main source of heavy metals is production of chemicals and fertilizers electrochemical, paper, glass and ceramic industry, as well as oil refineries (Kabata-Pendias and Pendias 1999). Particularly, large amounts of metals accumulate in the vicinity of roads, mines and steelworks (Kabała and Singh 2001, Pasieczna 2002). Together with fumes, dust and wastewater, the metals may reach soil solution and watercourses and might be easily absorbed by plants (Domagala-Świątkiewicz 2003). Agriculture can also be a potential source of a significant amount of heavy metals in soil. In this group, the largest percentage of metal emissions are pesticides, food preservatives, wastes from pig and poultry farms, as well municipal wastewater (Kabata-Pendias and Pendias 1999). In addition, fertilizers are often derived from minerals, and may also contain small amounts of heavy metals such as lead, zinc, copper and cadmium. The largest amounts of these elements are transferred to soil with phosphate and calcium-magnesium fertilizers (Kociołek-Balawejder and Stanisławska 2012).

Research conducted by IUNG has revealed that 97% of Polish soils belong to 0 and I degree-class in the scale of heavy metal contamination. These degrees correspond respectively to uncontaminated soils, which are suitable for cultivation of plants consumed by humans and animals, and soils with increased level of metal concentration, suitable for cultivation of plants but not for consumption purposes. In the above-described scale of heavy metal contamination, the remaining 3% of Polish soils range between III and V degree, but those soils belong to highly industrialised areas, mines and steelworks, mainly located in Silesia (Mocek 2004). The scale of contamination with heavy metals, proposed by the Institute of Soil Science and Plant Cultivation (IUNG) is presented in Table 2.

Table 2. Scale of pollution with heavy metals in soils

Degree of contamination	Description
0	Soils uncontaminated. They are suitable for cultivation of all plants, especially intended for consumption of infants and children
I	Soils with increased metal content. They are suitable for cultivation of all field plants with limitation of vegetables intended for direct consumption by children and intended for preserves
II	Slightly contaminated soils. Plants, which are cultivated on these soils may be chemically contaminated and therefore cultivation of such plants like spinach or lettuce should be excluded. Cereals, root crops and fodder plants can grow on such soils
III	Soils contaminated in medium degree. Plants can be contaminated with heavy metals, so only cultivation of cereal, forage and root plants is recommended on these soils, however with controlling metal content in both consumer and fodder parts of plants
IV	Heavily contaminated soils. It is recommended to cultivate industrial plants, such as hemp, flax, rape for technician oil. The use of such soils as grasslands should be limited
V	Extremely contaminated soils. These soils should be excluded from agricultural production and subjected to reclamation. Reclamation should be conducted by soil improving including agricultural processes which can limit mobile forms of metals

Significant accumulation of heavy metals in soil is classified as chemical degradation. It should be noted that, contrary to organic pollution, metals introduced into soil are permanent contamination. In this case only metal form is variable, however the concentration remains unchanged. In addition, heavy metals introduced by humans are usually a more soluble and thus more available form for living organisms than metals of natural origin that are in the form of various minerals. Processes occurring in the soil system highly affect the toxicity of heavy metals introduced into the soil. Changing weather conditions, air-water soil relationships, pH, temperature and concentration of other components of soil, may result in releasing high amounts of accumulated heavy metals to soil solution, making them easily available and therefore toxic to plants, animals and humans.

4.2. Micro- and macronutrients

Metals contained in soil, depending on the amount necessary for the growth and development of plants, are divided into micro- and macronutrients. The group of metals playing the role of macronutrients includes potassium, calcium and magnesium. Excess of these elements in soil is generally not harmful. Deficiency is often noticeable in the case of potassium. Polish soils contain app. 0.01-1.2% of this element and its main sources are minerals and soil bedrock. Slightly larger amounts in soil are attributed to magnesium ions which mainly can be found in the forms of dolomite, magnesite, carnallite and serpentinite. Calcium is present in soil in much larger amounts, from 0.02 to 27%, and it derives primarily from weathering of calcite and dolomite. At low pH it can be also transformed to well-soluble bicarbonates.

Trace elements which are necessary for plants only in small amounts include, among others: molybdenum, copper and zinc, while it should be noted that too large amounts of these metals may have toxic effect on plant growth (Bednarek *et al.* 2004). Natural sources of these elements are usually bedrocks and organic substance. The smallest amounts are found in soils formed from sands, sandstones and acid igneous rocks. On the other hand, significant amounts of metals belonging to micronutrients have been found in soils derived from basic igneous rocks - clays and shale clays (Zawadzki 1999). Average content of selected microelements and other selected trace elements in soils is presented in Table 3.

Table 3. Total contents of selected metals in lithosphere and in soils (Swaine 1977)

Element	Average in lithosphere (mg dm^{-3})	Typical range in soils (mg dm^{-3})
Mn	1000	200-2000
Mo	2	0.2-5
Cu	70	2-100
Zn	80	10-300
Fe	50000	50000-300000
Ti	4400	1000-10000
Cr	200	5-1000
Co	40	1-70
Ni	100	10-1000
Cd	–	0.01-7

4.3. Factors determining mobility of metals in soil

Mobility of metals in the soil environment is determined by a number of factors. In general, circulation of metals in the natural environment depends on their chemical form, composition and physicochemical properties of soil, climate conditions, and individual biogeochemical characteristics.

Influence of soil pH

One of the most significant factors for the mobility and form of metals is soil pH. More precisely, acidification of soils is the main problem which refers to current toxicity of heavy metals in soils of Poland and many other industrialised and developed countries. A majority of soils have slightly acidic and acidic pH resulting from natural but also from anthropogenic reasons. Only a dozen or so percent of soils have neutral or alkaline pH. The main reason of increasing soil acidity is, among other factors: agriculture (fertilizers, pesticides), industry (dusts and gases, for example sulphur compounds falling on the surface of soil and causing its degradation) and forest management. Reduction of soil pH causes dissolution of minerals and hydrated oxides of iron, manganese and aluminium, whose surface others heavy metal ions are often adsorbed on. In consequence, the mobility of heavy metals as well as their phyto-availability increases (Kabata-Pendias and Pendias 1999). Reduction processes in these conditions may be presented by the following relations:

$$Fe^{3+} \rightarrow Fe^{2+}$$
$$Mn^{4+} \rightarrow Mn^{2+}$$

It should be stressed that the properties of individual metals are different to such extent that pH at which a particular element is made mobile is also varied (Kociołek-Balawejder and Stanisławska 2012), e.g.:

Cd ~ 6.5,
Zn ~ 6.0,
Cu and Ni ~ 5.5,
Hg, Pb and Cr (III) from 4.5 to 5.0.

Increasing of pH may lead to the appearance of complex or hydrolysed forms as well as to oxidation of cations from lower to higher oxidation degree:

$$Fe^{2+} \rightarrow Fe^{3+}$$

Cations of nickel, zinc, mercury and lead are not so responsive to pH like aluminum and beryllium. Some metals exist in anion form. The availability of selenium, vanadium, arsenic or chromium anions decreases along with pH increase. However, pH influence is rather less significant for anions than for cations (Bohn *et al.* 1979).

pH affects also the distribution of metals in different soil layers. E.g., zinc and cadmium are present in the smallest amounts in layers with the lowest pH, which is a result of their high mobility under acidic conditions. The content of lead and copper does not reveal any relationship with pH, which is probably connected with a high accumulation of these elements in the most acidic organic horizons (Kocowicz 2002). Liming of soils limits the mobility and availability of heavy metals to some extent. High pH may cause the formation of complex ions of metals, negatively charged, and metal complexes with organic ligands as well as ammonium ions (Karczewska 2002).

Chemical properties of metals

The mobility and chemical forms of metals in soils are also conditioned by the physicochemical properties of metals:

- redox potential,
- degree of oxidation,
- volatility,
- solubility,
- affinity of metal to specific ligands and stability of their complexes.

In general, increase in the mobility of metal ions is observed at lower oxygen content. Decrease of Eh potential may cause a reduction of some soil micronutrients. In such conditions ions Mn^{2+} or Fe^{2+} as well as molibdate anions: $MoSO_2^{2-}$, $MoSO_4^{2-}$ may be released to soil solution due to the dissolution of hydrated oxides. This might be a reason of phyto-availability increase of those elements. In consequence, soil system contains both oxidised and reduced forms of elements where the ratio of given forms stays in dynamic equilibrium and together with pH determines the value of soil redox potential (Bednarek *et al.* 2004). Under reduction conditions also heavy metals sorbed and occluded on hydroxyoxides are released to soil solution. In highly reducing environment, released heavy metals can be precipitated as sulphides due to reduction of sulphates (Karczewska 2002).

Time

Time is also an important factor which determines metals mobility. Generally, the availability of metal ions decreases in time. Together with time, the degree of metal diffusion to the strongest sorption centres is higher, as well as metals are built into the crystalline network and also into the amorphous structure of the mineral parts of soils (Bohn *et al.* 1979).

Humus substances

Humus substance content also has a significant influence on the mobility of metals in soil. However, binding of metals by fulvic and humic acids is a complex problem. The most interesting compounds are connections of organic ligands including metal with creation of chelating bonds. Taking into account the fact that such compounds may be highly soluble in water, they are simultaneously more available for plants and, contrary to simple metal cations, complex forms of metals are not as easily washed out from upper part of soil profile as acid-form ion.

The effect of organic substances, particularly specific humus substances, on the mobility of metals in the soil environment is highly complex. In comparison to the influence of simple and well-known compounds, fulvic and humic acids as well as humins do not have predefined structures. This fact implicates various and complex chemical behaviour in relation to other soil components like metal ions. Therefore, this problem is a main goal of this document and is described in detail in subsequent sections covering interaction of humic acids with metal ions.

4.4. Metal forms in soils

Metal forms are determined by factors described in the previous section. Generally, metals can be present in the soil environment in the following, most common forms:

- Inorganic compounds,
- Organic compounds,
- Ions in soil solution,
- Metals bound to the solid phase of soil.

The greatest availability is rather typical for simple cationic metal forms, complex ions, aqua complexes, organic and inorganic complexes and ions adsorbed exchangeably. Forms of metals less available generally include compounds where metal is strongly connected with mineral or organic part of structure. Release of metal from such a connection can require pH or redox potential change. Between metals contained in soil solution and contained in unavailable forms, chemical equilibrium is present, and this balance is continuously subject to dynamic shifts as a result of soil processes (Łabętowicz and Rutkowska 2002), including, among others:

- Exchangeable sorption,
- Precipitation and co-precipitation,
- Complexation,
- Dissolution,
- Oxidation,
- Reduction,
- Mineralisation.

In the processes of adsorption, co-precipitation and precipitation, metals can be immobilised. Immobilisation is often conditioned by the presence of iron and manganese oxides. These elements create thin oxide layers on the surface of clay minerals and in result obtain highly developed surface area. In addition to the abovementioned processes, metals may also be transported to plant cells or form volatile chemical connections. In respect to phytoavailability, forms of heavy metals may also be classified as labile and stable (Karczewska 2002). Table 4 presents examples of metal compounds characterised by strong and weak availability.

Table 4. Examples of metal compounds characterised by high and low availability

Element	Weakly available forms	Easily available forms
Potassium	Illite, feldspar, mica	Chlorides, sulphates, carbonates, potassium exchangeable from soil colloids
Magnesium	Biotite, augite, olivine, chlorite, montmorillonite, vermiculite and dolomite	Magnesium exchangeable from soil colloids, soluble magnesium salts
Calcium	Pyroxene, apatite, gypsum, calcite, dolomite	Calcium exchangeable from soil colloids, soluble calcium salts
Iron	Sulphides, carbonates, phosphates, silicates	Chlorides, nitrates, sulphates
Copper	Sulphides, carbonates, phosphates, silicates	Chlorine, nitrites, nitrates, sulphates
Manganese	Sulphides, sulphites, carbonates,	Chloride, nitrite, nitrate, thiosulphate, sulphate
Aluminium	Sulphides, sulphites, carbonates, phosphates	Chloride, nitrite, nitrate, sulphate, thiosulphate

Available forms of trace elements constitute only a dozen or so percent of their total content in soil. High availability is desirable for sorption of micro and macro nutrients by plants. However, nutrients may also include certain heavy metals such as Zn and Cu, necessary only in small amounts. Another part of heavy metals, such as As, Cd, do not present any benefits for living organisms and their available forms and, even in small concentrations, are extremely dangerous and might lead to many diseases similar to the ones observed in the case of excessive levels of heavy metals playing the role of micronutrients (Bernacka and Pawłowska 2000). Therefore, some of the key goals of today's ecology is to neutralise toxic forms of heavy metals or their excessive amounts by transforming them into unavailable forms, or even utter removal and recovery. For this purpose, it is necessary to understand the mechanisms of interactions between metals and other soil components.

5. INTERACTIONS OF METALS WITH HUMIC ACIDS

Humic acids and their functional acidic groups are the main factors responsible for binding metal cations and, consequently, for their state and concentration in soils (Lux 1993). In soil environment the most significant are soluble combinations of HA with metal ions (Antoniadis and Alloway 2002, Sokołowska and Warchulska 2009, Tipping 2002). According to some theories, metals from such compounds may be more available for plants rather than from insoluble forms or simple ionic forms (Brady and Weil 1999, Clemens *et al.* 1990, Havlin *et al.* 1999). Simultaneously, humic acids have limited sorption capacity and too large a dose of metal or other mineral component which can be sorbed on humic acids may cause saturation of negative, surface charge of humic acids leading to coagulation of soluble compounds. Thereby, there is a risk of higher accumulation of metals, including also heavy metals precipitated as humus-insoluble salts. Such processes may lead to soil degradation and cause micronutrient deficiency for plants.

5.1. Research problem

There is no clear explanation of interactions between humic substances and heavy metals (Golonka *et al.* 2005, Guclu and Apak 2003, Trtić-Petrović and Jönsson 2002), whereas studies of these interactions reveal a still actual problem of circulation and remediation of metals in the environment (Melekhova *et al.* 2006). It is generally known that the mobility of metals depends on a great deal of factors on pH environment (Cao *et al.* 2004, Pullin and Cabaniss 2003), as well as on organic matter contained in soil, so that in consequence it depends also on humic acids (Erdogan *et al.* 2007, Lux 1993). Therefore, despite the fact that this topic is a continuation of the previous elaborations, these problems are still a burning and open scientific issue, not only on the Polish scale, but also for the entire world, and require additional investigations.

The need of further detailed studies can be explained in the following points of research problems which are still not clear and contain a lot of question marks:

- **Sort of binding functional groups in humic acids**

According to the literature, the problem of influence of metals on humic substances was investigated by some scientists (Dobranskyte *et al.* 2006, Lippold *et al.* 2007, Plaza *et al*. 2005 b and c). However, it should be stressed that there are still a lot of unclear, contrary opinions concerning the metal – humic acid interaction mechanisms as well as the influence of humic acid properties on these interactions (Cao *et al.* 2004, Lippold *et al.* 2007, Zhou *et al.* 2005). Especially unclear pieces of information relate to the way of binding humic acids with metal ion. Some scientists claim that carboxylic groups of humic acids are responsible for binding processes with metal ions (Rahman *et al.* 2010). Some scientific literature reports indicate a complex nature of both carboxylic and phenolic functional groups of humic acids (Pehlivan and Arslan 2006). Some scientists suggest also a possibility of binding of metal to humic acid particles by nitrogen atoms (Alberts and Filip 1998, Senesi *et al.* 1987). Despite the fact that this element occurs in small amounts in the structure of humic acids (about 3%), its role in the complexing processes can be significant, because according to Pearson theory complexes created between, for example, copper ions and nitrogen should be more stable than complexes formed from copper ions and oxygen atoms from carboxylic groups (Perdue 1998).

Apart from the possibility of metal binding by carboxylic and phenolic groups as well as nitrogen-containing structures like amine and amide groups, it is also believed that one of the factors responsible for metal binding by humic acids may also be the presence of hydroxyl, enol, carbonyl groups or groups which contain sulphur (sulphide and sulphhydryl groups) (Kowalkowski and Buszewski 2002). However, these opinions and conclusions are still varied and divided both in terms of various metals as well as humic acids of different properties, and in relation to various environmental conditions under which the interactions take place.

- **Ligand-metal ratio**

Previous studies have also not provided any precise answer to the question: what is the ratio of ligand to the central ion during complexing processes. Most of the studies are rather based on the assumption of model ratio of complexation between metal ions and functional groups of HA, usually 1:1 (the simplest model) (Stevenson 1994, Tipping 2002).

- **Complex chemical nature of different metal ions and wide spectrum of humic acid properties**

Additional aspects, which make studies more complex, are the varied chemical properties of metals: valence, ionic radius, hydrate ratio, electronegativity, as well as varied physicochemical nature of humic acids: different molecular weight, degree of structure transformation, content of aromatic rings, aliphatic chains or functional groups. Simultaneously, it should be added that the form, mobility and reactivity of both humic acids and metal ions in the soil environment strongly depend on multiple factors like concentration, ionic strength, pH (Cao *et al.* 2004, Pullin and Cabaniss 2003).. Additionally, humic acids have strong affinity to link metals due to their large sorption capacity. For this reason, the cumulative problem of studying humic acids and metals interactions is much more complex in scientific terms and still needs to be continued.

- **Insufficient number of studies on interactions of humic acids with metal ions in comprehensive way**

Previous studies conducted on humic acids and metals were focused rather on analysis of a single aspect of a particular problem, e.g. influence of pH or valence of metal, ratio of ligand to central ion, way of binding formation, or the studies were focused on a single, selected property of humic acids. There are not many studies with a comprehensive approach to the problem. There are no reports on attempts to consider a wider range of factors with application of different and the newest instrumental techniques.

- **Burning civilisation problem: heavy metals in soils**

Concentration of heavy metals in soils permanently increases along with development of cosmetics, agricultural and chemical industry, whereas methods of neutralisation or removal of heavy metals are still unsatisfactory (Melekhova *et al.* 2006).

- **Deficiency of micronutrients in soils**

A majority of light metals and a part of heavy metals constitute important nutrients for plants. Although their surplus in the soil may be harmful, their deficit may have a negative impact on growth and development of plants. Availability of these elements to plants and their mobility in environment, water and ground, depend mainly on the content of humus compounds. In other words, this problem is particularly noticeable in soils rich in humus compounds, like organic soils, and on the other hand in soils poor in humus, with low cation exchange capacity, where the most significant problem is too easy leaching of nutrients from the soil.

- **There are still not enough studies on relationship between reaction mechanisms of humic acids with metals and properties of humic acids or soil from which humic acids originate**

It should also be stressed that there is insufficient information in literature about studies on significant relations between properties of clear humic acids and soils which humic acids are isolated from – especially in relation to humic acids originating from a single kind of soil, for example from different peats. In connection with the above, attempts at referring the mechanisms between humic acids and metals to the simple parameters describing clear humic acids or soil are only occasional. However, results of such studies reported by some scientists indicate the presence of significant variability of physicochemical properties of soils even in the same type of soil (Gawlik 1992, Matyka-Sarzyńska and Sokołowska 2002, Sokołowska *et al.* 2005), which could be translated into diversity of properties of organic matter contained in soils. Finding significant correlation between reaction mechanism and properties of humic acid or the whole soil would enable easy assessment of the ability of humic substances to connect to metal ions, as well as the prediction of the direction of such a reaction.

- **Aspect of soils rich in organic substances: Problems with adoption of organic soils for agricultural purposes and appropriate mineral economy on such soils**

Constantly intensifying economy contributes to an increasing interest in more effective usage of organic soils, such as peat-muck soils, by agriculture. However, the properties of these soils make them difficult to be adopted to agricultural use and, in consequence, efforts towards developing ways of achieving such soils high yields with good quality do not always terminate successfully (Gawlik and Harkot 2000), and despite numerous attempts many areas of these soils remain still unused. The need to increase the productive values of muck soils is connected in multiple cases with the use of chemicals, among which metals may have a great impact on humic acids. The largest natural concentration of heavy metals occurs in the surface layer of soil and is connected with immobilisation by humus compounds, but may also indicate anthropogenic factors such as deposition of metals in the vicinity of transportation routes from fuels, oils and automotive supplies (Węglarzy 2001). In consequence, agricultural practices and reclamation works carried out on hydrogenic soils in order to acquire new agricultural land, and land for the construction of new roads, cause disruption of natural air-water relationships, changing the direction of pedogenic processes. Simultaneously, melioration processes contribute to inhibition of accumulation of organic matter and, consequently, to intensification of decesion processes where mucking is present. These conditions favour the acidification and mineralisation of soil, and thus release heavy metals – readily available to plants, animals and humans (Nicia *et al.* 2010).

- **Aspect of soils rich in organic substances: metals in unique ecosystems of peatlands**

Investigations of metals cycle in soils rich in organic substances like soils originated from peats (for example mucks) are essential because knowledge about mineral state of non-degraded peatlands can also contribute to better protection of such areas (Boguta *et al.* 2011). Biodiversity of fauna and flora, as well as specific landscape of Polish bogs, situate these areas among the most valuable natural resources of our country. However, both in Poland and in other parts of the world, peatlands occupy only a small area. In addition, these regions for several years have faced degradation problems. For the biggest part it has been connected with inappropriate treatment measures causing water drainage from wetlands and drying processes resulting from steady agriculture intensification. Only in the period of 1952-1992, 73%, i.e about 12800 ha of bogs in Poland were drained for farmland. However, research has revealed that attempts to use bogs and wetlands contribute to significant disturbances in carbon economy in ecosystems (Waddington and Price 2000, Waddington and Warner 2001). Therefore, from the environmental point of view, protection of peatlands should be an ecological priority. This problem is particularly important due to the fact that peatland areas situated usually in short distance to muck soils and other agricultural land may be exposed to the influence of metal ions, mainly from pesticides and fertilizers. Organic substance contained in peat can interact with metal ions, leading to a disturbance of balance in peat ecosystem, which in turn may contribute to its degradation, destroying biota specific for this area.

For reasons mentioned above, there is still a strong necessity to conduct monitoring of the state of organic soils environment, especially in relation to metal ion content.

5.2. Measurable effects of studies conducted on humic acids-metal ions systems

Problems mentioned above demonstrate that research on interactions between humic acids and metals should be continued. Despite the fact that current research is a continuation of previously conducted studies by other scientists, it should be stressed that this subject takes into account still new measurement possibilities and still new aspects of environmental problems linked to civilisation development. This should provide innovative, interesting, supplemental information to the current state of knowledge. Results of the research are the most valuable in various areas which could be indicated as:

- **Providing significant information to ecological knowledge**

Investigation on the behaviour of metal ions under humic acids influence reveals still existing problem of circulation and remediation of metals in the environment**.** It is generally known that the mobility of metals depends on multiple factors, such as the pH of the environment, as well as organic matter contained in soil, and in consequence depends also on humic acids**.** Therefore, these problems are still a burning and open scientific issue, not only on the Polish scale, but also for the whole world, and need additional investigation. Knowledge about interactions between heavy metals and soil organic matter characterised by varied physicochemical properties should allow the prediction of possible consequences of the metals emission to the environment. Simultaneously, this knowledge should contribute to development of more effective methods of heavy metal remediation.

