

VYSOKÉ UČENÍ TECHNICKÉ V BRNĚ

BRNO UNIVERSITY OF TECHNOLOGY

FAKULTA CHEMICKÁ

ÚSTAV FYZIKÁLNÍ A SPOTŘEBNÍ CHEMIE

FACULTY OF CHEMISTRY

INSTITUTE OF PHYSICAL AND APPLIED CHEMISTRY

STABILITY OF COMPLEXES OF HUMIC ACIDS WITH HEAVY METALS

DIPLOMOVÁ PRÁCE

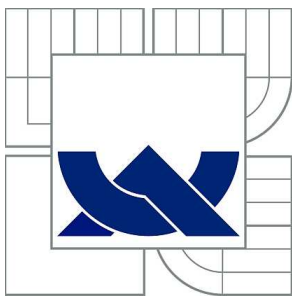
MASTER'S THESIS

AUTOR PRÁCE

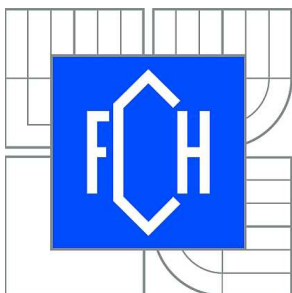
AUTHOR

Bc. KRISTÝNA NOVÁČKOVÁ

BRNO 2011



VYSOKÉ UČENÍ TECHNICKÉ V BRNĚ
BRNO UNIVERSITY OF TECHNOLOGY



FAKULTA CHEMICKÁ
ÚSTAV FYZIKÁLNÍ A SPOTŘEBNÍ CHEMIE
FACULTY OF CHEMISTRY
INSTITUTE OF PHYSICAL AND APPLIED CHEMISTRY

STABILITY OF COMPLEXES OF HUMIC ACIDS WITH HEAVY METALS

STABILITA KOMPLEXŮ HUMINOVÝCH KYSELIN S TĚŽKÝMI KOVY

DIPLOMOVÁ PRÁCE
MASTER'S THESIS

AUTOR PRÁCE
AUTHOR

Bc. KRISTÝNA NOVÁČKOVÁ

VEDOUCÍ PRÁCE
SUPERVISOR

doc. Ing. MARTINA KLUČÁKOVÁ,
Ph.D.

BRNO 2011



Vysoké učení technické v Brně
Fakulta chemická
Purkyňova 464/118, 61200 Brno 12

Zadání diplomové práce

Číslo diplomové práce:	FCH-DIP0508/2010	Akademický rok: 2010/2011
Ústav:	Ústav fyzikální a spotřební chemie	
Student(ka):	Bc. Kristýna Nováčková	
Studijní program:	Spotřební chemie (N2806)	
Studijní obor:	Spotřební chemie (2806T002)	
Vedoucí práce	doc. Ing. Martina Klučáková, Ph.D.	
Konzultanti:	Ing. Jiří Kislinger, Ph.D.	

Název diplomové práce:

Stabilita komplexů huminových kyselin s těžkými kovy

Zadání diplomové práce:

prostudovat stabilitu komplexů huminových kyselin s těžkými kovy

Termín odevzdání diplomové práce: 13.5.2011

Diplomová práce se odevzdává ve třech exemplářích na sekretariát ústavu a v elektronické formě vedoucímu diplomové práce. Toto zadání je přílohou diplomové práce.

Bc. Kristýna Nováčková
Student(ka)

doc. Ing. Martina Klučáková, Ph.D.
Vedoucí práce

prof. Ing. Miloslav Pekař, CSc.
Ředitel ústavu

V Brně, dne 15.1.2011

prof. Ing. Jaromír Havlica, DrSc.
Děkan fakulty

ABSTRACT

This diploma thesis focuses on comparison of thermo-oxidative and chemical stability of metal complexes with humic acids. Three elements representing the group of heavy metals were selected for these experiments (cobalt, copper, nickel). The utilized humic acid was extracted from South-Moravian lignite. Differently concentrated solutions of metals were used for complexes preparation, in order to observe the influence of their initial concentration on both studied stabilities of prepared complexes. Chemical stability of metal complexes was assessed in term of metal ions release from the humic acid structure into two different extraction agents (HCl, $MgCl_2$). Thermo-oxidative behaviour was investigated employing methods of thermal analysis: differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) in particular. The results of this work bring deeper insight into the realm of metal interaction with the humified part of soil organic matter; and at the same time try to shed light on the fate of metal pollutants in the environment and help in the desirable knowledge of treatments such as soil decontamination and remediation.