Better understanding of molecular mechanisms of interactions between humic acids and heavy metals will be the initial but valuable point in prediction of their mobility in soil and in soil protection, especially in relation to organic matter in such unique ecosystems rich in organic substance like peatlands, peaty-muck and muck soils. Such soils are especially exposed to the effect of heavy metals.

Studies devoted to the influence of various factors (for example metal ions or pH) and carried out on soils rich in humic carbon may be critical in reduction of processes where greenhouse gases are released.

- **Benefits for agriculture**

Research which connects weakly examined soil organic matter and its interactions with soil components may contribute to the development of agricultural chemistry. Such studies might be helpful in conducting optimal and rational usage of artificial fertlizers and other chemical compounds containing metals. The results can also be valuable for agricultural industry. Recently it has been revealed that there is a significant interest in production of new forms of artificial fertilizers containing an addition of organic compounds including humic acids.

Moreover, studies of interactions between humic acids and metals may also bring some knowledge on the development of new agricultural areas. From agriculture point of view, detailed investigations conducted on soils rich in organic substance, like peats, peaty-muck and muck soils, as well as on humic acids isolated from them, will be helpful in enhancement of effectiveness of agricultural application of above areas degraded by draining processes as well as in protection of non-degraded unique peats areas.

Summing up, in consequence such studies will contribute to improvement of soil cultivation, ecological and integrated tillage, while minimising the use of chemical, toxic substances.

- **Scientific contribution in investigations concerned with one of the most valuable soil substances – humic acids**

These compounds are still not well known, despite numerous studies. It is a result of the complex structure of humic substances. This fact implies varied chemical and physical properties as well as complex behaviour in connection with other soil substances. Therefore, the application of the newest instrumental techniques, research on the molecular chemistry level, investigation of soil processes in micro- and nano-scale: analysis of coagulation processes, electrostatic interactions or chemical bonds creation, will enable to reveal new scientific facts.

- **Additional research value might be an attempt to find relationship between mechanisms of humic acids–metals interaction and simple parameters determined for soils or humic acids isolated from them.**

Such relationships could help in the evaluation and prediction of soil behaviour under metal stress based on measurement of a simple parameter (property) of soil.

5.3. Interactions with “light” metals

Interactions of light metal ions with humic acids are usually based on simple reactions leading to the formation of ionic bond. In result, salts called “humates” are created. Replacement of hydrogen with metal ion is prompt. This process is mainly participated by functional groups such as carboxylic and hydroxyl of phenols. Therefore, the degree of ion exchange depends largely on individual groups dissociation and thus also on pH value. Generally, potentiometric studies reveal an increase in exchange degree along with pH increase up to 10-12, where all COOH and OH groups are dissociated. At neutral pH, protons are exchanged mainly from carboxylic groups of humic acids.

Elements of this group involved in ion exchange with humic acid radicals are cations very common in nature:

- Calcium: Ca^{2+},
- Magnesium: Mg^{2+},
- Sodium Na^{+},
- Potassium: K^{+}.

Humates of monovalent metals like sodium or potassium are well soluble. Soluble salts make metal more mobile in the soil environment. The exchange reaction can be schematically described as depicted below:

$$\text{—COOH} + K^{+} \longrightarrow \text{—COOK} + H_2O \longrightarrow \text{—COO}^{-}\ldots\ldots K^{+}$$

High solubility of humates of monovalent elements causes that a deficiency of such metals as potassium is observed, especially in soils rich in organic matter (Boguta *at al.* 2011, Brandyk *et al.* 2008, Piaścik and Łachacz 2001, Sigua *et al.* 2006). Additionally, studies performed on different peaty-muck soils indicate that the most degraded peat-muck soils contain the lowest concentration of potassium. This relationship is presented in Figure 8.

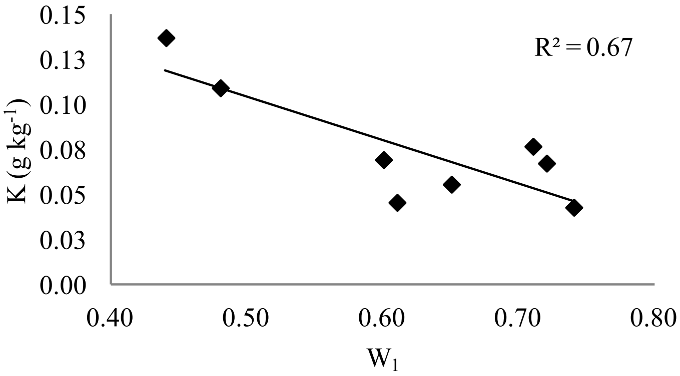


Fig. 8. Total concentration of potassium (g kg^{-1}) in peat-muck soils in relation to secondary transformation index (Boguta *et al.* 2011)

Based on these results it could be concluded that the amount of potassium might undergo decrease in soils with higher degree of degradation. The relationship displayed in Figure 8 could also result from the fact that potassium is a chemical element which may be easily displaced by other cations like for example calcium or magnesium.

Humates of divalent metals, for example calcium and magnesium, are insoluble in water and their formation can be described by schematic reaction presented in Figure 9.

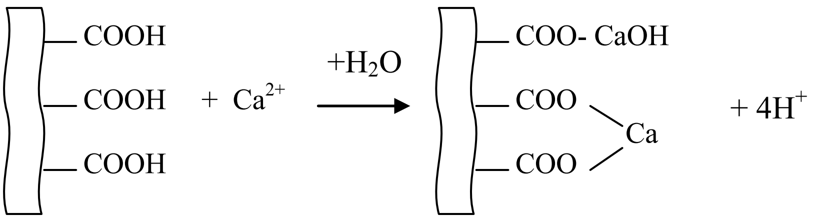


Fig. 9. Schematic reaction of humic acid molecule with calcium cation

Calcium has coagulation properties – it immobilises humic acids in the form of insoluble calcium humates (Sokołowska and Warchulska 2009). Sokołowska and Warchulska noted during their investigation of the influence of sodium phosphate on calcium humates at different pH that the process of organic matter release increases along with pH increase as well as increasing amount of sodium monohydrogen (ortho) phosphate added to humates. Sodium with HA creates soluble salts making it more mobile.

Most often the above salts of HA are present in soils as mixtures of aluminium and iron hydroxides or in the form of complex compounds connected with these hydroxides (Dobrzański and Zawadzki 1995). Magnesium and calcium ions are probably bonded on the surface of HA by carboxylic groups, whereas the diffusion layer contains also ions of monovalent metals, for example sodium cations. In the case of humic acids, the amount of ions bonded in particular layers is directly dependent on pH conditions. This factor influences the dissociation of surface functional groups of humic acids (Campitelli *et al.* 2006).

The processes of above mentioned ion exchange are also extremely important in soil acidification. It is especially well visible in the case of soils undergoing mineralisation because the amount of exchangeable hydrogen increases during decay stage. These hydrogen ions displace calcium ions bonded with solid phase of soil. Calcium is transferred to soil solution and in consequence is leached, and the soil obtains a more acidic character. This phenomenon is particularly well visible in peat bogs areas. Low content of this element (0.05-0.2% in transitional peat and 3-6% in low peat) contributes to lower pH and fertility decline in peat (Szajdak 2002). In some cases, in addition to ion exchange, molecular sorption is possible, for example in reaction with hydroxides: $Ca(OH)_2$ and $Ba(OH)_2$. Studies have revealed that in consequence of interactions of light metals with soil HA, plants may change the degree of sorptivity of different elements. Results indicate that addition of HA to soil causes a decrease in sorption of sodium, potassium and calcium ions by plants, whereas magnesium sorption increases (Gaur 1964, Sanchez-Conde and Ortega 1968). Tan (1978) revealed that humic and fulvic acids are also capable for releasing potassium fixed in illite and montmorillonite.

Studies also indicate a possible coagulation influence of magnesium and calcium ions in relation to HA and other organic substances present in soil. These processes may occur at highly alkaline pH (about 9.5 for calcium and over 10.5 for magnesium), because under these conditions calcium carbonates and magnesium hydroxides are precipitated, and organic contaminants including humic complexes may be adsorbed on these precipitated metal particles. However, these circumstances (alkaline) are untypical in the environment. Therefore, such processes are uncommon. Reduced solubility of HA and enhanced removal of organic pollutants in the presence of calcium and magnesium ions have been also confirmed at low pH range, from 2.0 to 4.0 (Świderska-Bróż 1984). In turn, under these conditions, HA structure is more susceptible to coagulation.

In general, high affinity of calcium and magnesium to HA can be confirmed by high amount of these elements in soils rich in humus compounds. According to studies conducted by Boguta *et al.* (2011) regarding amount of elements in peaty-muck soils, calcium and magnesium concentration in studied samples were quite high. In the case of calcium, positive correlation between metal concentration and secondary transformation degree has been found, similarly as in case of potassium. This relationship is presented for eight selected peaty-muck soils in Figure 10.

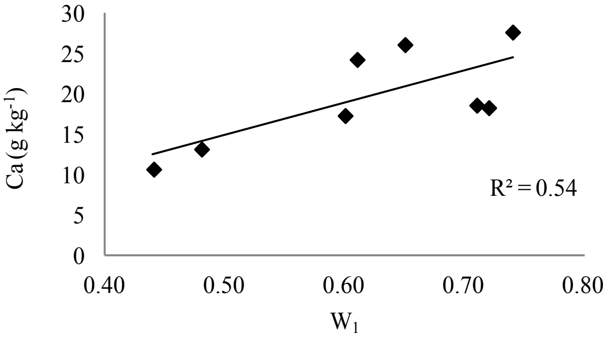


Fig. 10. Total concentration of calcium (g kg^{-1}) in peat-muck soils in relation to secondary transformation index (W_1) (Boguta *et al.* 2011)

This could result from increased HA/FA ratio because, due to transformation, "younger" compounds like lignins and fulvic acids are transformed to more mature compounds like HA (Kovaleva and Kovalev 2009). It could be favourable for forming not well-soluble bonds with calcium ions and, in consequence, for concentration of higher amounts of this element in soil. However, such simple correlation has not been noted for magnesium content.

5.4. Interactions with heavy metals

The mechanism of chemical bonds creation in the case of interactions of humic acids with heavy metals ions may be much more complicated in comparison to elements from groups I and II of the periodic table, where bonding reactions with humic acids could be expressed by simple reactions of ion exchange. In the case of heavy metals, the most difficult aspect which complicates interpretation of studies results are strongly varied chemical properties of heavy metals, particularly valence, ionic radius, hydration radius, electronegativity. On the other hand, there are varied properties of different HA: molecular mass, degree of structure transformation, content of aromatic units, aliphatic chains or functional groups.

Among the most common mechanisms of interactions for this group of metals, reactions of complexation can be distinguished in the first place. In addition, other processes are also possible, e.g.: adsorption, coagulation and peptisation. Ion exchange is also possible, but to a smaller extent and it is an untypical process for heavy metals. In general, one can say that the adsorption processes occurring on solid HA molecules and complexation processes take place with participation of dissolved fractions of HA – compounds included in dissolved fraction of organic carbon called shortly DOC (*dissolved organic carbon*). Studies have proven that these two processes are determined by the presence of surface functional groups and, for example, for copper and zinc adsorption on HA, stability constants form similar row-like lines of the stability constants of complexes of these metals with HA (Ashley 1996). DOC fraction is generally recognised as that fraction which increases the mobility of metals in soil solution, whereas solid fraction of soil organic matter has usually immobilising impact (Römkens *et al.* 1999). Thus, DOC fraction is a very important factor in determining metal flow in aqueous-ground environment (Antoniadis and Alloway 2002, Tipping, 2002), affecting ion availability to plants. Release of soluble carbon compounds results from multiple factors, especially sorption and desorption processes, pH increase, temperature and water content, as well as ionic strength drop. Studies confirm that understanding of the influence of these factors on mobilisation of organic matter is a key to the explanation of interactions between metals and soil organic matter (Zhao *et al.* 2007).

Complexation processes

Complexation processes of heavy metals assume different mechanisms:

- Complexation in which radical of humic acid is connected by one coordinate bond with central ion (metal).
- Complexation reactions with creation of chelating connections. Radical of humic acids in compounds of such type is connected with central ion by several coordinate bonds. According to Kononowa (1966), in this case metal ion is bonded to two functional groups located closely in the structure of humic acids. Compounds of such type have a significant influence on the podsolisation and weathering processes.

The distinction between complexation processes and ion exchange may be carried out on basis of changes in pH (drop of pH may be an indicator of complexation), solubility, oxidation potential, electrical conductance or chemical behaviour of analysed system. Inability of exchangable cations such as Ba^{2+} or K^+ to replace all Cu^{2+} and Zn^{2+} from HA can be an evidence of complex formation.

Complexation reaction of heavy metal is gradual. Ligands, in turn, bind to central atom, forming series of intermediate complexes, leading to the formation of coordinately saturated complex. Complexation reactions of humic acid with metal ions leading to the formation of simple complexes and chelates are based on displacement of hydrogen ions from functional groups of HA (HA):

$$M^{n+} + HA \rightarrow MA^{n-1} + H^+$$
$$MA^{n-1} + HA \rightarrow MA_2^{n-2} + H^+$$
$$M^{n+} + HmA \rightarrow MA^{n-m} + mH^+$$

The complex chemical structure of humic acids provides great potential for varied ways of metal ions complexation. In connection to the above, among studies conducted until now there are different findings concerning the ratio of ligand to central ion in the complexation process. Most of the researches rather based on the assumption of reaction model of metal and functional groups of humic acids in given proportions (Stevenson 1994, Tipping 2002). According to Senesi (2001), major complexing sites for metal ions are salicylate- and phtalic-type sites in 1:1 complexes. However, metals can also coordinate two or more particles of humic acids forming 2:1 complexes or chelates. According to Świderska-Bróż (1978, 1985), complexes of heavy metals with humus substances are in a large part multicore chelates. Ashok *et al.* (2000), on the basis of their studies, reported that at pH 3.5 1 mole of humic acid complexed 1 mol of Cu and 1 mol of Fe, 2 moles of Ni, Co, Cd, Mn, Zn and Mg, whereas 4 moles of humic acid complexed 5 moles of Pb and 3 moles of humic acid complexed 4 moles of Ca. They mentioned two mechanisms of interactions of humic acids with metal: by COOH+OH groups or only by COOH. Complexes formed at ratio 1:1 are more stable than 1:2.

Chelate compounds of humus substances with elements like copper, iron, manganese or zinc can cause, in particular conditions, an increase of solubility and availability of these metals for plants (Clemens *et al.* 1990, Havlin *et al.* 1999). Chelate compounds “hold” metal in soil solution and, in consequence, it can easily diffuse to plant roots. This is probably due to special ring structure of chelate compounds, in which metal ion is surrounded by a molecule like for example humic compound. Thus, metal cations are protected against reactions with other components of soil (Brady and Weil, 1999), and after reaching the surface of root can be released from chelate structures and penetrate into the root or penetrate in the form of chelate and disintegrate inside the root. In both mechanisms, organic molecule is "returned" to the soil solution and can chelate next cations of metal. An example of chelating reaction can be presented as interaction of iron ions with humic acid molecule, which can be written as in Figure 11:

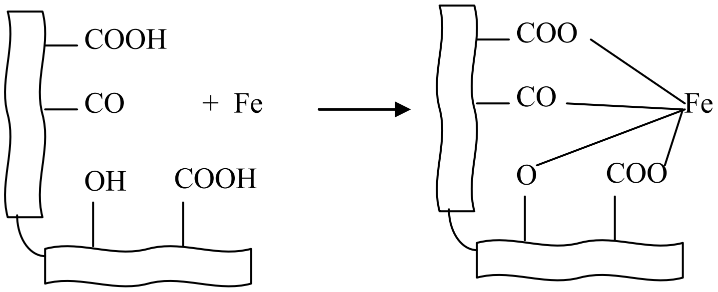


Fig. 11. Scheme of chelation mechanism of humic acid molecule by iron (III) ion

Processes of chelation are especially important for soils with high and even alkaline pH. It is commonly known that metal availability is significantly reduced in such conditions, and in consequence deficiencies of micronutrients for plants are observed. Chelating compounds can increase the mobility of metals by forming soluble complexes. Chelation processes in such soils can be improved by addition of chelating agents like EDTA or by increasing organic matter concentration. Above studies show that knowledge of chelating mechanisms can have a positive impact on intentional increasing of mobility of micro- and macronutrients required for plant growth. On the other hand, according to Janowska and Szymański (2005), chelating complexes as well as metals associated in another way with organic matter or precipitated on the surface of soil particles and built into the structure of minerals can be in some conditions difficult to assimilate by plants. Accordingly, if a large amount of humic acids is formed during decomposition of organic substance, sorption of metals by plants could be weaker. Erdogan *et al.* (2007) note that due to the presence of functional groups in humic substances they are important in metal trapping in the environment. Cation complexation by humic substances could reduce bioavailability. The increasing level of heavy metals in the environment is connected with a serious threat to human health and living resources.

There are few but differing answers in professional literature concerning the mechanisms of interaction of functional groups of humic acids with heavy metal ions. Researchers consider the following possibilities of binding of heavy metals:

- **Functional groups containing oxygen.** It is believed that the main factor responsible for the binding of metals by humic acids is the presence of a number of surface, oxygen-containing functional groups like carboxyl, phenolic, phenyl, hydroxyl, ketonic or enolic groups (Kowalkowski and Buszewski 2002). However, many scientists say that mainly carboxyl groups of humic acids are responsible for the processes of connection with metal ions. There are also reports of a complexing effect of both carboxyl and hydroxyl functional groups (Erdogan *et al.* 2007), but opinions are still divided. Oxygen atom fulfil in this case the role of ligand atom. Metal complexes with carboxyl groups are more stable than complexes with weakly acidic phenolic groups (Senesi 2001).
- **Nitrogen atoms in humic acids.** Some researchers also suggest the possibility of metal contact with humic acids by nitrogen atom (Alberts and Philip 1998, Senesi *et al.* 1987), for example in amine or amide functional groups. Despite the fact that this element is present in small quantities in the structure of humic acids (~ 3%), its contribution to complexation processes may be important because, according to Pearson's theory, complexes formed e.g. between copper ions and nitrogen atoms should be more stable than those formed in combination with the oxygen atoms of carboxyl groups (Perdue 1998).
- **Chemical connections through sulphur atom.** Mainly there are functional groups which contain this element, like sulphides and sulfhydryl groups (Kowalkowski and Buszewski 2002).
- **Direct chemical connections with humic acid carbon.** There are some news that heavy metals can be bonded directly to carbon atoms in chains or aromatic rings of humic acids, through very strong and stable bonds. In consequence, difficult-soluble metal-organic compounds are formed and in this case metals can be accumulated in soil (Świderska-Bróż 1985).
- **Organic-mineral compounds**. Metal ions can play the role of bridges in complexes of humus compounds with clay minerals.

The Irving-Williams series shows the order of stability of complexes with different metals:

$Pb^{2+} > Cu^{2+} > Ni^{2+} > Co^{2+} > Zn^{2+} > Cd^{2+} > Fe^{2+} > Mn^{2+} > Mg^{2+}$

Van Dijk (1971) created a similar order for meta-humic complexes acid at pH 5:

$Zn^{2+}>Fe^{2+}>Ni^{2+}>Co^{2+}>Mn^{2+}>Mg^{2+}>Ca^{2+}>Ba^{2+}$

According to Świderska-Bróż (1985), the sequence of entrance energy of a given metal to complex with humic acid could be as follows:

H>Pb, Cu, Fe^{3+}>Zn, Fe^{2+}>Ni>Co>Mn>Mg>Ca>Ba.

Energy of exit from complex of humic acids is identical but in reverse direction. From above point of view it could be concluded that humic acids have a significant influence on the dynamics of magnesium and calcium in soils and in waters.

Above orders were also confirmed with small changes depending on the conditions of studies by different scientists working with different humic acids. Stability of creating complexes is also in great part determined by the kind of functional groups, or more precisely by donor atom which takes part in forming a coordinate bond. The most important chelating functional groups in order of decreasing affinity for metal (Figure 12) are:

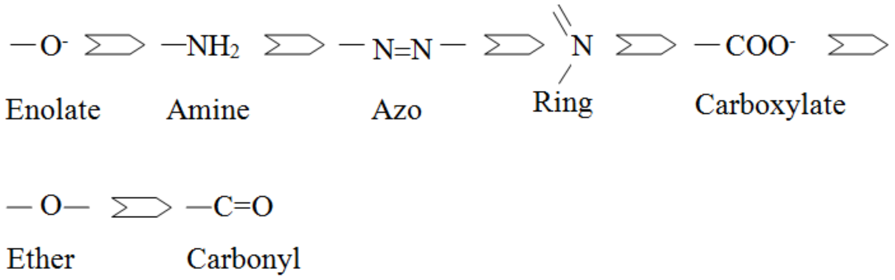


Fig. 12. Chelating groups of humic acids

Other donor functional groups may also be: sulphonic acids (-SO_2OH), phosphoric acid (-$PO(OH)_2$), hydroxyl (-OH), sulphydryl (-SH) groups (Schnitzer and Khan 1972).

Processes with solid phase participation: adsorption and coagulation

One of the mechanisms of interactions of heavy metals with humic acids on the border of solid and liquid phase is coagulation. Colloidal systems of humic acids are very unstable because the surface of dispersed molecules is well-developed and molecules tend to decrease the specific surface area through coagulation and then flocculation. In general, coagulation process is stimulated by the addition of electrolytes, different fillers (strongly crumbled solid phase), increase of temperature and chemical reactions. Stability of colloidal system of humic acids is determined by repulsion of particles, which can be expressed by zeta potential. Coagulation process is accelerated at decreasing potential and at so-called critical potential quick coagulation takes place. Zeta potential also depends on electrolyte addition, and more precisely on the charge of electrolytes.

Interaction of humic acids with metal ions, leading to coagulation of organic molecules, is important in ecology. Thanks to above process, it is possible to remove humic acids and their complexes with heavy metals, but also other organic contaminants from water. The degree of removal of metals forming connections with organic molecules is directly proportional to the amount of removed organic ligands.

It is commonly known that trivalent ions are more effective in such processes than divalent and than monovalent metal ions. This is in agreement with Schulze-Hardy rule. Similar bahaviour is noted for anions. Sulphates coagulate more effectively than for example chlorides and nitrates.

The general rule for calculation of critical concentrations of metal for starting coagulation is that the critical concentration of metal is inversely proportional to the sixth power of metal valency. So ratios of metals with different valency are as follow:

$$(1/1)^6 : (1/2)^6 : (1/3)^6$$

When valency is the same, coagulation is dependent on ionic radius and is more effective for cations with bigger radius.

In the case of interactions of humic acids with metals, two pH-dependent coagulation mechanisms can be distinguished:

1. Electrostatic interaction of positively charged coagulant particles with negatively charged sites of humic acids

This mechanism predominates at pH less than 6. Under these conditions, almost all of carboxyl functional groups of humic acids are dissociated and give negative charge on the surface. Hydrolysis products of coagulants are positively charged. Thus effectively coagulation is possible through the system destabilisation on the way of electrostatic interactions. This process is characteristic for low doses of coagulant. High efficiency of coagulation by using copper ions at pH 5 for removing humic acids described by different degrees of humification, different aromaticity and sorption capacity, was confirmed by studies of Boguta and Sokołowska (2013). However, it should be stressed that in the case of each coagulation test (different humic acids and different metal concentration) very small part of the copper and humic carbon stayed in the liquid phase after coagulation, which could indicate the presence of dissolved complex compounds or free copper ions and not complexed, dissociated humic acids.

2. Adsorption of humic acids on precipitated metal hydroxides

Increase in pH from neutral to alkaline can lead to the formation of metal hydroxides such as iron and aluminium and, in consequence, adsorption will be the process that will dominate and determine humic acid removal. This mechanism dominates at high doses of coagulant. Świderska-Bróż (1984), on the basis of her studies, proposed three characteristic pH ranges related to humus acids removal:

- pH 2-5: precipitation of humic and hymatomelanic acids
- pH 5-9: low efficiency of coagulation, part of molecules is present as difficult-sedimenting particles
- pH > 9: high efficiency of coagulation

Studies at pH higher than 11 enabled to remove additional amounts of humus compounds.

Interactions of selected heavy metals with humic acids

In recent years chemical connections of heavy metals with humic acids become an increasingly important environmental problem. This is due to the fact that once these metals were present in trace amounts, and their presence was not so dangerous for the environment. However, continuous development of economy and industry caused that the amount of heavy metals emitted to environment increased dramatically. It becomes very important to know the mechanisms that exist between heavy metals and one of the most reactive fractions of the soil environment i.e. humic acids. Therefore, this chapter provides an overview of research on the interactions of selected heavy metals with humic acids.

COPPER

Copper occurs in soil in the form of simple divalent cations Cu^{2+}, but also as a series of complexes, mainly also divalent. Monovalent forms are rather rarely met. Transformations of divalent copper form to monovalent copper and vice versa are possible at participation of oxidation reactions of phenols in the presence of phenolases (Szajdak 2002). Copper in soil stays mainly in combination with organic substance and clay minerals. Copper sulphides and carbonates are insoluble forms that are retained in the soil profile.

It is believed that humic acids bind copper by sulphydryl, carboxyl, phenyl and hydroxyl groups (McBirige and Martinez 2000). The use of FTIR spectroscopy showed that the major contribution to interaction with copper ions is that of carboxyl groups of humic acids. Schmitt *et al.* (1996) found that binding of copper to humic acids occurs in a variety of active sites. During studies of humic acids flocculation they obtained results indicating participation of carboxyl and phenolic functional groups. It is also interesting that at low concentration of copper (before the flocculation started), changes in the electrophoretic properties were observed, in contrary to titration of humic acid by iron (III) ions wherein the electrophoretic mobility of organic compounds did not undergo significant change. According to Perdue (1998), determination of capacity of humic acids for complexation of copper ions by carboxyl groups often lead to erroneous estimation because sediment can occur at a time when not all of the carboxyl groups are complexed. Similar conclusions were presented by Plaza *et al.* (2005c). Complexation capacity of humic acids for Cu (calculated from fluorescence spectra), were 2-5 times lower than it could result from the content of functional groups. The reason could be that some of the functional groups were unavailable for copper due to undissociated protons (competitiveness of H and Me) or due to electrostatic effect consisting in the fact that copper ions complexed in one place reduce the complexation capacity of neighbouring groups for complexing other copper ions. One of the reasons for lower capacity may also be the presence of bonds in which Cu (II) is bonded with more than one carboxyl or phenolic group, for example by forming bidentate complexes or 2:1 complexes in which copper acts as a bridge between macromolecules of humic acids. Complexation capacities determined from studies did not correlate well with such humic acids properties as: content of functional groups C, H, N, S, O, C/N, C/H, O/C, total acidity, COOH, OH, E_4/E_6. This suggests that complexation capacity is affected not only by the functional groups but also the whole chemical structure, spatial factors, aromaticity and degree of humification can have an influence on complexation capacity.

Moreover, it was proved that complexation of metal ions can also take place through nitrogen atoms because, according to HSAB theory (hard and soft acids and bases), these atoms can create very stable bonds with copper ions. Similarly, copper can be bonded by functional groups of humic acids which contain sulphur (Evangelou *et al.* 2002). Interactions of copper with humic acids can be with participation of such processes as adsorption, occlusion, precipitation, chelation or creation of simple complexing compounds.

Bonding of copper by humic acids is dependent in a great degree on pH, humic acid concentration, form of humic acid, and can lead to the formation of both mobile and insoluble compounds. Cao *et al.* (2004) reported that copper forms the most stable complexes when it is at low concentration in relation to humic acid concentration, because firstly the places with the strongest sorptive properties are occupied. When these places are saturated, copper is bonded to weaker complexing groups and these groups become an important factor which determines stability constants of the complex. The influence of copper/humic acids concentration ratio was also studied by Lu and Johnson (1997). The results of their studies showed that the complex was very sensitive to pH changes and did not exist above pH 7 (precipitation) at humic acid:copper ratio of 0.003. It was complex HS-Cu-H_2O. At this ratio, about 60% of copper was complexed whereas the rest of copper stays in solution as Cu^{2+} (pH 3-7). Above pH 7 copper hydrolysis and complex is precipitated. Adsorption processes become dominant with participation of copper hydroxides. Ratio HS:Cu = 0.6 leads to the formation of complex resistant to pH till pH 10. It implicates that at low concentration of humic acid, percent of created complex decreases with increasing pH, and above pH 7 complex stops to exist and at higher humic acids concentration complexes have higher stability and are the dominating form in the solution till pH 10. According to Plaza (Plaza *et al.* 2005 b,c), complexes of humic acids with copper ions can change the electron structure depending on the metal amount. It was proved by studies with application of fluorescence spectrometry. Fluorescence intensity decreased in studied humic acids during copper ions addition, and maximum of emission was shifted toward lower wavelengths. Maximum of wavelength of excitation did not change. It could be evidence of electron reorganisation in the structure of forming complexes.

Above results indicate that, apart from humic acids and metal concentration, interactions of humic acids with metal are strongly affected by pH. Figure 13 shows the scheme of copper ions forms in relation to pH.

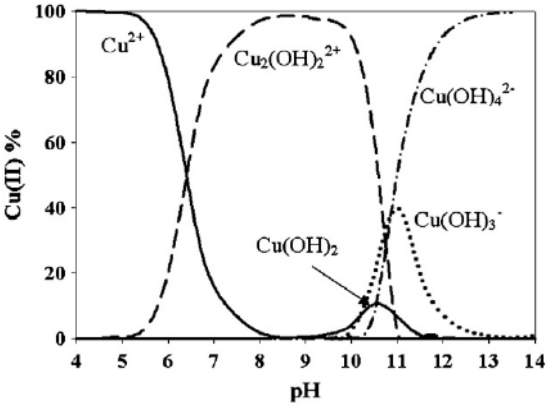


Fig. 13. Distribution of copper (II) ions forms in the function of pH, at Cu concentration 10^{-2} M and ionic strength 1 M (Nosrati *et al.* 2012)

Studies show that at pH 4 and 5 almost 99% of copper is bonded by clear humic acids and humins. Reaction of bonding is quick and bonded copper can be recovered in 90% by extraction using 0.1 M HCl. Alleoni *et al.* (2001) reported that copper was totally adsorbed on soil at pH 7.5 without and with incubation (4 and 12 weeks), whereas at pH 4.5 adsorption of copper was lower without incubation. Adsorption for incubated samples was almost total, independent of the surface charge or pH. Gondar *et al.* (2005) claim that copper bonding by humic acids increases with increase in pH as a result of stronger electrostatic interactions connected with higher charge and lower Metal-Proton competitiveness at active sites. Their results additionally showed that copper is bonded stronger to humic acids than to fulvic acids, independent of pH. Results of interactions were soluble complexes. Studies of Iglesias *et al.* (2001) showed that, in relation to pH, copper ions can compete with others elements, for example with calcium cation, for sorption places of humic acids. Calcium binding was dominated by carboxylic functional groups, whereas copper binding was connected with the presence of carboxylic and phenolic groups. Carboxylic groups played a significant role at high concentration of copper and at low pH, whereas phenolic groups were important at higher pH. So, competition of calcium and copper ions at lower pH (5.5) was stronger than at higher pH (6.5). Studies of Karczewska (2002) inform also about increasing solubility of copper compounds at strongly alkaline pH. Above case could be related also to other metals which in alkaline environment can create negatively charged complex ions of $M[OH]^{n-}$ type, complex connections with ammonium ions and with low-molecular organic particles.

Cao *et al.* (2004) used different instrumental methods: ISE, ASV and FQ for the study of complexation of copper ions by organic ligands. The binding constants of copper for humus substances, including humic acids from various sources, were calculated using a discrete single site model. The scientists noted that stability constants of humic acids-Cu complexes (logK) increase with an increase in pH (ion strength of 0.1 N), due to increasing dissociation of functional groups. Increasing negative charge produces electric field attracting copper ions, and simultaneously the competitiveness level of protons and copper ions is decreased causing growth of stability constants of complexes. Increasing ionic strength causes a decrease in the ability of copper for binding with humic acids. It was well seen at pH 4, when the log k values decreased with increase in the supporting electrolyte concentration and total copper ion addition. Complexation in one place of humic acids structure decreases the tendency for complexation of metals in neighbouring functional groups of humic acids. Thus, condensation of opposite ions in double electric layer increases and this phenomen also causes a decrease in ability for complexation. In consequence, the higher the ionic strength, the weaker the complexation processes of metal by humic acids.

In general, dissociated soluble forms of humic acids create with copper ions and other metals soluble and highly-mobile complex compounds, while humic acid particles suspended in solution can adsorb various substances on their surface. Solubility of the created compounds is additionally determined by pH, ionic strength, and number of ion-exchangeable active sites (Plaza *et al.* 2005c).

Copper interactions with humic acids can also undergo coagulation. Boguta and Sokołowska (2013), in their study carried out at pH 5, showed that this metal has a strong affinity to humic acids characterised by a wide range of physicochemical properties. The results indicated that low doses of copper caused complexation by humic acids and creation of water-soluble chemical connections. At the higher doses, coagulating copper concentration was found for studied humic acids. These concentrations were not identical for humic acids having different physicochemical properties. Measurements of carbon concentration in series of solutions are presented in Figure 14 as a function of organic carbon in liquid phase vs. amount of copper concentration in each solution in series. The process of coagulation of HA under copper (II) ions was also investigated by measurements of absorbance at 465 nm in liquid phase after 24 hours from copper addition. These results are presented in Figure 15 as a graph of copper concentration remaining in the liquid phase in the function of copper amount introduced at the start.

On the basis of the relationships shown graphically in Figure 14, critical copper concentrations affecting coagulation have been determined: C_{crI} and C_{crII}, which marked the beginning and end of coagulation, respectively. Low correlation coefficients were found between these parameters and sorption properties of HA and they were statistically insignificant. However, the research covered a small number of samples and more extensive studies on this subject are necessary for better evaluation of these relationships.

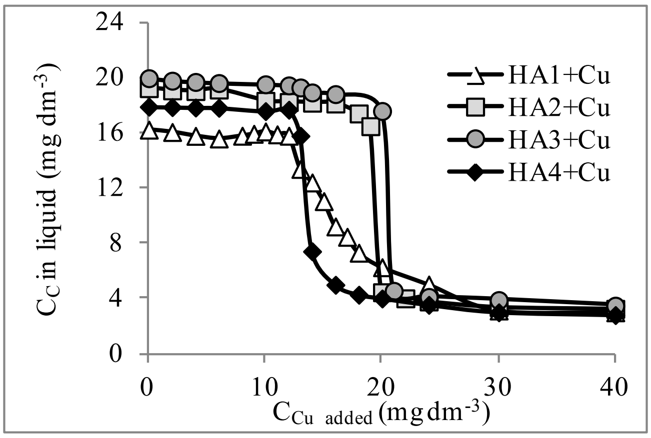


Fig. 14. Humic carbon content (mg dm^{-3}) in liquid phase in function of copper amount introduced initially (mg dm^{-3}) for systems HA-Cu at pH 5 (Boguta and Sokołowska 2013)

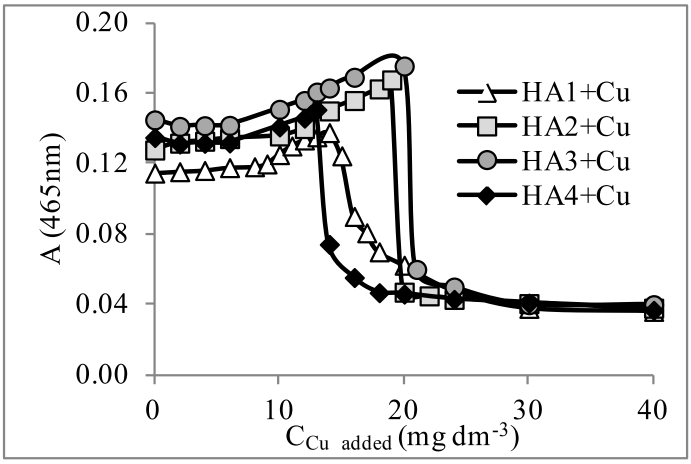


Fig. 15. Absorbance values of solutions of humic acids at pH 5 with increasing copper concentrations in function of copper amount (mg dm^{-3}) added to the solution at the start of experiment (Boguta and Sokołowska 2013)

IRON

Iron (II) and (III) can be complexed in soil solution by the soluble humic acids fraction. Soluble and mobile chemical connections are formed in this case. There is also a possibility of creation of not well soluble compounds, resulting in the accumulation of iron and organic compounds, mostly in upper layers of soil. Studies have shown that the mobility of iron and manganese is strongly dependent on the redox conditions in the soil environment. The solubility of iron decreases with the intensification of the processes of oxidation and increasing of pH. Furthermore, Fe(II) can be oxidised, and Fe(III) can be reduced. Reduction processes of iron by soluble organic matter can be catalysed by light or temperature (Emmenegger *et al.* 2001). Some studies have also confirmed the reduction of iron in the presence of fulvic acids without access to light (Voelker and Sulzberger 1996, Pullin and Cabaniss 2003). Reductive processes promote dissolution of hydrated iron and manganese oxides, and transition of metals to soil solution in the form of Mn^{2+} and Fe^{2+}. In reducing conditions, other metals previously occluded in hydroxyoxides can also be released to solutions. However, in strongly reducing conditions, metals can undergo chemisorption to difficult-available forms such as CuS, MnS, CdS. The dependence between the mobility of iron and redox conditions and pH is usually used in the process of coagulation of humic substances.

Coagulation by iron ions

High affinity of iron ions to humic acids is used in the processes of coagulation and flocculation of humic acids. The application of coagulants, both ferric and aluminium, is associated with the fact that iron charge in natural pH of waters is positive, so there are possibilities for interactions with negatively charged surface of humic acid. However, the main disadvantages of these coagulants are the danger of formation of colour and water-soluble complexes with humus compounds at low doses of iron or aluminium coagulant or at improper pH (Kaleta 2004). Schnitzer and Khan (1972) reported that excess of humic acid added to a solution of iron (III) or aluminium can cause some solubilisation of Fe^{3+} and Al^{3+}.

Generally, the problem of humic acids removal from water by application of Fe-coagulants has still much controversy as to whether humic acids coagulation occurs as a result of charge compensation of humic acids by iron ions (Cheng and Chi 2002, Jung *et al.* 2005, Schmitt *et al.* 1996, Vilge´-Ritter *et al.* 1999) or humic acids are precipitated from the liquid phase by adsorption on colloidal precipitated hydroxides of iron (Tseng *et al.* 1989, Ying *et al.* 1988). These two processes look very similar. Studies of Krasner *et al.* (1995) show that coagulation of humic acids due to addition of iron ions is possible only when a significant part of humic acids is complexed. Similar conclusions were presented by Lippold *et al.* (2005, 2007). According to those researchers, colloidal stability of humic acid is determined by the ratio of Fe(III) /humic acids, and the concentration of Fe necessary for flocculation depends on the concentration of humic acids. Agglomeration and flocculation take place when negative charge of the colloid humic acid is compensed. So, iron ions are needed for flocculation in higher amount at pH 6 than at pH 4 since at higher pH a greater amount of functional groups of humic acids is deprotonated. In addition, at higher pH, charge of iron is reduced due to the hydrolysis of iron ions. Flocculating humic acid simultaneously with adsorbed iron can also cause adsorption of other metals from the solution.

Coagulation of humic acids based on prior complexation by iron (III) ions is also confirmed by the results of Cheng (Cheng 2002). According to that author, removal of humic acids is effective at pH of 4-7 because there are more functional groups dissociated on the surface of humic acids and more iron (III) ions are present in the solution with a high positive charge. pH conditions below 4.5 do not belong to optimum ones, since a small amount of Fe-HA complex alters its charge to the opposite, protecting complex against removal from the solution, while simultaneously humic acids also dissociates protons weakly in this pH. At high pH the amount of coagulant required to remove of humic acids is more, since both the humic acid is better dissociated and enlarges more negative charge, as well as iron loses its positive charge by transforming into hydroxide forms. Of course, humic acid particles may be still neutralised in such circumstances, but to a weaker degree, and negative charge is not saturated to the end. In addition, at high pH, apart from creating $Fe(OH)_3$ particles, negative hydroxy ions $Fe(OH)_4^-$ are also produced and these ions prevent iron combining with humic acids. Aguiar *et al.* (1996) examined coagulation of organic pollutants from waters using iron chloride (III) as coagulant. They determined that the optimal pH was between 4 and 5. In the case of necessity of obtaining the lowest iron amount remaining in water after coagulation, it was determined that the optimum pH was in the range of 5.0-6.0. Removal efficiency of humic substances that could be obtained was in the range of 60 to 80%. Strong dependence of binding of iron ions by humic acids in relation to pH was confirmed by studies of Weber *et al.* (2006). Their results show that the maximum of sorption was at pH 3.5. Below this pH, dissociated iron becomes dominant, and the amount of complexed iron drops. Above pH 3.5 iron precipitates (Weber *et al.* 2006). In another work, Lippold *et al.* (2005) describe interaction of humic acids with iron as a chemical binding of iron in polycore form to humic acids molecules. Polystructural iron is degraded with time and more and more acidic places of humic acid are occupied by iron breaking down onto smaller particles (it is proved by increase in pH after a while). The presence of intermolecular forms of iron is the reason of variable amount of iron in complexes with humic acids and the lack of regularity between content of iron and cation sorptivity (Pastuszko 2007). According to Lippold *et al.* (2007), the process has the character of charge compensation because colloidal iron (III) at pH 6 should flocculate, whereas iron (III) ions in the presence of humic acids excess do not become destabilised. Those authors excluded the possibility of Fe (III) reduction to Fe (II) under the conditions of their experiment.

According to some studies, the dominant process of humic acids coagulation in the presence of iron ions can be adsorption on colloidal forms of metal. Among others, Warren and Haak (2001) and Allard *et al.* (2004) report that it is possible to create colloidal iron forms having a high influence on the mobility and bioavailability of other pollutions in soil, especially organic compounds. The process of coagulation by iron ions is the most effective in pH range of 4-6.

Coagulation of humic acids by iron ions is most often carried out with the use of simple compounds of iron, such as iron (III) chlorides, and also polymeric iron forms, like polysulphates of iron (III) (Cheng 2002).

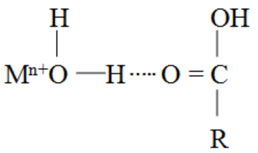
In natural conditions, especially in drained soils where the amount of clay admixtures is insignificant, humus compounds are almost always connected with multivalent cations. In mucks, as a result of drainage, iron is present mainly in the form of hydroxides and this metal creates complex connections with organic substance (Pastuszko 2007). Studies of Piaścik (1996) show that iron is very strongly bonded in muck layers and much more weakly in peat (1.5-3 times). It is evidence of intensive redox processes as well as intensive mineralisation and accumulation of iron by humus compounds, the amounts of which are much higher in mucks than in peats. These results showed that mobile iron was only 2-7% of its total content. Gondek and Filipek-Mazur (2004) also proved that high-molecular humus compounds produced during composting process have a significant influence on iron accumulation. However, those scientists also claimed that iron (III) with humus compounds can form, in some conditions, complexes with low stability constants and, in consequence, precipitation of iron is prevented in oxidative conditions and simultaneously this metal is quite easily sorbed by plants.

Organo-mineral connections with humic acids

Results of some studies proved that iron can also be included in organomineral connections with humus compounds and clay minerals. These connections are still weakly investigated. There are some suggestions for probable mechanisms of their formation:

- Formation of “bridges” by ions: Fe^{3+}, Al^{3+}, Mg^{2+} and $Ca^{2+,}$
- Formation of Van Der Waals’ bonds,
- With participation of hydrogen bond,
- Through association with hydrated oxides.

First theories indicated the formation of above compounds by “bridges” from cations Ca, Al and Mg, and a possibility of penetration of humic acids particles to the crystal structure of clay minerals. This idea was not ideal because the diameter of humus compounds could be higher than distances in the crystal network of clay minerals. Newer, the theory says about the formation of “bridges” between clay minerals and humic acids with the use of trioxides of aluminium and iron. These bridges are formed from complex compounds: Fe-HA and Al-HA formed through interactions of nonsilicate form of trivalent metals with humic acids. Bonding with the surface of clay minerals is based on processes of dehydration and forming of intermolecular bonds. Relatively stable complexes of clay minerals and HA can be formed with participation of hydrogen bonding. So-called “water bridges” can link polar organic molecules (RCOOH) with exchangeable metal cations (M^{n+}) in the following manner:



$$M^{n+}\overset{\displaystyle H}{\overset{|}{O}} - H \cdots O = \underset{\displaystyle R}{\underset{|}{\overset{\displaystyle OH}{\overset{|}{C}}}}$$

Metals in organo-mineral compounds are very strongly connected and they are difficult for extraction. These compounds in heavy soils can constitute almost 50% of total content of humus compounds, contrary to simple soils: humates and fulvates, the amount of which in soils is much more lower. Dominant factor which can determine interactions between HA and clay minerals are the properties of organic compounds, water content, chemical nature of cations on the surface of clay minerals, and properties of clay minerals.

MANGANESE

Manganese creates with humic acids complexing compounds. This metal can be in organo-mineral complexes as exchangeable ion on +2, +4, +6 and +7 degree of oxidation. Compounds containing manganese of high oxidation degree are usually not easily soluble (Szajdak 2002). In general, high moisture and low amount of air in soil favours the presence of reduction conditions in which manganese is on low degree of oxidation and is highly mobile in soil. In oxidation circumstances, Mn^{2+} is transformed to Mn^{4+} form, difficult-soluble in water and weakly available for plants. Studies show that manganese is weakly bonded by organic matter, contrary to others metals. Mobility of manganese is a consequence of a lot of factors, however pH increase causes decreases in solubility of manganese compounds. Exemplary distribution of stability of different manganese forms is depicted in Figure 16.

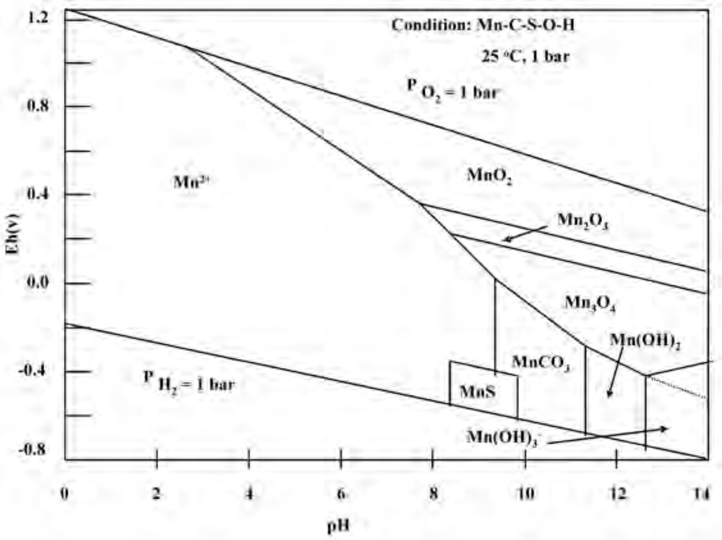


Fig. 16. Relationship Eh-pH of stability of different manganese forms (Adriano 2001)

Manganese, similar to copper, iron and zinc, can form chelate connections with humic acids (Dobrzański and Zawadzki 1995, Kaleta 2004). In this way, metal loses its ionic properties and, as a chelate, does not easily react with other soil components. As a result of this, manganese is protected from precipitation and can be easily sorbed by plants. Binding of manganese by humic acids has a stoichiometric nature, which was proved in studies of Ghabbour *et al.* (2006). In this case, neutarlisation of carboxylic functional groups took place. Similar conclusions were proposed for interactions of humic acids with iron (III) and lead (II). Participation of carboxylic –COOH, and hydroxyl groups –OH in heavy metal binding is also confirmed by Chassapis *et al.* (2009). According to those authors, binding of manganese as well as lead, cadmium, copper or zinc by humic acids takes place at main participation of carboxylic groups and, in weaker degree, participation of OH groups. The remaining ligands present in the structure of humic acids have a lower importance. The authors report that manganese indicated the weakest properties for complexation among all studied metals. However, during the experiment a decrease of pH after addition of each metal was observed. It was evidence of complexation processes. Analyses of potentiometric titration curves of humic acids-metal systems showed that manganese complexation was the weakest (sigmoidal potentiometric curve). Potentiometric titration curves with 2 degree of inflection were recorded for other metals, which suggested the presence of more than one way of complexation. Manganese can also form organo-mineral compounds (Kaleta 2004).

Forming of complexes compounds with humus molecules as well as weak chemical affinity to humic acids cause that manganese displays great mobility in organic soils in comparison to other multivalent metals like aluminium or iron. For this reason manganese ions can be easily exchanged by others and its concentration in soil can decrease. High mobility of manganese is additionally conditioned by pH. In acidic organic soils, this element is present in form Mn^{2+}, therefore its amount in soil could be low. An exception to this could be the surface layer of soil, where oxidising processes take place and manganese can have higher local concentration (Kalembasa and Pakuła 2008).

In studies of Boguta *et al.* (2011), the amount of manganese in peaty-muck soils decreased with increase of secondary transformation degree. This relationship is presented in Figure 17.

It could result from transformation of humus substances during secondary transformation processes. According to literature (Szajdak *et al.* 2007), increase of secondary transformation degree causes a decrease of nitrogen and organic carbon amount in ground waters, which could result from the fact that under secondary transformation processes organic matter is changed from hydrophilic to hydrophobic (Sokołowska *et al.* 2005). These changes are a consequence of humus substances transformation which goes toward the formation of humic acids that complex manganese ions weakly. As a result, the amount of manganese can decrease in soil.

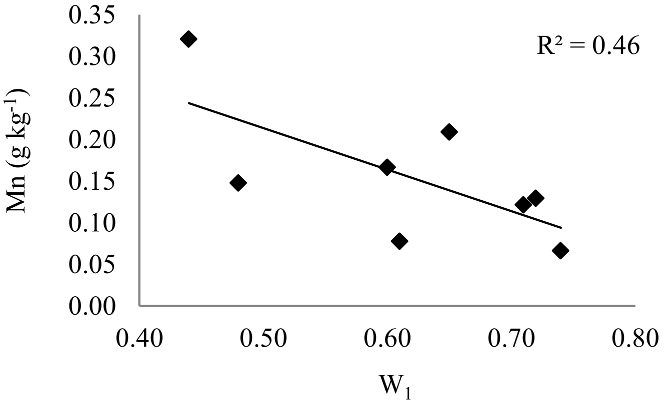


Fig. 17. Total concentration of manganese (g kg^{-1}) in peat-muck soils in relation to secondary transformation index (Boguta *et al.* 2011)

LEAD

Lead in the soil environment is present mainly as galena (PbS) and pyromorphite [Pb5(PO4)$_3$(Cl, OH, F)]. Lead is weakly mobile and weakly available for plants in crystaline form. Changes of redox conditions can cause transition of lead to more mobile forms and, in consequence, migration to the soil solution and pollution of waters (Templeton *et al.* 2001). Studies confirm also a relationship between the presence of lead and the amount of humus substances of soil. The highest concentration of this metal is present in top layer of soil, which can indicate lead binding by humus substances. Lead in soil has three main forms: as Pb^{2+} ion and as complex ions $PbOH^+$ and $Pb(OH)_4$. Intensive sorption of this element can be also connected with binding on the surface of hydroxides of trivalent metals and by clay minerals. In acidic soil, lead is present in the form of simple cation and partly is also bound by organic compounds forming complexes. Soil organo-mineral complexes with lead are very stable, especially at pH about neutral (Dobrzański and Zawadzki 1995). Above pH 6.5 lead can be accumulated and the main factors which determine this phenomenon are processes of precipitation of lead carbonates and phosphates (Dziadek and Wacławek 2005). In turn, Świderska-Bróż (1999) reports that complexes of humus substances with lead or copper at pH values below 8.0 increase the degree of humus substances removal. Stabilisation of humus compounds was observed at alkaline pH ranges, and the process of pollutants removal was inhibited in some degree.

ZINC

Zinc ions, like copper ions, are mainly present as divalent simple cations as well as negative or positive complexed ions.

Studies indicate quite a strong influence of organic matter on zinc ions. These interactions lead to the formation of stable and weakly mobile compounds which are accumulated in the top layer of soil. However, it is possible that the influence of zinc on humic acids can be dependent on humic acids concentration. Vaughan and McDonald (1976) showed that addition of humic acids slightly inhibited Zn uptake by beet roots cut in discs when HA concentration exceeded 25mg/dm$^{3.}$ Lower concentration did not affect Zn uptake. It could be evidence of formation of complexes with low mobility in soil. Decrease of toxic effect of zinc, cadmium and manganese by forming insoluble compounds with organic matter (1.2% and 3.8%) was also proved by White and Chaney (1980). Studies of Dąbkowska-Naskręt (2001) also showed that significant amounts of zinc are associated with soil organic matter, particularly in the upper soil horizon where humic acids concentration is the highest. However, there are also studies stating other facts. Jalali and Takkar (1979) reported that the uptake of Fe, Cu and Zn by rice plant was enhanced by addition of organic matter.

Mechanism of interactions between humic acids and zinc ions is significantly determined by pH. According to Janowska and Szymański (2005), at low pH ranges, zinc ions, like other mineral connections and organic compounds, indicate in general a high mobility in soil. However, the presence of other soil components can cause accumulation of zinc ions even in such circumstances. Organic substance including humic acids, but also inorganic constituents such as sulphides ions or iron and manganese ions, binds zinc ions easily, causing that the metal is accumulated in upper soil horizon.

Increasing pH causes a decrease of zinc desorption. However, at alkaline pH, zinc can transform to anionic complex forms as well as to organo-mineral compounds, so its mobility can be increased in such cases.

CADMIUM

Cadmium is present on +2 oxidation degree in chemical compounds but it has a capacity of creation of different anionic and cationic forms. Cadmium is commonly met as complexed ions, for example $CdOH^+$, $CdHCO_3^-$, $CdCl^-$, $Cd(OH)_4^-$ and in the form of organic chelates. This metal displays high mobility in low pH ranges, especially at pH 4.5-5.5. Increasing pH causes that this metal comes into the composition of difficult-soluble carbonates (Dziadek and Wacławek 2005). Solubility of cadmium compounds is also decreased in the presence of phosphorous because this element causes the transition of cadmium to insoluble organic compounds, oxides and carbonates. At high pH, cadmium can form positive and mobile hydroxy ions $CdOH^+$.

Cadmium form, dependent on pH, affects also its connections with humic and fulvic acids. Complexes of this metal show high mobility and, together with other mobile forms of cadmium like simple ions Cd^{2+} or hydrated ions, are relatively easily sorbed by plants.

In general, the highest content of cadmium in polluted soils is observed for exchangeable fraction and in carbonates, whereas participation of oxides and organic forms of cadmium is lower than the content of other forms (Filipek and Domańska 2002).

Apart from surface negatively charged functional groups of humic acids, carbonates and phosphates, cadmium displays also affinity to other ligands like sulphydryl groups, and thus it can form stable connections with cysteine and proteins (Kabata-Pendias and Pendias 1999). In spite of low content of cadmium in Polish soils (0.03-0.22 ppm), the highly mobile forms of this element make this metal extremely toxic for plants and living organisms.

CHROMIUM

Chromium is found in soil on a number of oxidation degrees. The most popular are chromium (II) and chromium (VI). Lower oxidation degree is represented by hydroxides and oxides and at higher pH values: as mixture of hydroxides of chromium and iron. There are also inorganic salts of chromium such as chlorides or sulphates. Humus acids, including humic acids fraction, bind with chromium ions and form soluble complexes.

Chromium (VI) displays toxic properties what is especially dangerous because its compounds, both in alkaline and acidic environment, have high mobility. Humic acids, similar to clay minerals and hydroxides of aluminium and iron, can sorb ions of chromium present on +6 oxidation degree (as chromates VI) (Janowska and Szymański 2005). This process has a significant meaning in ecology because organic matter contributes in great degree to detoxification of very harmful chromates (VI) ions (Bednarek *et al.* 2004).

NICKEL

Nickel can be found in soil solutions in different forms: the simple cation form Ni^{2+} as well as complex ions with various charge: $NiOH^+$, $HNiO_2^-$, $Ni(OH)_3^-$,

$NiHCO^+$. In the presence of humus compounds, nickel is present as mobile chelate compounds. Solubility of nickel compounds increases with acidification, however in the case of organic soils, where nickel displays high affinity to humus compounds, nickel shows high mobility even at higher pH (Kabata-Pendias and Pendias 1999). Świderska-Bróż (1985), reports also that removal of nickel and zinc ions in the presence of humus compounds does not have any practical meaning due to the lack of metal binding to the insoluble chemical forms.

Results obtained by Zhou *et al.* (2005) showed that binding of nickel by humic acids is strongly determined by the presence of calcium ions which compete for exchangeable places of humic acids. Model studies assuming the presence of two types of binding sites in humic acids: carboxylic and phenolic functional groups, at both low and high pH conditions, confirmed that complexation of nickel ions can be inhibited by calcium ions. pH increase can decrease competitiveness of nickel and calcium ions. Increase of pH values favours also intensification of sorption of nickel on iron and manganese oxides. However, these connections can also be characterised by significant mobility (Dziadek and Wacławek 2005).

MERCURY

Mercury is bound by humus substances at low pH values whereas at higher pH the amount of available forms of mercury increases. Mercury vapours can also be sorbed on organic substance of soils. In this way, mercury can pollute the soil environment and, in consequence, sources of this element can originate not only from soil but also from water and air.

MOLYBDENUM

Molybdenum plays an important role in the soil chemistry, and together with humic substances determines the fertility of soil and quality of water. It is also a key element in the activity of nitrogenase. Interactions of molybdenum with humic acids depend in great measure on pH of soil environment. Increase of pH from acid to alkaline increases the mobility of molybdenum due to the fact of MoO_4^{2-}anion formation. In peats, this anion can be sorbed by electropositive soil colloids coming from aluminium and iron hydroxides (Szajdak 2002).

Mercê *et al.* (2006) assayed the affinity of solutions of salicylic acid models of humic and nitrohumic acids toward molybdenum ions by using electrochemical methods and UV-VIS spectroscopy. Their studies showed that in pH range of 6.3 to 8.0 complexed species of molybdenum existed in at least one form for all models of humic acids. The authors reported that previous studies showed also complexed species for a model with phthalic and nitrophthalic, and also humic and nitrohumic acids, however it worked only for pH below 6.5. The calculated formation constants showed that the substitution of the nitro group in the *orto* position was less favoured than in the *para* substitution, probably due to a steric hindrance in the former, which was clearly seen in the double substituted salicylic nitro derivative.

5.5. Interactions with selected metaloids

Studies showed that humic acids display also strong affinity to some elements of the "p" block of the periodic table which are not typical metals, but show some properties characteristic for metals.

Metaloids such as arsenic can easily bind with humic acids and can form chemical connections which reduce toxicity of these metals even up to 100%. Results of studies of Jones and Huang (2003) proved that high affinity of humic acids to arsenic ions can also have a positive meaning at immobilisation of other toxic substances in soil, such as pesticides which can contain arsenic atoms in their structure.

In turn, aluminium ions with humic acids can create both complexed and soluble chemical connections, as well as insoluble ones with colloidal character. The formation of soluble complexes of aluminium with humic acids is possible at a suitable ratio of individual components concentration. Zhou *et al.* (2005) studied complexation of humic acids by simultaneous addition of Al, Ni and Ca to humic acids. They wanted to simulate the competitive complexation. For this purpose they assumed the presence of two types of binding sites in humic acid, carboxylic and phenolic functional groups, at low and high pH conditions. Results showed that the diffuse-layer model (DLM) could fit the experimental data well under different experimental conditions. Ni and Ca ions strongly competed with each other for reactions with the humic acid but Al showed little influence on the complexation of either Ni or Ca due to its hydrolysis and precipitation at pH 5.

Humic acids, by forming colloidal complexes, decrease the toxicity of aluminium ions for aqueous microorganisms (Dobranskyte *et al.* 2006) as well as plants and soil microorganisms (Szajdak 2002). Dobranskyte *et al.* (2006) studied the influence of humic acids of waters on the presence of Al at neutral pH. Results showed that humic acids significantly reduced the loss of Al from the water and increased the fraction of filterable Al, although this was a relatively small fraction of total Al. The authors checked also the change of toxicity of Al for Lymnaea Stagnalis after interaction with humic acids. It was observed that toxicity was partly reduced as observed by a reduction in behavioural suppression, however addition of humic acids did not affect Al concentration in tissues. These results suggest that humic acids maintain Al in a colloidal form that is bioavailable to L.

stagnalis. However, these colloidal Al-humic acids species were less toxic since behavioural toxicity was reduced. Complexes of humic acids with Al can also reduce toxicity of this metal for fishes (Lydersen *et al.* 1990).

Aluminium ions are often used in coagulation processes of substances polluting waters and giving a colour or turbidity. Humic acids are included among such substances. The compound mostly applied in this purpose is hydrated aluminium sulphate [$Al_2(SO_4)_3 \cdot 18H_2O$]. The process of hydrolysis runs quickly at pH of natural waters and the products of hydrolysis are linked together, forming chemical compounds with different solubility, called hydroxycomplexes, polycations or hydroxypolymers. The main factor which determines the coagulation process is pH. At the range of pH 4-6, dissociation of carboxylic functional groups of humic acids takes place and simultaneously coagulant particles appear in the solution in the form of positively charged products of coagulant hydrolysis. In connection with the above, the removal of organic pollutants consists in precipitation during interactions with negatively charged functional groups of humic acids.

At lower pH, interactions relate to monomeric forms of aluminium, which can be described by the following equation:

$$\text{(HA)} + \text{aluminium monomers} \rightarrow \text{(HA – Al monomers)}_{\text{(solid)}}$$

On the other hand, at higher pH, coagulation is based on interactions with polymeric, hydrolysed forms of the metal (Dempsey *et al.* 1984):

$$\text{(HA)} + \text{aluminium polymers} \rightarrow \text{(HA – Al polymers)}_{\text{(solid)}}$$

Coagulation taking place with participation of negatively charged carboxylic groups of humic acids has a stoichiometric nature in relation to coagulant concentration and is similar to complexation reaction. If pH is closer to neutral, coagulation processes by both aluminium and iron ions are determined by adsorption of colloidal humic acids particles on the surface of metal hydroxides. The amount of coagulant needed to remove organic pollutants from the solution is also higher. It is a result of increasing dissociation degree of organic compounds as well as formation of polymeric metal forms. Coagulation is more effective when particles of coagulant are bigger, which results from their better adsorption properties in these circumstances. Huang and Shiu (1996), report in that the removal of organic pollutants is directly connected with molecular weight of humus substances and the results confirm stoichiometric relationships between initial concentration of humic acids and optimal dose of coagulant. At pH above 7, HA interact with hydrated forms of aluminium and coagulation reactions can be written as:

$\text{(HA)} + \text{Al(OH)}_{3\text{(solid)}} \rightarrow \text{(adsorption)}$,

$\text{(HA–Al polymers)} + \text{Al(OH)}_{3\text{(solid)}} \rightarrow \text{(adsorption)}$.

Removal of humic acids by aluminium coagulants causes a decrease of total carbon concentration in water by about 50%. Due to better removal of particles with higher molecular weight, the use of oxidation before coagulation processes is pointless because splitted particles are more difficult to remove from water and there is a possibility of THMs formation (Turkiewicz 2005).

5.6. Factors affecting interactions of humic acids with metals

Direction and kind of interactions of humic acids with metal ions, as well as kind of chemical bonds, stability of created connections or degree of saturation by metal cation are dependent on a number of factors. The relationship between the amount of metal adsorbed and the composition of soil is due to solid phase heterogeneity and influence of soil solution chemistry (Appel and Ma 2002, Diatta *et al.* 2003, Zhou De-Zhi *et al.* 1991). Thus these processes are determined by properties of metal and properties of humic acids as well as by external conditions like pH, temperature or ionic strength. This last group of factors can be controlled in some degree. In consequence, the above processes and factors and the kind of compounds formed in particular conditions determine the mobility of soil components, development of other soil processes and soil profiles.

pH influence

The main factor influencing the interactions of humic acids with metals, mobility and reactivity of heavy metals in the soil environment is pH (Cao *et al.* 2004, Pullin and Cabaniss 2003, Sokołowska and Warchulska 2009). Reactivity of humic acids depends on their dissociation degree. Humic acids are chemically heterogeneous compounds having different types of functional groups in different proportions and configurations. They contain carboxyl (–COOH), amine ($-NH_2$), hydroxyl (–OH), and phenol (Ar–OH) groups, as well many other functional groups which can develop negative charge on its surface in weakly acidic to basic media because of deprotonation (Evangelou *et al.* 2002). In consequence, binding of metals by these groups can take place on the way of neutralisation of negative charge by positive cations of metals. However, availability of functional groups for interactions with metals is strongly dependent on pH. Hydroxyl groups can react at higher pH (neutral and alkaline), therefore higher participation in metal binding is assigned to carboxylic groups which start dissociation at low pH. The scheme of dissociation and negative charge formation of soil organic matter functional groups in relation to OH^-/H^+ content (pH) is shown in Figure 18 (Matyka-Sarzyńska and Sokołowska 2005b). Decrease of pH which is observed during metal binding can serve both as an indicator of complexation reaction and as a measure of stability of metal complexes. At higher pH protons are also dissociated from water molecules covalently bonded to metal ion and, in consequence, the formation of hydroxy complexes can be possible.

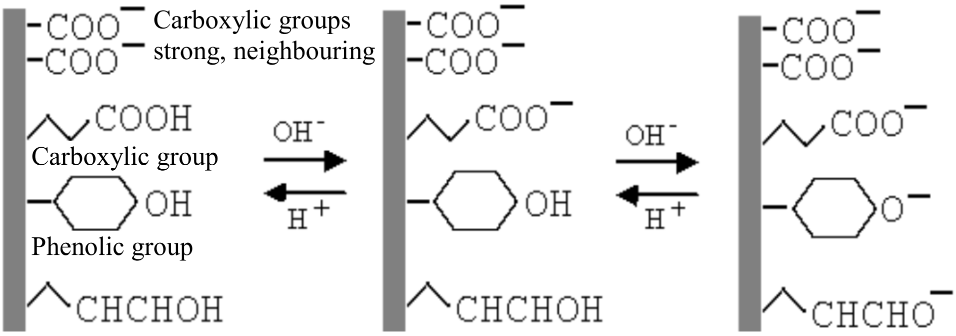


Fig. 18. Formation of negative variable charge on soil organic matter (Matyka-Sarzyńska and Sokołowska 2005b)

pH affects interactions of humic acids with metal ions also by change of the structure of organic compound. Low values of pH cause not only a decrease of functional groups dissociation, but also a decrease of ζ potential of colloidal system. Humic and fulvic acids have, in such circumstances, a tendency to aggregation and to the formation of coiled fibres, which is an additional difficulty in access of metal cations to the surface of humic acids molecules. Increase of pH stabilises colloidal system, increases dissociation degree of functional groups of humic acids, deepens water colour, but decreases oxidation of structure.

The parameter which is directly connected with pH of the soil environment and which simultaneously informs about the possibilities of metal binding by the sorption complex is CEC (cation exchange capacity). This parameter is determined by the content of clay minerals fraction, organic matter and pH. Most soils have negative charge of surface, so cation exchange capacity is higher than anion exchange capacity. Soils with higher CEC are able to absorb higher amount of metal ions such as Ca^{2+} or K^+ to the exchange sites of clay and organic matter particle surfaces. Buffer capacity of such soils is also higher than, for example, for sandy or silted soils, which prevents the whole system against sudden changes of pH. Cation exchange capacity increases with pH increase. In these conditions, more and more functional groups are dissociated, the amount of negative charge on the surface also increases, and binding of metal ions is possible. Cation H^+ of the functional groups of humic acids competes with other cations for exchange sites. In conditions when pH is high, more base cations will be bonded by exchange sites and thus these cations will be less susceptible to leaching. On the other hand, when the soil pH is lower and in consequence the concentration of hydrogen cations is high, alkali cations can be easier exchanged by H^+. As a result, base cations are more mobile and can be transported to soil water or even can be removed from upper parts of soil. Chemical reactions in the sorption complex can be described by equations of ionic exchange with selective and irreversible character. In the case of presence of a number of different metals or influence of physicochemical properties (pH changes, moisture, salinity), the competitiveness phenomenon is visible and metals with lower binding energy are displaced. Thus, the processes of sorption in the sorption complex are associated in parallel with the desorption processes (Łabętowicz and Rutkowska 2002).

A second of parameter which is also connected with the sorption abilities of humic acids in relation to metals and other components of the soil environment is their buffer capacity. Buffer properties of soils or some soil components such as humic acids can be defined as the ability to resist sudden pH changes under the influence of acids or bases. Buffering is connected with maintenance of a state of equilibrium between soil solution and hydrogen ions (and aluminum) contained in the soil sorption complex. When alkaline agents appear in a soil, hydrogen ions are removed from the soil solution (due to neutralisation) and supplemented from the soil sorption complex. On the other hand, when hydrogen ions are introduced to the soil solution, they are transferred to the sorption complex. In both cases, soil pH does not change significantly until saturation of the sorption complex by those ions. Buffer properties of soils are strongly positively correlated with their sorption capacity. HA contained in soils exhibit high cation–exchange capacities. Consequently, the buffer capacity of these compounds and HA-rich soils is also high (Lavelle and Spain 2002, Zawadzki 1999). However, excessive amounts of strongly alkalified or acidified agents in the environment and over-saturation of the soil complex with ions can exceed the natural buffer capacity of the soil system, resulting in a soil pH change, or changing the natural buffer capacity into a buffer capacity characteristic for the newly introduced components.

Buffer capacity, β, is expressed as the number of moles of a strong acid or base which, when introduced to 1 dm^3 of a buffer solution, changes its pH by one unit:

$$\beta = \mathrm{d}c/\mathrm{d}pH \approx \Delta c/\Delta pH \qquad (1)$$

where:

Δc – number of strong acid or base moles added to the buffer solution,

ΔpH – change in pH value.

The mobility of humic acids influences significantly the type of compounds forming with metals. Generally, studies show that the fraction of dissolved humic acids has a tendency to create soluble metal complexes. Sokołowska *et al.* (2009b) reported that dissolution of soil organic matter was significantly affected by pH. The amounts of soil DOM measured in water extracts at different pH are shown in Figure 19.

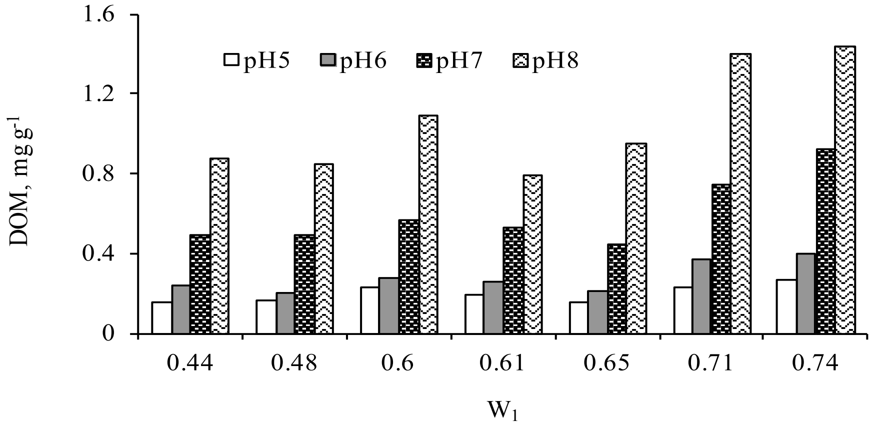


Fig. 19. Concentration of dissolved organic matter released at different pH vs. secondary transformation index of studied mucks (Sokołowska *et al.* 2009b)

Data in Figure 19 show that dissolved organic matter concentration was low at low pH values and high at alkaline conditions. Releasing of humic acids from soils to solution in relation to pH is presented in Figure 20.

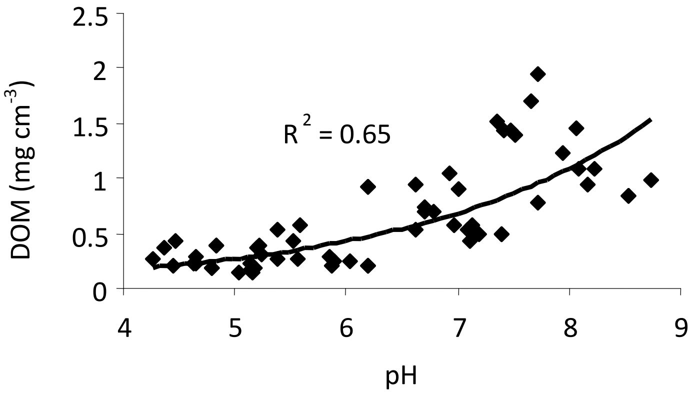


Fig. 20. Releasing of humic acids from soils to solution in relation to pH (Matyka-Sarzyńska and Sokołowska 2005)

Additionally, the results from Figure 19 show that the amount of organic matter released from soils increased not only with pH but also with secondary transformation (marked in the Figure as W_I), however this relationship was not so clear. Previous researches of those authors showed that the organic matter dissolution process during alkalisation could be satisfactorily described with the following equation (Matyka-Sarzyńska and Sokołowska 2005):

$$\mathrm{DOM(pH)} = 0.01\ \exp(b_I\,\mathrm{pH}) \tag{2}$$

where b_1 is the parameter which could be considered as quantitative index of OM release process under the experimental conditions applied.

It was proved that parameter of the equation – b_I – increases with secondary transformation degree. The highest concentration of DOM was obtained for the strongly secondary transformed sample ($W_I = 0.74$). The opposite was obtained for the weakly secondary transformed sample ($W_I = 0.48$). The tendency of increasing of DOM with the state of secondary transformation could indicate that partial disruption of humus aggregates took place during the secondary humification. As a consequence of the above, different substances of lower molecular weight, *e.g.* fulvic acids, were formed.

The form of metals has a significant influence on humic acids interactions with metal ions. Binding of these elements is the easiest for available forms with high mobility. In general, metal macronutrients (K, Ca, Mg) are more available within pH range of 6.5 to 8, while the majority of micronutrients (Cu, Fe, Mn, Ni, and Zn) are more available within pH range of 5 to 7. These metals are very strongly bound to the soil at high pH and are therefore more available at low pH levels than high pH levels. Cations Ca, K, Mg are more weakly bound to the soil and are prone to leaching at low pH. In soil with pH considerably above 7 (alkaline and carbonate soils of high sorption capacity), the transfer of heavy metals into non-soluble forms takes place without any additional agrotechnical treatments (Davies and Ballingen 1990).

Influence of ionic strength

Salinity (ionic strength) of soil solution has a significant meaning in interactions of humic acids with metal ions. In most cases, binding of metals is inhibited at high values of ionic strength. It can be seen for example in sea waters, however this phenomenon is not the same for all metals. In this case the dominant role is played by processes taking place in the range of double electric layer. Generally, addition of salts causes a change in the structure of humic acids. Some investigations (Wandruszka *et al.* 1997) have shown that aqueous solutions of humic acids respond to the addition of cations by forming intramolecular and intermolecular aggregates, compact structures with relatively hydrophobic interiors and hydrophilic surfaces. Stretched configuration of humic acids is transformed and tends to shrink or contract, as well as favuors the coiling of chains. In such conditions water molecules can be removed and humic acids colloids obtain hydrophobic character. Increase of ionic strength can increase peptisation pH from 3 even to 5. This is thought to be due to two mechanisms:charge neutralisation and functional group bridging. Functional group bridging enhances this effect, especially with multivalent cations, by drawing together various groups on the humic acids chain. Cao *et al.* (2004) reported that increasing ionic strength causes decreases in copper ability to bind with humic acids. Complexing in one place of humic acids structure decreases the tendency for complexing others groups by next copper cations. Thus, condensation of oppositely charged ions increases in double electric layer and this situation does not favour complexation reactions. In consequence, complexing abilities are higher at low ionic strength.

Ionic strength is also a factor which can determine not only complexation but also coagulation processes. Studies carried out by Boguta and Sokołowska (2013), relating to coagulation of humic acids coming from different organic soils by using copper (II) ions at pH, revealed that humic acids coagulate and flocculate under copper (II) chloride addition. However, measurements of metal content in solutions after coagulation showed that the amount of copper which transited from the liquid phase to the precipitate decreased with increase of copper chloride introduced initially. It should be stressed that in those circumstances the measurements of organic carbon showed that almost all carbon was flocculated. This fact was probably connected with increasing ionic strength of the system, as a result of which the interactions of humic acids with copper ions were inhibited and a part of copper stayed in the liquid phase of the system.

Influence of temperature

The influence of temperature on interactions of humic acids with metal ions is related to changes of mobility of above components. This effect is especially visible in the case of humic acids. Sokołowska and Boguta (2010) write that the availability of organic matter and its mobility in the soil can be a consequence of changes in climate, especially temperature. Evidence for the importance of climatic factors comes from observations of seasonal variations in soil water, lakes, streams and rivers, in which summer and autumn maxima are found. These seasonal cycles are positively correlated to soil temperature and groundwater flow rates. Dissolution of organic matter from soils at elevated temperature seems to depend on the degree of the secondary transformation: the higher the degree of transformation the higher the dissolution rate (Sokołowska *et al.* 2006, Sokołowska and Boguta 2010). The process of dissolution of organic matter at 293 K and 393 K is agreed with the fist-order reaction model. The first-order rate constants for the dissolution of organic matter calculated at 293 K are lower than at 363 K. The results of the studies showed that the amount of released organic matter from studied organic soils was higher for 363 K than for 293 K. Processes of releasing organic matter from soils at above two temperatures are displayed in Figures 21 and 22 (Sokołowska *et al.* 2012). In the context of interactions with metals, higher temperature should favour the forming of soluble complexes because more dissolved organic matter is released to the solution.

The authors also noted that especially organic soils like peatlands or peaty mucks contain a number of labile organic compounds of known and unknown structure which are very sensitive for temperature change, and their dissolution in water depends on solubility product. Besides humic and fulvic acids, such sensitive compounds could be lignite, bitumines, peptides, enzymes and fats, as well as low molecular organic compounds like free amino acids, alkaloids, purine and pyrimidine bases, carbohydrates, vitamins, sugars, phenols, antibiotics, steroids, triterpenoids, β-sitosterol etc. All of these compounds could be sensitive to temperature changes and, in consequence, these changes can affect interaction with metal in different ways.

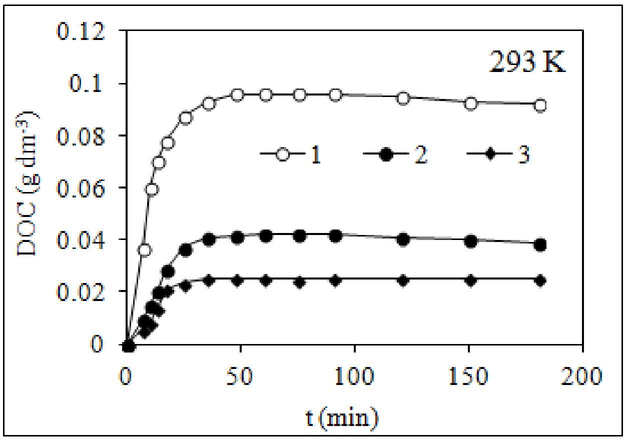


Fig. 21. Releasing of dissolved organic matter from three selected organic soils to the solution at temperature of 293 K. Abbreviations in figure refer to the number of studied organic soil (Sokołowska *et al.* 2012)

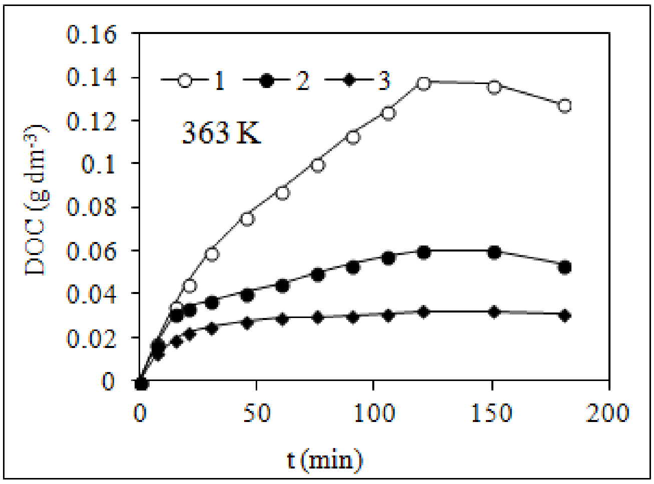


Fig. 22. Releasing of dissolved organic matter from three selected organic soils to the solution at temperature of 363 K. Abbreviations in figure refer to the number of studied organic soil (Sokołowska *et al.* 2012)

6. SELECTED METHODS OF STUDIES OF INTERACTIONS OF HUMIC ACIDS WITH METALS

The issue of interactions of humic acids with metal ions is still an important research problem. These mechanisms are not completely clear, and a lot of questions require additional research. Most recent investigation techniques allow analysing the interaction mechanisms already at the atomic level. The combination of various instrumental methods provides great opportunities for a comprehensive look at the reactions between metals and humic acids, taking into account different environmental conditions. Thanks to modern chemical and physicochemical methods, there is the possibility of analysis of bonds, their stability, spatial structure of forming compounds, sorption capacity of ligands, as well as observing the competitiveness of different ions and optical, electrochemical, and magnetic properties of investigated systems. Therefore, the following paragraphs present the selected research methods useful in the analysis of interactions of humic acids with metal ions.

6.1. Carbon content

Measurements of carbon content in solution can be used for the study of coagulation and flocculation processes of humic acids by metal ions. Experiment is based on preparation of a series of solutions with soluble humic acid at adjusted pH. The series contains increasing concentration of added metal ions covering points before coagulation, coagulation, flocculation and some points after flocculation. Samples of supernatant are collected from the solutions after a set time and organic carbon content is measured. On the basis of obtained results, charts of organic carbon content remaining in liquid phase in relation to the amount of metal added initially are compiled. From these relationships, two threshold concentrations of copper (C_{Cr}I-point of coagulation start, C_{Cr}II-point of coagulation end) and distance between the thresholds (ΔC_{cr}) were determined in graphic way (Boguta and Sokołowska 2013) by simplification of way proposed by Khil'ko *et al.* (2001). Scheme of above parameters determination is shown in Figure 23.

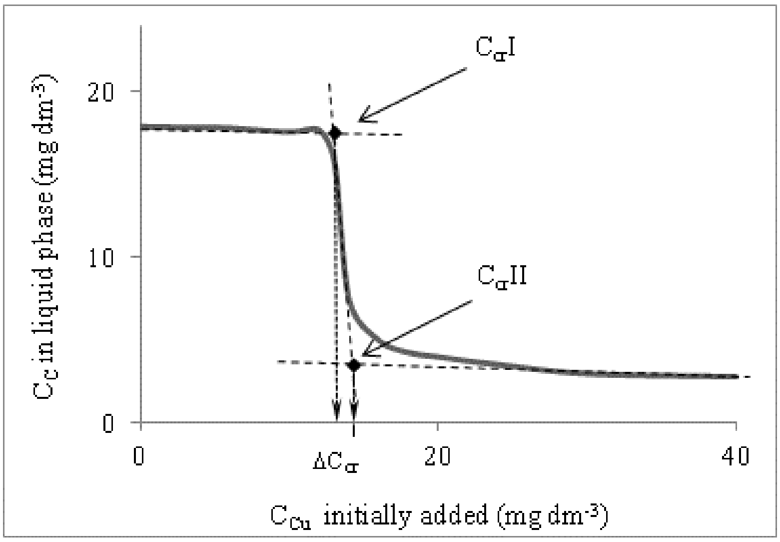


Fig. 23. Scheme of determination of critical copper concentration at which coagulation was observed: C_{cr}I – point of coagulation start, C_{cr}II – point of coagulation end, ΔC_{cr} – difference between C_{cr}II and C_{cr}I (Boguta and Sokołowska 2013)

Measurements of organic carbon content are also important from the point of view of assessing the quality and fertility of soil. Carbon is the main component of soil organic matter, including humic acids, where it builds aliphatic structures, aromatic cores, and functional groups. Thus, organic carbon content of soils, especially of organic soils, is often considered as an indicator of humus compounds content. Researches of Kalembasa and Becher (2009) show that the content of organic carbon of peat-muck soils in the muck horizon (Mt) varies from 312 to 381 g kg^{-1} of dry mass. Total content of organic carbon in organic soils is sometimes identified with the organic carbon content, which is rather simplistic and can lead to overestimated conclusions. This is due to the fact that, at the time of transition from the accumulation phase to decesion phase, mineralisation processes begin to dominate in organic soils. Such soils become a source of carbon dioxide in the atmosphere (5-fold higher CO_2 emissions than from mineral soils) but these soils can also enrich the inorganic carbon compounds (Miatkowski *et al.* 2010). Therefore, in the case of studies on peaty muck soils and other organic soils, information about the content of inorganic carbon and its relation with organic carbon content is important. This information is valuable from the point of view of assessing the agricultural suitability of soils because carbon compounds determine the biological and chemical properties of the soil, its fertility and, in consequence, abilities of nutrients sorption in which metals play the main role. In peaty-muck soils, where organic matter content is more than twice higher than in mineral soils, organic carbon is also a valuable source of information on the dynamics of chemical and biological transformations.

6.2. Atomic absorption spectrometry (AAS)

One of the most frequently used instrumental method serving for investigation of metal content is atomic absorption spectrometry (AAS). This method enables to measure the content of ~70 elements, both liquid and solid samples (most often after mineralisation). The advantages of the method include high selectivity, short time of analyses, small amount of material needed for measurements, and possibility of analysis of metal at low concentration, even in the range of $\mu g\ kg^{-1}$. The idea of analysis of metal content in a sample is reduced to sample nebulisation, then atomisation of ions contained in the sample by flame or electrothermic method, cold vapours of mercury or hydrides method.

Atoms of elements can absorb photons of radiation in ground state, thereby it is transited into excited state. Energy levels can have only defined energies, so in connection with the above, the differences between ground and excited level are also strictly defined, which is translated into defined wavelengths – specific to given element and corresponding to energy of given quanta. Excited state is unstable, and after a short time (10^{-8} s), radiation emission to the ground state takes place. According to Kirchhoff's law, this means that an atom can absorb electromagnetic radiation only at the wavelength at which it can emit this radiation at excited state. Such radiation is characteristic for a given element (Cygański 2002). This phenomenon is the basis of quantitative analysis of elements by atomic absorption spectrometry. It could be described by the fundamental laws of absorption:

$$A = \lg \frac{I_0}{I} \tag{3}$$

where:

A – absorbance of electromagnetic radiation by free atoms,

I_0 – intensity of incident beam,

I – intensity of not-absorbed radiation after passing by “cloud” of atoms.

Lambert-Beer’s law says about proportionality of absorbance to the number of absorbing atoms:

$$A = \varepsilon_{\lambda} \cdot b \cdot N \tag{4}$$

where:

ε_{λ} – molar absorption index,

b – optical path length,

N – amount of free atoms.

The amount of free atoms can be converted to the concentration of atoms in the sample:

$$A = a \cdot c \tag{5}$$

where:

a – proportionality index,

c – element concentration.

Metal content of soil material is one of the basic characteristics for evaluating quality of soil, agricultural utility (content of mineral components: micro-and macroelements), as well as degree of pollution and degradation by heavy metals. Metal content is also important in studies of soil organic matter, because humic compounds, including humic and fulvic acids, are the main components of the soil environment responsible for its sorption properties, and therefore also for the mobility of metals.

Measurements of metal content by atomic absorption spectrometry provide information on the total metal content in solution, regardless of its form or chemical compound in which metal is involved. Additional use of other methods such as ion-selective electrodes allows separate measurement of complexed and ionic forms of metal ions, makes it possible to analyse the process of complexation and to observe the amount of metal bonded by ligands in relation to the total amount of initial uncomplexed element.

Measurement of metal content in solution is also a convenient tool for investigating the process of humic acid coagulation by given metal ion. In this case, series of humic acids solutions are prepared with adjusted pH. Concentration of humic acids is constant in each solution. Metal ions are added to the solutions in increasing amount. Analyses of metals are carried out in solutions of humic acids samples both before and after coagulation. On the basis of the results, interesting information can be obtained: whether metal ions precipitate in the same point as organic carbon as well as whether metal concentration after coagulation decreases to zero or metal stays partly in solution? This information is very valuable, since it supplements the results obtained from measurements of absorbance and from carbon measurements during coagulation processes. Metal content before and after coagulation can give information whether coagulation of humic acids was due to the complexation of metal ion and saturation of negative surface charge, or whether coagulation process was due to destabilisation followed by electrolyte particles. Figure 24 shows an example of measurements of metal (in this case copper) during coagulation of humic acids at pH 5 (Boguta and Sokołowska 2013).

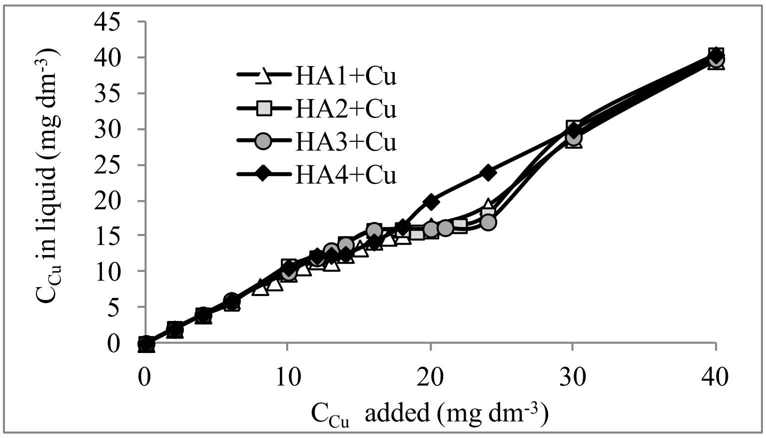


Fig. 24. Content of copper ions remaining in liquid phase (mg dm^{-3}) in the function of copper concentration added at start of experiment (mg dm^{-3}) for HA-Cu systems at pH 5 (Boguta and Sokołowska 2013)

In above systems, coagulation of humic acids started at copper concentration of about 12-20 mg dm^{-3} depending on humic acid sample. Humic acids were almost totally transferred to the precipitate. Measurement of copper ions in the solutions showed that only a small part of metal is transited to humic acids precipitate, and at high concentration of introduced copper (II) chloride the amount of metal is practically stable, which can be an indicator of humic acids coagulation under the influence of increasing electrolyte concentrations.

6.3. Content of functional groups

The structure of humic acids can be defined as a polymer consisting of an aromatic core, aliphatic chains and functional groups. Functional groups determine the reactivity of humic acids particles to a great degree, and therefore they have a large influence on the physicochemical and chemical properties of humic acids. The most frequently encountered groups include carboxyl, phenolic, alcoholic, quinone, methoxyl, carbonyl and, in smaller quantities, also groups containing nitrogen or sulphur (Kwiatkowska 2007, Pastuszko 2007, Stevenson 1994). Their presence determines, among other things, the hydrophilicity, acidity and ion exchange capacity of humic acids. It is well known that larger amounts of acidic groups are present in fulvic acids (900-1400 meq $(100\ g)^{-1}$) than in humic acids (400-870 meq $(100\ g)^{-1}$).

From the point of view of interaction with metal ions, probably oxygen-containing functional groups are the most important, mainly due to a large amount of this element in the structure of humic acids as well as because oxygen atoms have the properties of good electron donors. These can be carboxylic groups COOH and OH groups derived from phenols, but also from alcohols, enols and others. The effect of COOH and OH functional groups on the value of sorption capacity (depending on pH) is stronger by a few to several times than the influence of other mineral components of the sorption complex. The mechanism of interaction of individual functional groups may be varied, depending on concentration and valence of metal ion, concentration of humic acid, pH and ionic strength of soil solution. However, mainly the mechanisms of ion exchange, complexation, chelation and adsorption processes should be taken into consideration in the case of humic acids (Zheng *et al.* 2010). In connection with the above, measurement of the most important functional groups belongs to necessary analysis in the case of studying interactions of metal ions with the fractions of humus compounds.

One of the methods for studying functional groups is alkacymetric titration. Kucharenko and Dragunowa (Polish Soil Society 1990) proposed determination of carboxylic and phenolic groups by using a method based on the ability of humic acids for reacting with metal ions. Sample of humic acids is dissolved in NaOH solution. After 30 minutes, $BaCl_2$ solution is added and whole solution is filled to known volume by distilled water. Simultaneously, blind sample is prepared. Filtered solutions are titrated by HCl solution at the presence of phenolphthalein. Content of functional groups (meq $(100\ g\ KH)^{-1}$) is calculated on the basis of the difference of acid volume needed for titration of blind and measured sample. Carboxylic groups are determined using the ability of carboxylic groups for reaction with metals in neutral pH. For this purpose, calcium acetate is applied. According to Kucharenko, reaction of humic acids with acetates could be described by the following equation:

$$\mathrm{HA–(COOH)_{2m} + m(CH_3COO)_2Ca \rightarrow HA–[(COO)_2Ca]_m + 2mCH_3COOH} \qquad (6)$$

The amount of carboxylic groups is determined by titration of acetic acid formed during the reaction. The amount of phenolic groups is calculated from the difference: total acidity – content of carboxylic groups.

Total acidity and quantity of carboxyl and phenolic groups can be also measured by $Ba(OH)_2$ and Ca-acetate methods of Schnitzer and Gupta (1965). In this method, the quantity of phenolic OH groups is calculated as difference of total acidity and amount of carboxyl groups.

In qualitative determination of behaviour of functional groups, spectroscopic techniques are also useful, among others infrared spectroscopy (see chapter 6.7). Determination of distribution function of dissociation constants by potentiometric titration also enables quantitative analysis of dissociating groups in given pH conditions.

6.4. Potentiometric titration

Potentiometric titration is one of the most important methods for studying interactions of humic acids with metal ions and other substances found in the soil environment. Potentiometric titration method is also a valuable tool for obtaining a lot of information on the surface and sorption properties of humic acids. The great possibilities of potentiometric titration are due to the fact that humic acids molecules contain high amounts of functional groups exhibiting different acidity.

Surface charge and distribution function of dissociation constants

Back titration method can be applied for qualitative and quantitative analysis of the dependence of variable surface charge (Q) on given pH, as well as for evaluation of the distribution functions of apparent surface dissociation constants and the amount of particular functional groups (Campitelli *et al.* 2006, Gondar *et al.* 2005, Janos *et al.* 2008, Matyka-Sarzyńska *et al.* 2000). Because of dominant participation of carboxylic and phenolic groups, signal which is observed in this method during titration is related to changes of negative charge of humic acids surface. On the other hand, value of humic acids charge is strongly pH-dependent, since different types of functional groups dissociate in various ranges of pH, making molecules charged negatively. Full dissociation of humic acids occurs at pH> 8, above which OH groups are also deprotonated. COOH groups dissociate in pH range of 4.6-9.0 (Kaleta 2004). Therefore, potentiometric titration is a very convenient method for studying changes of charge, and hence changes of humic acids reactivity, because obtained dependencies allow the interpretation of above properties in a wide range of pH.

According to this method one measures the titration curves of the natural soil suspension and its equilibrium solution. The titration curve of the solid phase is determined by subtraction of the pH curves of equilibrium solution from the pH curve of the suspension. The amount of base used for neutralisation of humic acids charge is connected with neutralisation of hydrogen cations of surface acidic functional groups. The sffect of this is presence of negatively charged groups and, in consequence, increase of negative charge of solid phase. Thus, the value of surface charge within pH range of measurement can be calculated on the basis of the amount of base used in titration.

Determination of distribution function of dissociation constants derived from above functional groups of humic acid is based on measurement of concentration of hydrogen ions in solution of mixture of weak acid and its salt formed after the addition of a defined amount of strong base to a known amount of weak acid.

The following equations show the theoretical basis used for potentiometric titration of organic substances (Benegas *et al.* 2003, Józefaciuk and Shin 1996a,b, Matyka-Sarzyńska *et al.* 2000, Nederlof *et al.* 1993):

Reaction of dissociation of surface functional groups (SOH), where S represents the surface, can be defined as:

$$\mathrm{SOH} \leftrightarrow \mathrm{SO}^{-} + \mathrm{H}^{+} \tag{7}$$

Equilibrium constant has the form:

$$K = \frac{a_{H^+} \cdot a_{SO^-}}{a_{SOH}} = a_{H^+} \cdot \frac{c_{SO^-} \cdot f_{SO^-}}{c_{SOH} \cdot f_{SOH}} \tag{8}$$

where:

a – activity,

f – activity factor.

Taking into account that surface activity of protons is related to their activity in solution, above equation can be logarithmed and equation for pK can be obtained:

$$\mathrm{p}K = \mathrm{p}H - \log \frac{c_{SO^-}}{c_{SOH}} - \log \frac{f_{SO^-}}{f_{SOH}} \tag{9}$$

Using Debye-Huckel equation the following function can be obtained:

$$\mathrm{p}K = \mathrm{p}H - \log \frac{c_{SO^-}}{c_{SOH}} + A\sqrt{I} \tag{10}$$

where: A – Debye constant,
I – ionic strength of solution.

SOH groups are partly neutralised, so initial activity of acid can be expressed as:

$$a = c_{SOH} + c_{SO^-} \tag{11}$$

Using equation no 10 and 11 the pK will be as follows:

$$\mathrm{p}K = \mathrm{p}H - \log \frac{c_{SO^-}}{a - c_{SO^-}} + A\sqrt{I} \tag{12}$$

When concentration of strong base is marked as “b”, titration equation can be written in the form:

$$\mathrm{p}K = \mathrm{p}H - \log \frac{b + c_{H^+} - c_{OH^-}}{a - (b + c_{H^+} - c_{OH^-})} + A\sqrt{I} \tag{13}$$

Difference $c_{H^+} - c_{OH^-}$ is negligible for mixtures with pH between 4 and 10. Additionally, for diluted solutions $\sqrt{I} \to 0$, so:

$$\mathrm{p}K = \mathrm{p}H - \log \frac{b}{a - b} \tag{14}$$

It means that pH of weak acid neutralised at half (b = 0.5sa), i.e. solution containing equilibrium amounts of acid and its salt, is equal to pK of given acid. So for the determination of dissociation constant of a weak acid, pH of this acid solution at half of neutralisation process should be found from titration curve. At given pH value, total amount of dissociated groups is summary participation of individual groups. So, the curve obtained from titration can be approximately treated as combination of individual titration curves of monobasic acids with subsequent values of constant K_i. In consequence, the amount of surface charge originating from dissociation of all surface groups at given pH value, SO^-(pH), is equal:

$$SO^-(pH) = \sum_{i=1}^{n} SO_i^-(K_i,pH) \qquad (15)$$

At above assumption, participation of particular surface functional groups $f(\mathrm{p}K_{,i})$ at given value of pK_i can be calculated as:

$$f(\mathrm{p}K_i) = \frac{1}{Q}\,\frac{[\Delta SO^-(\mathrm{p}H_{i+1}) - \Delta SO^-(\mathrm{p}H_i)]}{\mathrm{p}K_{i+1} - \mathrm{p}K_i} \qquad (16)$$

where: Q – total amount of base used for solid phase titration in measurement conditions.

Values of $f(\mathrm{p}K_{,i})$ shown in relation to pK_i give the distribution function of dissociation constants. Assuming that the sum of participation of functional groups equals 1, average value of pK can be calculated for investigated range:

$$\mathrm{p}K_{av} = \sum_{i=1}^{i=N} \mathrm{p}K_i \cdot \mathrm{f}(\mathrm{p}K_i) \qquad (17)$$

This parameter has direct relationship with average energy of protons binding by individual functional groups of studied humus substances.

Electrochemical measurements like potentiometric titration can also be used to perform the analysis of buffer properties of soils and soil components like, for example, humic acids (Boguta and Sokołowska 2012, Garcia-Gil *et al.* 2004, Lavelle and Spain 2002, Pertusatti and Prado 2007, Prado *et al.* 2006, Sokołowska *et al.* 2011). Knowledge of this parameter and, more precisely, knowledge of distribution of buffer capacity in function of pH is very valuable information because it indicates how much humic acids can bind a given component without changing pH and without disturbing acid-base balance in soil environment. Potentiometric titration method is very suitable for this purpose because obtained signal is very clear and the whole apparatus is very precise and sensitive to signal change. Studies of Boguta and Sokołowska (2012) showed that even at the presence of strong buffering substances like phosphates it is possible to observe and determine buffer capacity of humic acids. It is also possible to find subtle changes in buffering properties of humic acids isolated from different soils. However, the scientists noted that for such observations some procedures of optimisation of the potentiometric titration process are necessary (Boguta and Sokołowska 2010). According to these studies, optimisation should be performed by selection of an appropriate dose step, titrant concentration, rate of titrant supply, potential drift value and sample concentration to the measurements. Figure 25 shows comparison of titration curves of humic acid titrated at two different potential drift values and volumes of titration step. In this case, low value of potential drift caused that titration process was slower but sorption of CO_2 was greater, which was visible on the relationships of calculated apparent surface dissociation constant. Higher concentration of humic acids and smaller volume step of titrant resulted in potentiometric titration curves with lower level of noise.

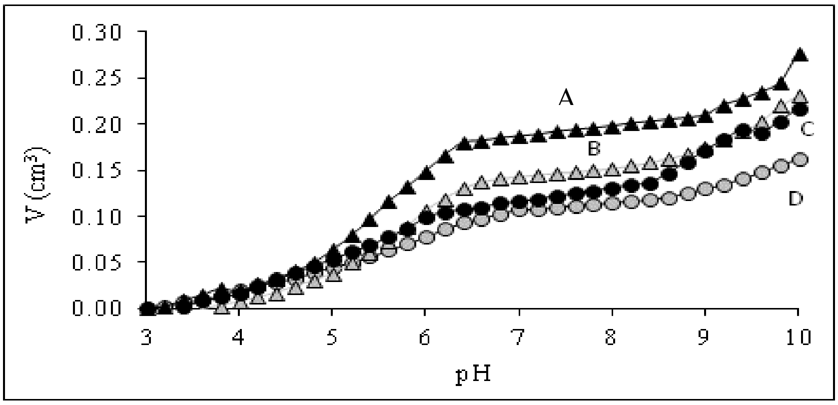


Fig. 25. Relationship between potential drift value and titration step during titration of humic acid (500 mg dm^{-3}); Drift potential 70 mV min^{-1}: A, B; 10 mV min^{-1}: C, D. Titration step 0.1 ml: A, C; 0.01 ml: B, D (Boguta and Sokołowska 2010)

Complexation

Potentiometric titration of humic acids with metals can indicate complex formation as well as other processes like coagulation, adsorption and formation of hydroxides. When a small amount of metal is added to the solution of humic acids, the metal is bonded to ligands with forming soluble complex compound, protons are released and more volume of alkaline titrant is needed for neutralisation. Potentiometric titration curves are more flat. Higher amount of metals, especially transition metals and Al, can give some inflection points resulting from formation of metal hydroxides and processes of hydrolysis. Lu and Johnson (1997) noted such behaviour of pH during addition of copper ions to organic ligands.

Examples of potentiometric titration curves of manganese ions 2, 6 and 10 mg dm^{-3} and humic acid (40 mg dm^{-3}) with 2, 6 and 10 mg dm^{-3} of manganese are presented in Figures 26 and 27 (Boguta P., data not published).

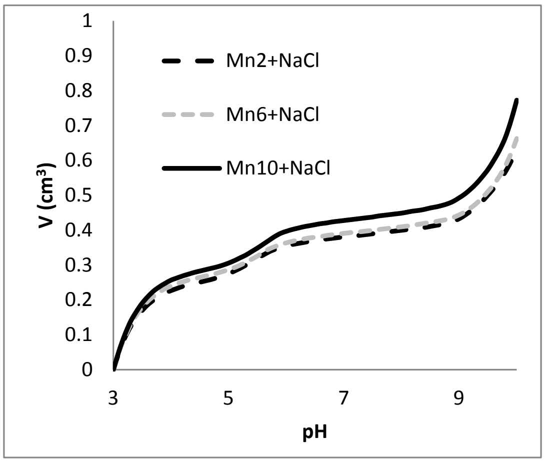


Fig. 26. Potentiometric titration curves of manganese ions at 2, 6 and 10 mg dm^{-3} (Boguta P., data not published)

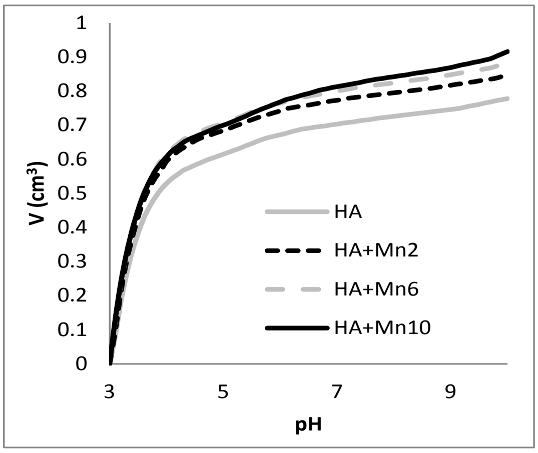


Fig. 27. Potentiometric titration curves of humic acid (40 mg dm^{-3}) with addition of manganese ions at 2, 6 and 10 mg dm^{-3} (Boguta P., data not published)

Complexation of humic acids with metal can be also studied by using ion-selective electrodes. Plaza *et al.* (2005b) studied the complexation of humic and fulvic acids by copper ions using an automatic potentiometric titrator equipped with Cu(II) ion-selective electrode, at pH adjusted to 5. They measured the concentration of free-Cu(II) after each addition of titrant. On the basis of obtained results they calculated complexation capacities and stability constants. In this case modelling of Cu(II) binding was based on the assumption that Cu(II) binding occurs at identical and independent binding sites or ligands and that only 1:1 metal:ligand complexes are formed. Complexation of metal (*M*) by functional groups of humic acids (*L*) at given pH and established ionic strength can be described as conditional stability constant

$$K = \frac{[ML]}{[M][L]} \tag{18}$$

where:

[M] – free-metal molar concentration,

[L] – molar concentration of all forms of the ligand that are not bound to M,

[ML] – molar concentration of ML complex.

Mass balance can be expressed as follows:

$$C_L = [L] + [ML] \tag{19}$$

where:

C_L – stoichiometric concentration of ligand sites which is related to the total concentration of humic substance, $(HS)_{total}$, by

$$C_L = HS_{total} \cdot CC \tag{20}$$

where:

CC – complexation capacity.

On the basis of equations (18)-(20), fraction of ligand bound (v) can be calculated in terms of complexing capacity, stability constant and free metal ion concentration:

$$v = \frac{[ML]}{(HS)_{total}} = \frac{K[M]}{1 + K[M]} CC \tag{21}$$

When ν and *[M]* are determined for each titration point, the optimum set of model parameters (i.e., *CC* and *K*) for each HA and FA sample was estimated by iteratively varying the adjustable parameter values until the sum of squares of the differences between observed and fitted values of ν was minimised. Full, unconstrained optimisation was achieved using the Quasi-Newton algorithm (Plaza *et al.* 2005b). Cao *et al.* (2004) compared different methods for the calculation of stability constants and complexation capacity of humic acids. They used, among others, ISE electrodes, anodic stripping voltammetry as well as fluorescence quenching. Obtained parameters were different due to the fact of different amount of added copper. ISE was the most applicable as long as the concentration of copper was above 10^{-7} mol dm^{-1}.

6.5. Turbidity and uv-vis spectroscopy

Application of UV-VIS and turbidimetry methods for the study of interactions of humic acids with metal ions consists in observation of changes in absorbance or transmittance. These changes can come from both the humic acids and from metal ions.

Change in absorbance from the metal side can result from processes of hydrolysis and precipitation of hydroxides as well as, in some cases, absorbance changes enable also spectrophotometric determination of metal content by using special compounds addition for creation of colour complexes with metal cations. It should be stressed that colour of concentrated humic acids gives high influence in such measurements, so metal concentration can be determined by UV-VIS only in diluted solutions of humic acids. Kislenko and Oliynyk (2004) investigated concentrations of chromium and ferric ions in solutions after interactions with humic acids at pH close to 3. In the case of ferric ions, ammonium rhodanate was added and the optical density of formed ferric rhodanate complex was measured at 364 nm. The concentration of chromium ions in solution was found after its oxidation by perchloric acid and formation of a complex with 1,5-diphenyl carboxyazide. Absorbance of the complex was recorded at 540 nm. Amount of elements bound to humic acids was calculated from the difference of initial and final metal concentration in the solution. These are only two examples of such complexes but, generally, d-electrons metal can create more colour complexes which can be determined by using UV-VIS spectroscopy.

In the case of humic acids, the method can be used because humic acids have special structures with units called "chromophores". These structures are responsible for the absorbance and they consist of conjugated double bonds and unbonded electrons like those associated with oxygen, sulphur, and halogen atoms. Absorbance is probably mainly due to the aromatic ring structure (Bloom and

Leenheer 1989). Most frequently, the observed change in absorbance results from internal vibration and rotation of molecules and intermolecular interactions, changes of pH, aromaticity and molecular weight, but also from such processes as coagulation and flocculation of humic acid particles after saturation of the surface functional groups. Interactions of humic acids with metals can be also observed by application of nephelometric measurements of aqueous HA solution turbidity (Anđelković *et al.* 2004). The intensity of scattering light, according to Rayleigh, is shown in the equation (22):

$$I = k \cdot a \cdot v^2 \qquad (22)$$

where:

I – intensity of scattered light,
v – volume of particle,
a – number of particles,
k – constant.

When particle size in an aqueous system increases, turbidity of whole solutions also increases, so in consequence, by measuring intensity change of scattered light it could be possible to study the interactions of humic acids with metal cations. Concentration of colloid is constant in this model. According to Anđelković *et al.* (2004), these studies should enable to monitor the behaviour of humic acids solutions under the influence of cation valence of selected metals. The authors showed that results were in agreement with the Schulz-Hardy rule. Problems with colour interferences were eliminated by measurements of relative values of turbidity, not absolute. For total dispersion of humic acids, samples had to be first alkalised to pH 10 and after this lower pH was established. This high pH range is rather avoided in humic acids investigations considering the fact of structure changes possibility, however, in this type of measurements such treatments are necessary. The authors gave the increasing effectiveness in turbidity increase as:

$$Na^+ < NH^{4+} < K^+ < Mg^{2+} < Ca^{2+} < Ce^{3+}$$

Above sequence proved that the cation with the biggest ionic radius is the most effective coagulant.

Observation of absorption changes of humic acids under the influence of metals enables to study coagulation and flocculation processes. These studies can be alternative to carbon measurements in solution (chapter 6.1) and can be carried out at wavelengths characteristic for humic acids. Examples of relationships between absorbance of humic acid and concentration of added metal are presented in Figures 28 and 29 (Boguta P., data not published).

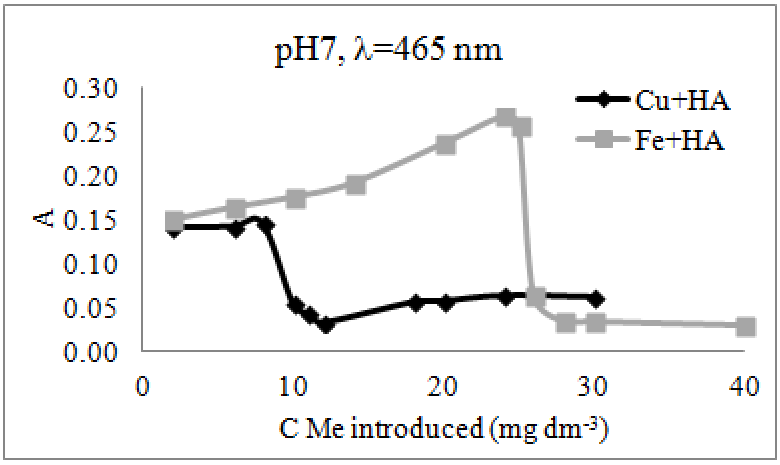


Fig. 28. Changes of absorbance (A) at 465 nm in series on solutions of humic acid (40 mg dm^{-3}) and increasing concentration of copper and iron at pH 7 (Boguta P., data not published)

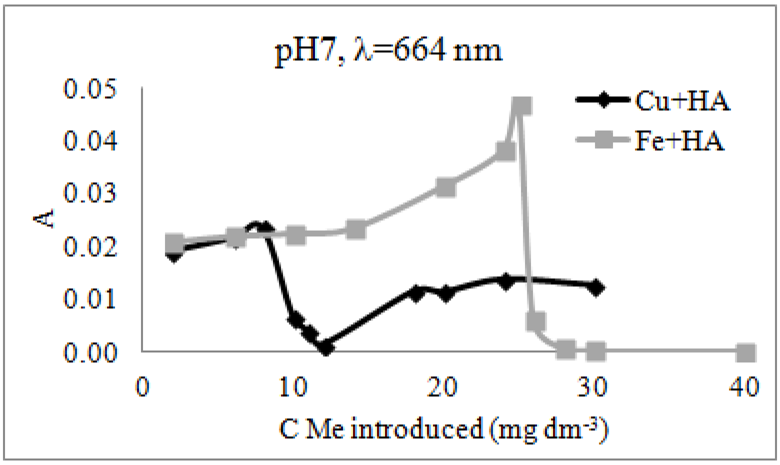


Fig. 29. Changes of absorbance (A) at 664 nm in series on solutions of humic acid (40 mg dm^{-3}) and increasing concentration of copper and iron at pH 7 (Boguta P., data not published)

Coagulation points visible as sudden drops of absorbance are almost the same for measurements at different wavelengths (Figure 28 and 29) and simultaneously are very close to the inflection points obtained from carbon measurements in liquid phase after coagulation (see Figure 14 and 15). However, it should be stressed that the choice of wavelength is important because given wavelengths relate to different kinds of structure units. Absorbance at 260-280 nm is typical for compounds like lignin and quinone moieties, i.e. for material at the very beginning of transformation. Signals at 460-480 nm reflect organic material at the beginning of humification, and absorbance at 600-670 nm indicates strongly humified material with a high degree of aromatic condensed groups (Kononova 1968, Zbytniewski and Buszewski 2005). Lippold *et al.* (2007) proposed measurements of absorbance of sedimentation of flocculating humic acids at 254 nm. On the basis of studies of interactions of humic acids with Fe (III) they reported that in the case of iron (III) interactions of humic acids can be very complex. One of the theories assumes binding of polymeric forms of iron with humic acids. UV-VIS spectroscopy can confirm this by measurement of iron spectrum, humic acid spectrum and spectrum of iron mixed with humic acid. If the sum of individual spectra of humic acid and spectra of iron are the same as spectrum of humic acid mixed with iron, iron is bound in oligomeric form (Lippold *et al.* 2007). Improvement of absorbance analyses or changes of carbon concentration in liquid phase during coagulation by metal ions can be simultaneously observation of metal concentration also in liquid phase over the precipitate.

UV-VIS gives also a possibility of evaluation of physicochemical properties of humic acids. These absorption spectra of humic acids had neither maxima nor minima, and the optical density usually decrease as the wavelength increased. However, the fourth derivative of UV-VIS spectra of humic acids shows unique peaks at wavelengths of ~280, 470 and 665 nm. These peaks are seen in Figures 30 and 31.

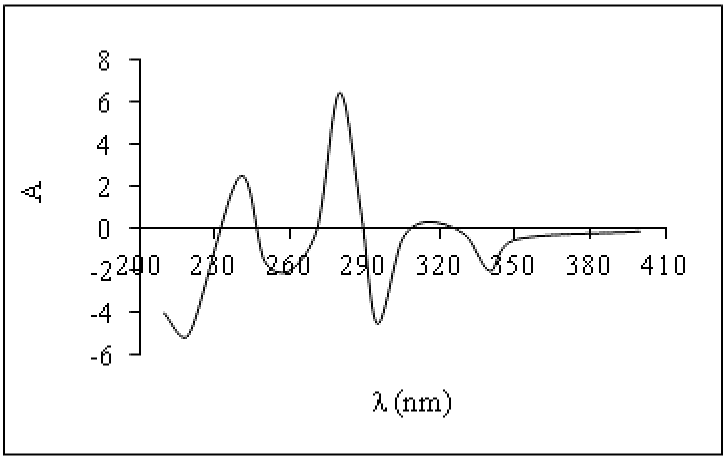


Fig. 30. Fourth derivative of humic acids UV-VIS spectrum in wavelength range of 200-410 nm (Matyka-Sarzyńska and Sokołowska 2005b)

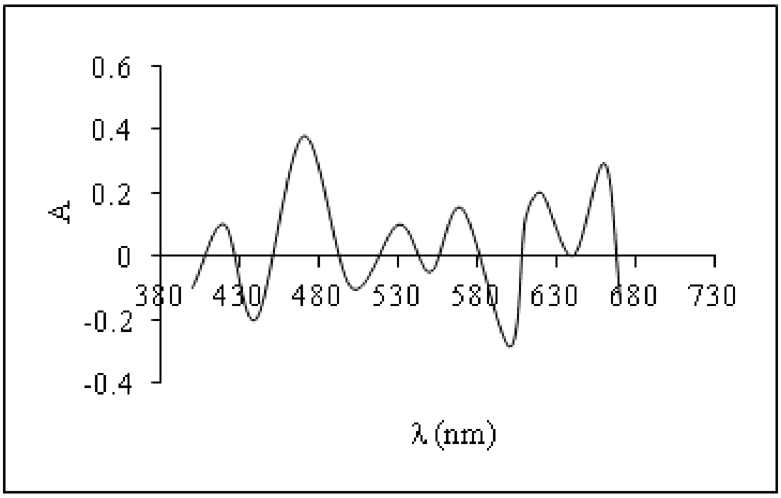


Fig. 31. Fourth derivative of humic acids UV-VIS spectrum in wavelength range of 380-730 nm (Matyka-Sarzyńska and Sokołowska 2005b)

In consequence, the composition of organic matter (including humic and fulvic acids) is frequently characterised by the ratio of absorbances at 465 nm and 665 nm (E_4/E_6), by 280/665 nm ratio as well as by 280/465 nm ratio. As the absorbance at 465 nm is due to smaller molecules, and at 665 nm to larger molecules, the E_4/E_6 ratio is expected to be larger for fulvic acids of low molecular weight and smaller for humic acids of greater molecular weight. The E_4/E_6 ratio is <6 for humic acids and 6-18.5 for fulvic acids (Kononowa 1966). Lower E_4/E_6 is associated with more aromatic components, higher degree of condensation, greater susceptibility to flocculation. This parameter is often called the humification index and is the most often calculated ratio. E_2/E_6 index characterises the content of compounds of lignin type, and denotes the relation between non-humified and strongly humified material. Similar interpretation is for E_2/E_4, however the content of lignin-type compounds is related to compounds in medium degree of transformation. Those ratios have been reported to vary for humic materials extracted from different soil types and to be independent of concentrations of humic materials (Kononowa 1966). Sokołowska *et al.* (2009b) tried to find a relationship between absorbances ratios of humic and fulvic acids mixture extracted from organic soils and between secondary transformations of original soils. These relationships are shown in Figure 32.

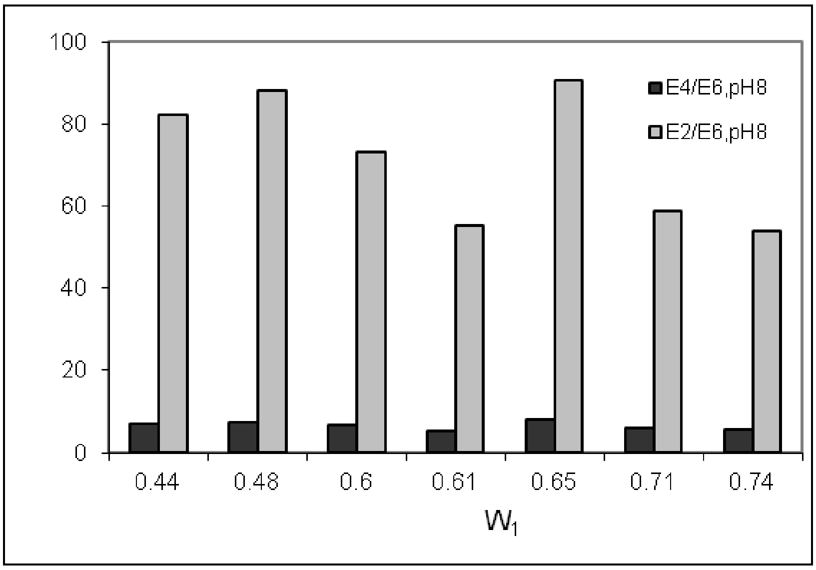


Fig. 32. Relationship between E_2/E_6, E_4/E_6 ratios of extracted organic matter and secondary transformation degree of the soils (Sokołowska *et al.* 2009b)

6.6. Fluorescence spectrometry

Specific structure of humic acids causes that these substances show fluorescence properties. Special chemical groups called fluorophores are responsible for this behaviour, for example groups like catechic, chinoic, phtalic, phenolic, amino or salicylic. Particles of substances which show fluorescence activity can be excited or quenched by absorption of radiation with short wavelengths, for example by UV radiation, and then they can emit absorbed energy in the form of fluorescence. The mechanism of above-mentioned phenomenon is based on photons emission in time when excited electron is transferred from the first excited singlet state to the singlet ground state. Transitions of such type are related to promotion of available n- or π-electrons on the orbital π^*. The time of fluorescence duration is $\sim 10^{-8}$s. Radiationless deactivation of higher oscillating states takes place still faster, so fluorescence can be observed from the lowest oscillating state of first electron excited state. Fluorescence, contrary to phosphorescence, does not result in multiplicity changes.

Fluorescence (in low ranges of fluorophores concentration) is proportional to the concentration of the whole fluorescing substance (in higher concentration, auto quenching can be present).

$$I_F = k \cdot I_0 \cdot c \cdot s \tag{23}$$

where:

k – proportionality constant,
I_0 – intensity of exciting radiation,
c – concentration,
s – thickness of the absorbing layer.

Electron mechanisms which describe fluorescence are highly probable in particles containing atoms with free electron pair, for example in oxygen, nitrogen or aromatic structures and/or in conjugated, unsaturated structural systems. Above described situations take place in humic acid particles. So it could be said that fluorescence intensity of humic acids (IF) is directly connected with the amount of fluorescing structures in humic acids particles.

The method of fluorescence spectrometry is very sensitive to various environmental factors: pH, ionic strength, solution type, temperature or interactions with metals. The possibility of fluorescence application in studies of interactions of humic acids with metal ions results from the fact that functional groups which are potentially responsible for metal binding are also responsible for fluorescence properties of humic acids (Elkins and Nelson 2001). In the case of metal ions binding by humic acid particles, fluorescence signal can be quenched or intensified. It depends on the metal bonded, and more precisely, on the mechanism of metal binding (Cheng and Chi 2002). In general, in most cases paramagnetic ions cause fluorescence quenching, whereas interactions with diamagnetic ions can influence fluorescence intensity in a different way. Senesi *et al.* (1991), Hernandez (2006) and Shin *et al.* (2001), in studies of humic acids interactions with metal ions, noted that fluorescence measured at higher wavelengths entailed presence of condensed structures with high conjugation of unsaturated bonds, high molecular mass and high humification degree. In particular, excitation-emission pairs of main peaks can indicate conjugated phenols, chinons, flavons and flavon-like substances. Inversely, fluorescence present at low wavelengths could be related to simple, low-molecular structures with low aromaticity degree.

Application of fluorescence spectrometry could be related with the following techniques:

- Emission spectra: exciting monochromator is set on given wavelength and emission monochromator analyses emitted light (λ_{exc} = constant, λ_{em} = is changed). These spectra are characterised by unique, wide fluorescence bands depending on the nature and origin of humic acids. Figure 33 presents an example of emission spectrum of solution of humic acids extracted from organic soil and humic acids with addition of metal.

- Excitation spectra: exciting monochromator goes through the wavelength of exciting radiation, whereas emission monochromator is set on a given wavelength in the range of fluorescence (λ_{em} = constant, λ_{exc} = is changed). The spectra are composed of greater amount of peaks than emission spectra. They are also better separated. Bands are situated in three ranges of wavelengths: long (480-440 nm), medium (400-380 nm) and short (360-300 nm), and describe particular groups of humus materials. Figure 34 presents an example of excitation spectrum of solution of humic acids extracted from organic soil and humic acids with addition of selected metal.

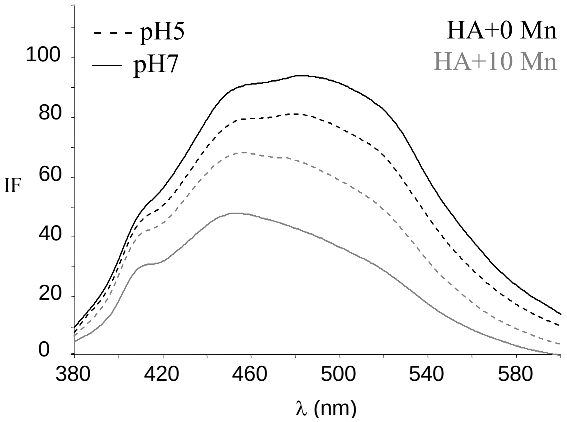


Fig. 33. Emission spectrum of humic acids (40 mg dm^{-3}) at pH 5 and pH 7 and humic acids with addition of 10 mg dm^{-3} of manganese (II) ions (Boguta P., data not published)

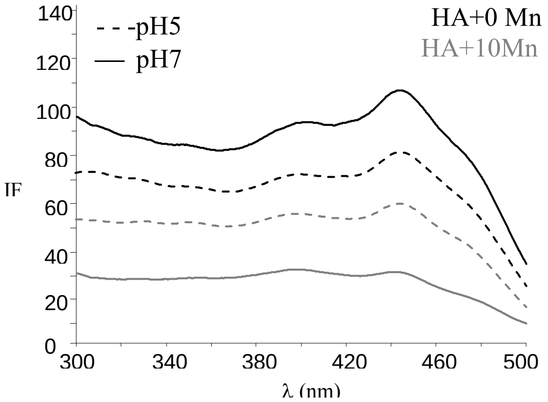


Fig. 34. Excitation spectrum of humic acids (40 mg dm^{-3}) at pH 5 and 7 and humic acids with addition of 10 mg dm^{-3} of manganese (II) ions (Boguta P., data not published)

- Synchronous spectra: Constant difference of wavelength is used between monochromators $\Delta\lambda = \lambda_{em.} - \lambda_{exc}$. This measurement enables to obtain still better separation of the spectra than in the case of emission or excitation spectra. In consequence it enables clearer differentiation of samples in terms of chemical properties, and better interpretation of particle structures responsible for fluorescence is possible. Figure 35 presents an example of synchronous spectrum of solution of humic acids extracted from organic soil and humic acids with addition of metal.

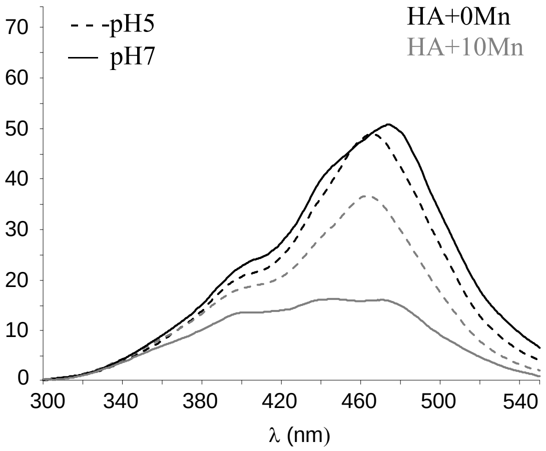


Fig. 35. Synchronous spectrum of humic acid (40 mg dm^{-3}) at pH 5 and 7 and humic acid with addition o 10 mg dm^{-3} of manganese (II) ions (Boguta P., data not published)

Today's measurement equipment enables also to obtain 3D fluorescence spectra. In this case, measurement is based on recording emission and excitation spectra in the form of a matrix of fluorescence intensities and wavelengths. These kind of measurements belong to quick, selective and sensitive analyses allowing observation of fluorescence changes in the full range of studied wavelengths. This kind of fluorescence technique is very convenient, because it gives more information about the chemical nature of humic acids. Examples of 3D emission spectra recorded for standard humic acid recommended by IHSS at pH 5 and 7 are shown in Figure 36 and 37.

High intensity of fluorescence in 3D emission spectra for main peaks can be evidence of electrondonor substituents such as hydroxyl, metoxyl, amino groups and others. Decrease of fluorescence signal can be the result of presence of electronacceptor structures such as carboxylic and carbonyl (Plaza *et al.* 2005c).

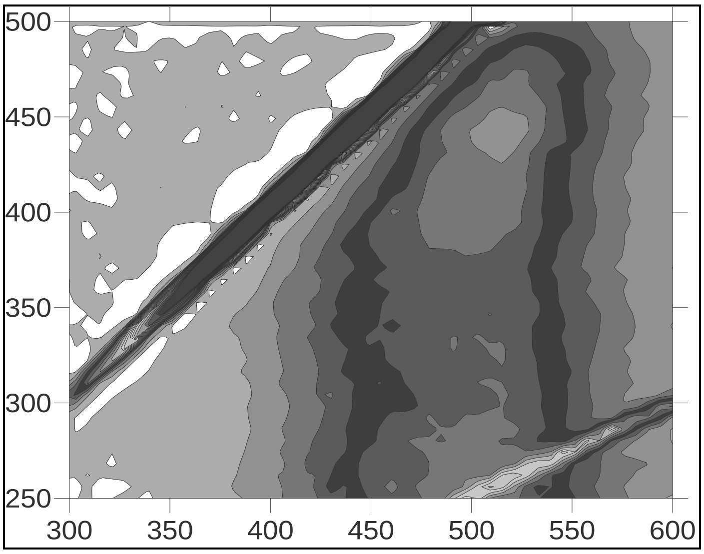


Fig. 36. Fluorescence emission-excitation matrices of IHSS standard humic acids (40 mg dm^{-3}) at pH 5 (Boguta P., data not published)

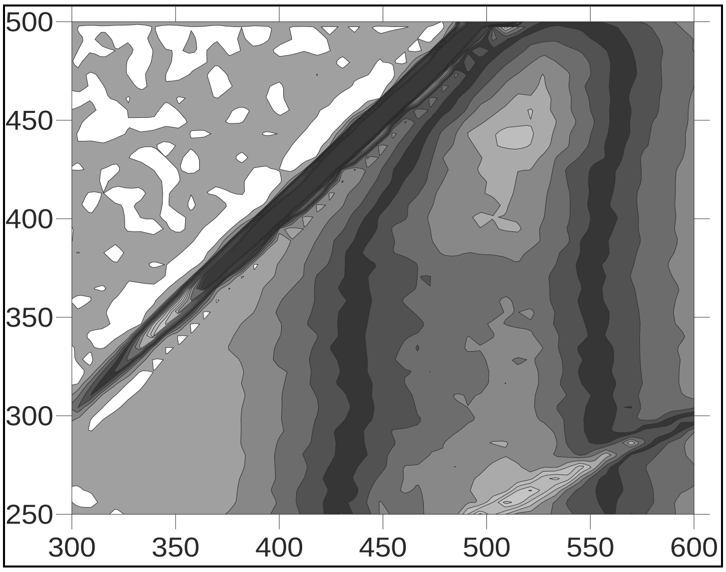


Fig. 37. Fluorescence emission-excitation matrices of IHSS standard humic acids (40 mg dm^{-3}) at pH 7 (Boguta P., data not published)

Application of fluorescence spectroscopy in studies of solutions of humic acids with metal ions allows also the calculation of complexing capacities (C_L) of humic acids in relation to given metal ion as well as stability constants of forming complexed compounds (K) (Ryan and Weber 1982). The model proposed for the calculations assumes proportionality of fluorescence to the ligands bonded with metal, complexing in identical and independent active places, at ligand: metal ratio 1:1. Complexing of metal ion (M) by organic ligands (L), in constant conditions of pH and ionic strength, i.e. complex formating (ML), can be described by the stability constant (K) and equation:

$$K = \frac{[ML]}{[M] \times [L]} \tag{24}$$

where: $[M]$ is molar metal concentration, $[L]$ is molar concentration of all ligand forms not bonded with metal, $[ML]$ is molar concentration of the complex. Mass equilibrium could be written as follows:

$$C_M = [M] + [ML] \tag{25}$$

$$C_L = [L] + [ML] \tag{26}$$

where C_M and C_L are stoichiometric amounts of metal and ligand.

Linking the equations (24) and (26) enables to obtain the amount of the fraction of bonded ligand (ν) expressed in the function of stability constant (K) and concentration of non-bonded metal:

$$\upsilon = \frac{ML}{CL} = \frac{K[M]}{1 + K[M]} \tag{27}$$

Linking of the equations (25) and (27) gives the relationship:

$$\upsilon = \frac{K(C_M - \upsilon C_L)}{1 + K[C_M - \upsilon C_L]} \tag{28}$$

Equation (28) can be solved towards ν:

$$\upsilon = (\frac{1}{2KC_L})(1 + KC_L + KC_M - \sqrt{(1 + KC_L + KC_M)^2 - 4K^2 C_L C_M} \tag{29}$$

In respect of the model, fluorescence intensity (I) changes in linear way together with the fraction of bonded ligand:

$$\upsilon = \frac{I_0 - I}{I_0 - I_{ML}} \tag{30}$$

where I_0 is fluorescence intensity of humic acids without addition of metal ions, and I_{ML} is fluorescence intensity which does not change in spite of addition of new metal ions portion (fluorescence intensity for saturated humic acids by metal ions). From the linking of equations (29) and (30) the following relationships can be obtained:

$$I = I_0 + (I_{ML} - I_0(\frac{1}{2KC_L})(1 + KC_L + KC_M - \sqrt{(1 + KC_L + KC_M)^2 - 4K^2 C_L C_M} \tag{31}$$

Knowing both fluorescence intensities from the experimental measurements carried out at different concentrations of metal ions and fluorescence intensities in the state of saturation of ligands by metal ions, the equation (31) can be solved towards K and C_L by using a minimising algorithm of squares sum between experimental values (I) and the values calculated by model.

6.7. FTIR spectroscopy

Interaction of metals with humic acids can be studied by FTIR spectroscopy. Using this method it is possible to identify absorbing functional groups involved in complexation and to provide information about the type of interaction. In particular, it is possible to observe the behaviour of such groups as carboxylic, phenolic, alcoholic, –N and –S ligands under metal influence (Senesi 2001).

Infrared spectra measured for substances with significant amounts of organic compounds are a valuable source of information about their structure and chemical properties. Peats, mucks and especially humic acids extracted from them can be also analysed by applying this method. They contain a lot of chemical structures absorbing in infrared range. In the FTIR spectroscopy, the observed spectrum is an image of specific absorption of infrared radiation by atom systems present in particles and, in consequence, increase of oscillate-rotate energy. It is possible because the frequency of infrared radiation is similar to the frequency of particles vibrations and simultaneously the energy of particles vibrations is quantised so only radiation with clearly defined energy is sorbed. Values of these energies are typical for functional groups present in studied samples. The following kinds of vibrations are distinguished in the description of a given spectrum:

Depending on the deflection symmetry:

- Symmetric (s),
- Asymmetric (as).

Depending on the changes of bond length or angles between bonds:

- In-plane,
- Out-of-plane,
- Stretching,
- Deformating,
  - Scissoring,
  - Wagging,
  - Rocking,
  - Twisting.

Characteristic features of FTIR spectra are the presence of absorption bands described by location, shape and intensity. Thanks to this, values of vibration frequencies can be collected in data bases, and on this basis functional groups can be determined and interpreted in studied material. The main areas of FTIR spectra interpretation are the following wavenumbers ranges:

- 4000-2500 cm^{-1} – vibrations of stretching bonds O-H, N-H, C-H, S-H,
- 2500-2000 cm^{-1} – C=C=C and triple bonds C≡N and C≡C,
- 2000-1500 cm^{-1} – double C=C, C=O, C=N, N=N, deformation vibrations of bonds N-H and O-H from water,
- 1500-650 cm^{-1} – fingerprint area, difficult for interpretation, valence vibrations of bonds C-C, C-N, C-O, deformation vibrations of C-H.

In the case of qualitative analysis, the variable most commonly used is shape and location, whereas in quantitative analysis: the height of band which describes the amount of energy absorbed by sample.

Absorption spectra in infrared are applied not only in evaluation of the structure of organic compounds but also in studies of chemical reactions and interactions of different substances which can react with functional groups of organic compounds. Soil organic matter, among others humic acids, contains in the structure numerous functional groups which have the possibility of interactions, for example with metal ions. The most important role in this case is probably played by groups rich in oxygen, like carboxylic, phenolic and alcoholic, which can be responsible for sorption of mineral components in the soil environment, acidity or buffering properties. In terms of interactions with metal ions, there are a lot of possibilities for bond creation, depending on external conditions, organic compound structure, and the ratio of its concentration to the metal ion concentration. Changes in FTIR spectra of humic acids under the influence of an exemplary metal are presented in Figure 39.

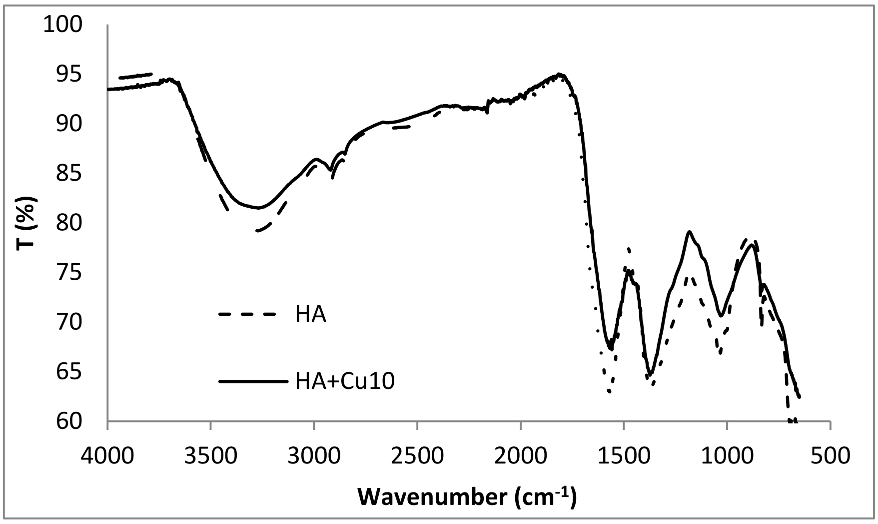


Fig. 39. FTIR spectra of humic acid, 40 mg dm^{-3} (originating from peaty-mucks) and complex of humic acid with copper (II) ions, 10 mg dm^{-3} at pH 7 (Boguta P., data not published)

Assessment of binding strength and stability of humic acid complexes with metal ions can be performed by analysing the shifting of antisymmetric band COO^- on FTIR spectrum. Shifting to a frequency of higher energy is evidence of higher participation of covalent bonds. Distance between the antisymmetric and symmetric vibration bands of COO^- also can be analysed. This parameter informs about the type of complex as well as its dentation. On the other hand, the ratio COO^-/COOH allows calculation of pK_{app} complexes. Evangelou *et al.* (2002), in studies of complexation of copper ions, found that change in pH does not cause shifting of antisymmetric bands COO^-. However, the intensity of this band increases with increasing pH from 4 to 7. This means that pH change does not causes changes in the binding mechanism, but the amount of bound copper increases. On this basis, it is also possible to conclude about electron changes and transformations of bonds in forming compounds.

The wavenumber at which the maximum of the band of a given functional group appears may undergo changes depending on humic acid kind, its purity, cation, ash or silicates content, and also depending on the amount of water, drying method and kind of metal which reacts with humic acid.

- ***Phenolic, alcoholic and carboxylic functional groups***

Absorption bands of OH groups of phenols and alcohols (coming from stretching vibrations) are placed in wavenumber range of 3000-3600 cm^{-1} (Sakellariadou 2006). Increase of band at ~3300 cm^{-1} can be caused by the binding of metal ions or by increased hydrogen bonding. For example, extended band about 3375-3426 cm^{-1} can come from hydration water (Martyniuk and Więckowska 2003). Decreasing intensity of band at 3440 cm^{-1} can be evidence of participation of oxygen atoms of phenolic groups in complexation processes of metal (Chassapis *et al.* 2010). OH groups of carboxylic acids can be visible at the range of 2500-3300 cm^{-1}. However, it should be remembered that absorption it this range can also originate from, for example, aromatic structures.

Interactions of carboxylic groups can be observed in the area of C=O groups vibrations. Elkins and Nelson (2001) reported that band at 1720 cm^{-1} is the most important band for fulvic acids. This broad peak originates from C=O asymmetric stretch of neutral –COOH groups. In the case of complexation by aluminium and terbium, this band decreases and a new one, with higher energy, appears at 1638 – 1636 cm^{-1} whereas for calcium at 1631 cm^{-1}. Band at 1720cm^{-1} is still present. There are some small differences in the location of the maximum of above band. Pospisilova *et al.* (2008) observed this band at 1718-1719 cm^{-1}. She noted that it is typical for carbonyl and carboxyl groups. Pajączkowska *et al.* (2003), as well as Sakellariadou (2006), found the maximum of this vibration at 1710 cm^{-1}. Jerzykiewicz *et al.* (1999) noted that the maximum of absorption of asymmetric vibrations C=O from carboxylic or keton groups can be placed at lower wavelengths (1712cm^{-1}). Licznar *et al.* (2008) showed that, in general, the band of asymmetric C=O can range even from 1600-1800 cm^{-1}.

The second spectral area on FTIR spectra where interactions with metals can be observed is for asymmetric vibrations of COO^-. According to Pajączkowska *et al.* (2003), this range can be visible at 1680-1630 cm^{-1} with a maximum at 1650 cm^{-1} (Jerzykiewicz *et al.* 1999). However, the location of this band is not as clear as for asymmetric C=O, because chemical connections of functional groups with metals cause that COO^- bands are shifted. On the basis of studies of different scientists, dissociated carboxylic groups can be observed from 1510 to 1650 cm^{-1} (Bronstein *et al.* 2007, Sedlacek and Klucakova 2009). Shifting of maximum depends on the kind of chemical bond, kind of bonded metal, or pH. For example, Yongqiang and Miller (2002) reported that asymmetric vibrations in bidentate zinc complex are present at 1550 cm^{-1}.

Symmetric vibrations of COO- are present at higher wavenumbers. Sakellariadou (2006) and Evangelou *et al.* (2002) claim that this band is present at 1385 cm^{-1}, but others scientists noted that above area can also exist at higher wavenumbers. For example, Martuniuk and Więckowska (2003) write about the range of 1370-1400 cm^{-1}. Jerzykiewicz *et al.* (1999, 2004) observed in their different studies that this band appeared simultaneously at 1420 cm^{-1} and in the range of 1414-1373 cm^{-1}. Yongqiang and Miller (2002) noted that symmetric vibrations of COO^- in complex with zinc are visible at 1456 cm^{-1}.

Another spectral region where complexation by metal can be observed is the area of COOH symmetric vibrations. This band is not so strong and not so broad as the asymmetric ones, but it is important in confirmation of humic acids complexation processes. Pospisilova *et al.* (2008) report presence of this band at the range of 1225-1223 cm^{-1}. Similar wavenumber (1220 cm^{-1}) is attributed to C-O stretching vibration and OH bending deformations coming from carboxyl groups by Martyniuk and Więckowska (2003) and by Sakellariadou (2006).

- ***Aliphatic hydrocarbons***

Humic acids structure consists also in a great amount of aliphatic units containing a number of methyl and methylene groups and they are visible on FTIR spectra. Generally, alcanes and cycloalcanes absorb in the range of 2850 – 3000 cm^{-1}. These bands are strong and broad and their location should not change by more than ±10 cm^{-1} (Jerzykiewicz *et al.* 1999, Licznar *et al.* 2008). Sakellariadou (2006) reports presence of these bands at 2920 and 2860 cm^{-1}. They belong, respectively, to asymmetric and symmetric stretching vibrations of aliphatic C-H bonds in methyl and/or methylene units. Results of Licznar *et al.* (2008), as well as Pospisilova *et al.* (2008), confirm these facts with high accuracy. Presence of these groups is also confirmed by absorption at the range of 1360-1420 cm^{-1}, which results from deformation vibrations of C-H methyl groups. Maximum is about 1390 cm^{-1} (Licznar *et al.* 2008).

- ***Aromatic hydrocarbons***

Presence of aromatic structures in humic acids can be visible as a weak band of stretching vibrations of C-H from 3060 to 3088 cm^{-1} (Jerzykiewicz *et al.* 1999). Bands of skeleton vibrations which cause stretching of C=C bonds located at 1450-1610 cm^{-1} can also indicate the presence of aromatic structures. Absorption band below 900 cm^{-1} can relate to out-of-plane C-H deforming vibrations of aromatic structures. On the basis of its location it is possible to conclude about the substitution type (*ortho-, meta-* or *para-*).

Nitrogen structures are much rarer than for example oxygen groups, however, complexation by these units could be also important in the case of humic acids. Sakellariadou (2006) and Pajączkowska *et al.* (2003) report that amide band, due to peptidic linkages and deformation vibrations of N-H, can be observed at 1540 cm^{-1}.

During analysis of interactions of humic acids with metal ions it should be taken into account that these are compounds showing different maturity and transformation degree. In connection with the above, analysis of absorption bands at low wavenumbers could be also valuable. Pospisilova *et al.* (2008) and Jerzykiewicz *et al.* (1999) report that the range of 1000-1200 cm^{-1} can contain absorption bands coming from C-O stretch vibration of aliphatic alcoholic groups, polysaccharides and various ether groups. Additionally to this, Sakellaradiou (2006) notes that alcoholic and polysaccharide CO stretch and OH deformation and, eventually, Si-OH band in silicate impurities can be observed at 1035 cm^{-1}.

Above arguments confirm that infrared spectroscopy, especially in conjunction with other instrumental methods, can be helpful in determination of the structures of forming compounds as well as in investigations of interaction mechanisms taking place between humus substances and metal ions.

6.8. Exchange resins

Complexes of humic acids with cations are mostly prepared by ion exchange HA-M in the solution of humic acids with addition of metal salt at given pH stabilised by buffer solution, for example acetate buffer. The suspension is magnetically stirred under nitrogen atmosphere and left for 24h for saturation with cation. Then, the complex is centrifuged or filtered, rinsed with distilled water and dried at room temperature in nitrogen atmosphere or lyophilised to the powder form (Erdogan *et al.* 2007). Such compounds can be analysed by FTIR, termogravimetry, fluorescence and other methods.

Stability constants can be also determined by using exchange resins. Dowex AG 50W-X8, 20-25 mesh Na^+ form was used by Ashok *et al.* (2000) to determine stability constants of complexes formed between humic acid (isolated from the soil) 50 + 250 μg ($\sim 3\cdot 10^{-5}$-$15\cdot 10^{-5}$ mol $liter^{-1}$ and metal salts solution 200 μg at pH 3.5. Mixtures of humic acids and metal cations were shaken with resin at pH 5. After 24 hours the resin was separated and analysed in terms of metal content by atomic absorption spectrometry. Filtered solution was investigated in the same way. Using such procedures, stability constants can be estimated according to the ion exchange equilibrium method of Schnitzer and Hansen (1970):

$$\log(\lambda_0/\lambda - 1) = \log K + x\log[HA] \qquad (32)$$

where:

λ_0 – distribution constant without humic acids,

λ – distribution constant in the presence of humic acids,

log K – logarithm of stability constant,

x – number of moles of HA that combine with 1-mol of metal,

$[HA]$ - concentration of humic acids (mol dm^{-3}).

From the experimental data distribution the constant without humic acids can be calculated:

$$\lambda_0 = \frac{\alpha_0 V}{(100-\alpha_0)g} \qquad (33)$$

where:

α_0 – percentage of total metal bound to exchange resin,

100-α_0 – percentage of total metal remaining in solution,

V – volume of solution,

g – weight of exchange resin,

x – obtained separately for each metal from the slope of the plot of ($\lambda_0/\lambda-1$) for different humic acid concentrations of each volume.

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8. SUMMARY

INTERACTIONS OF HUMIC ACIDS WITH METAL IONS

Despite numerous studies, humic acids are still not a well known fraction of humus substances. This results mainly from the complex structure of these compounds and, in consequence, from the varied chemical and physical properties as well as complex behaviour in connection with other soil substances. On the other hand, humic acids determine key features of soils: fertility, aggregate structure, suitable water-air ratios and microbial conditions. These substances belong to a group of chemical compounds characterised by strong sorption properties. It means that humic acids can interact with different soil substances, including heavy metals, which affects the mobility of cations in the environment.

The mechanisms of interactions of humic acids with metal ions belong to issues still unclear, complex and not fully explained. It should be stressed that

there are a lot of questions, unclear and contradictory opinions in scientific environment related to the reactivity of humic acids, and especially to their interactions with metals. The diversity of interpretations of above phenomena covers such subjects as the way of binding between humic acids and metal ion, type of created bonds, type of structure units of humic acids which take part in interactions, as well as the stability of formed compounds. Additional aspects which complicate studies are varied chemical properties of metal: valence, ionic radius, hydrate ratio, electronegativity as well as varied physicochemical nature of humic acids: different molecular weight, degree of structure transformation, content of aromatic rings, aliphatic chains or functional groups. Simultaneously, it should be added that the form, mobility and reactivity of both humic acids and metal ions in the soil environment strongly depend on pH. Depending on circumstances, created compounds can lead to mobilisation of metals and their leaching from upper parts of soils (which is disadvantageous in the case of fertilisation with microelements), or to the accumulation of heavy metals in soil in the form of insoluble humates, which is dangerous in the case of exceeding the concentrations at which microelements positively influence plants. Moreover, concentration of heavy metals in soils still increases together with the development of the cosmetics, agricultural and chemical industry, whereas methods of neutralisation or removing of heavy metals are still unsatisfactory.

For all these of reasons, the problem of the study of humic acids and their interactions with metals in soil is a very complex matter and difficult for realization. These problems are a still burning and open scientific issue, not only in Poland scale, but also for the entire world, and require additional investigations. Above study also brings up the still actual problem of circulation and remediation of metals in the environment.

Above paper is a comprehensive work about the interaction of metal ions with humic acids. The subject includes interactions with “light” and “heavy” metal ions, simultaneously taking into account its significance in agriculture and plant cultivation. The research problem relates also to the influence of different conditions of the environment, like pH, ionic strength, temperature, humic acids origin or chemical properties of different metals, such as ionic radius, oxidation degree, redox properties, chemical forms. The work includes chapters describing humic acids and metal ions as reactive components of the soil system. The most important characteristics of these components are shown, with emphasis on their sorption abilities. Additionally, the work covers description of the most important factors which can determine reactivity. For selected metals, especially important in soil, some subchapters were prepared and they contain literature review relating to their interactions with humic acids. The final chapters are about selected instrumental techniques, useful in the analysis of interactions of humic acids with metal ions.

Knowledge about interactions between heavy metals and soil organic matter characterised by varied physicochemical properties should let predict possible consequences of the emission of the metals to the environment. Simultaneously, this knowledge should contribute to development of more effective methods of heavy metal remediation. Such studies can contribute to improvement of soil cultivation, ecological and integrated tillage, and minimising the usage of chemical toxic substances.

Keywords: humic acids, metal ions in soil, soil sorptivity

9. STRESZCZENIE

ODDZIAŁYWANIA KWASÓW HUMINOWYCH Z JONAMI METALI

Kwasy huminowe stanowią jedną z najsłabiej poznanych frakcji związków próchniczych. Fakt ten wynika z ich skomplikowanej struktury, a w konsekwencji z różnych właściwości chemicznych i fizycznych, a także z różnego oddziaływania z innymi komponentami gleby. Z drugiej strony kwasy huminowe determinują kluczowe własności gleb tj. żyzność, agregatową strukturę, odpowiednie stosunki wodno-powietrzne i warunki mikrobiologiczne. Substancje te stanowią także grupę związków o bardzo silnych właściwościach sorpcyjnych. Oznacza to, że mogą one oddziaływać z różnymi składnikami glebowymi, w tym także z metalami ciężkimi, warunkując ich mobilność.

Mechanizmy oddziaływań związków próchniczych z jonami metali należą do zagadnień wciąż słabo poznanych i złożonych. W środowisku naukowym istnieje wciąż wiele pytań dotyczących reaktywności kwasów huminowych, a w szczególności sposobów ich oddziaływania z metalami. Różnorodność interpretacji powyższych zjawisk dotyczy zarówno sposobu wiązania pierwiastków, typu powstających wiązań, rodzaju struktur biorących udział w interakcjach, jak również stabilności tworzących się połączeń. Dodatkowym aspektem utrudniającym badania są wysoce zróżnicowane właściwości chemiczne metali: ich wartościowość, promień jonowy, hydratacyjny, elektroujemność, jak również właściwości fizykochemiczne i chemiczne samych kwasów huminowych, różna masa cząsteczkowa, stopień przeobrażenia struktury, zawartość pierścieni aromatycznych, łańcuchów alifatycznych czy grup funkcyjnych. Badania te są skomplikowane również ze względu na szereg czynników mających wpływ na procesy oddziaływania metali z glebową materią organiczną, np. pH czy stężenie i forma metali. W zależności od warunków, powstające połączenia mogą prowadzić do wzmożonego uruchamiania metali i ich wymywania z warstw ornych (co jest niekorzystne przy nawożeniu mikroelementami, do których należy także część metali ciężkich) lub też do zatężania metali ciężkich w glebie w postaci trudno rozpuszczalnych humianów, co z kolei jest niebezpieczne w przypadku przekroczenia stężeń, w których mikroelementy wpływają korzystnie na wzrost roślin. Ponadto, stężenie metali ciężkich w środowisku glebowym stale rośnie, co wiąże się z rozwojem różnych gałęzi przemysłu, np. rolnego, chemicznego czy kosmetycznego, podczas gdy metody remediacji gleb wciąż nie są w pełni satysfakcjonujące. Powyższe argumenty pokazują, iż problem badania kwasów huminowych i ich oddziaływań z jonami metali w glebie jest bardzo złożony, trudny do realizacji, a jednocześnie wciąż aktualny i otwarty. Aspekty wymienione powyżej skłaniają do podejmowania kolejnych prac w zakresie badania połączeń kwasów huminowych z jonami metali.

Niniejsza praca stanowi kompleksowe opracowanie zagadnienia oddziaływania jonów metali z kwasami huminowymi. Tematyka obejmuje interakcje z jonami metali lekkich jak i ciężkich, z jednoczesnym uwzględnieniem ich znaczenia w rolnictwie i odżywianiu roślin. Poruszany problem odnosi się również do wpływu różnych warunków środowiska, takich jak: pH, moc jonowa, temperatura, pochodzenie kwasów huminowych czy właściwości chemiczne różnych metali – ich promień jonowy, stopień utlenienia, właściwości redoks, formy chemiczne. Praca zawiera rozdziały opisujące kwasy huminowe i jony metali, jako reaktywne składniki układu glebowego. Pokazane są najważniejsze właściwości powyższych składników warunkujące ich możliwości sorpcyjne w glebie jak również omówione zostały czynniki, które mogą warunkować zmienność reaktywności. Dla wybranych, szczególnie istotnych w glebie metali, wyodrębnione zostały podrozdziały zawierające przegląd literaturowy odnoszący się do ich interakcji z kwasami huminowymi. Końcowe rozdziały ujmują również wybrane techniki instrumentalne, pomocne w analizowaniu oddziaływań kwasów huminowych z jonami metali.

Wiedza na temat interakcji pomiędzy metalami i glebową materią organiczną charakteryzującą się różnymi właściwościami fizykochemicznymi powinna przyczynić się do lepszego poznania i zaprognozowania możliwych konsekwencji emisji metali do środowiska. Jednocześnie wiedza ta może być pomocna w rozwoju bardziej efektywnych metod remediacji gleb, jak również w rozwoju ekologicznej i zrównoważonej uprawy roli i efektywnym wykorzystaniu chemicznych, a niekiedy toksycznych substancji.

Słowa kluczowe: kwasy huminowe, jony metali w glebie, sorpcyjność gleb

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