ABSTRAKT

Tato diplomová práce je zaměřena na porovnání termo-oxidační a chemické stability kovových komplexů huminových kyselin. Pro tyto experimenty byly vybrány tři prvky reprezentující skupinu těžkých kovů (kobalt, měď a nikl). Použitá huminová kyselina byla extrahována z Jihomoravského lignitu. Pro přípravu komplexů byly využito různě koncentrovaných roztoků kovů, aby bylo možné pozorovat vliv jejich počáteční koncentrace na obě studované stability připravených komplexů. Chemická stabilita kovových komplexů byla posuzována z hlediska uvolňování kovových iontů ze struktury huminové kyseliny do dvou různých extrakčních činidel (HCl, $MgCl_2$). Termo-oxidační chování bylo zkoumáno pomocí technik termické analýzy: diferenční kompenzační kalorimetrie (DSC) a zejména termogravimetrické analýzy (TGA). Výsledky této práce přinášejí hlubší poznatky o problematice interakce kovů s humifikovanou částí půdní organické hmoty, využitelné k osvětlení problematiky kovových polutantů v životním prostředí a prohloubení žádoucích znalostí procedur jako jsou dekontaminace půdy a remediac.

KEYWORDS

humic acids, complexes, stability, thermal analysis

KLÍČOVÁ SLOVA

huminové kyseliny, komplexy, stabilita, termická analýza

NOVÁČKOVÁ, K. *Stabilita komplexů huminových kyselin s těžkými kovy*. Brno: Vysoké učení technické v Brně, Fakulta chemická, 2011. 81 s. Vedoucí diplomové práce doc. Ing. Martina Klučáková, Ph.D.

DECLARATION

I declare that the diploma thesis has been worked out by myself and that all the quotations from the used literary sources are accurate and complete. The content of the diploma thesis is the property of the Faculty of Chemistry of Brno University of Technology and all commercial uses are allowed only if approved by both the supervisor and the dean of the Faculty of Chemistry, BUT.

PROHLÁŠENÍ

Prohlašuji, že jsem diplomovou práci vypracovala samostatně a že všechny použité literární zdroje jsem správně a úplně citovala. Diplomová práce je z hlediska obsahu majetkem Fakulty chemické VUT v Brně a může být využita ke komerčním účelům jen se souhlasem vedoucího diplomové práce a děkana FCH VUT.

.....
signature of diploma thesis author
podpis autora diplomové práce

Acknowledgement

I would like to say a word of thanks to my supervisor doc. Ing. Martina Klučáková, Ph.D. for her professional approach and willingness, my second thanks belong to my advisor Ing. Jiří Kislinger, Ph.D. for his help.

CONTENTS

1. INTRODUCTION	7
2. THEORETICAL BACKGROUND	8
2.1. Organic matter in soil.....	8
2.1.1. Soil-forming factors influencing organic matter in soil.....	9
2.1.1.1. The time factor.....	9
2.1.1.2. Influence of climate	9
2.1.1.3. Vegetation.....	9
2.1.1.4. Parent material	9
2.1.1.5. Topography.....	10
2.1.1.6. Effect of cropping	10
2.1.2. Function of organic matter in soil.....	10
2.1.3. Components of organic matter in soil.....	10
2.2. Humic substances.....	11
2.2.1. Basic information.....	11
2.2.2. Structure.....	12
2.3. Other sources of humic acids	15
2.4. Adsorption and Langmuir isotherm	18
2.5. Complexes.....	19
2.5.1. Complexes of humic substances – state of the art	19
2.6. Heavy metals and their toxicity.....	27
2.6.1. Cobalt.....	28
2.6.2. Copper.....	28
2.6.3. Nickel.....	29
2.7. Sequential extraction of heavy metal from soil and SOM	29
2.8. Thermal analysis (TA)	30
2.8.1. Generalities	30
2.8.2. Thermogravimetric Analysis (TGA)	30
2.8.2.1. Factors affecting thermogravimetric measurements.....	31
2.8.3. Differential Scanning Calorimetry (DSC)	32
2.8.3.1. Heat-Flux DSC	32
2.8.3.2. Power Compensation DSC	32
2.8.3.3. Advantages and drawbacks of DSC	33
2.9. Induced coupled plasma - mass spectrometry (ICP-MS).....	33
3. EXPERIMENTAL PART.....	34
3.1. Extraction of humic acid	34
3.2. Characterization of humic acid	34
3.3. Determination of acidic groups content	34
3.4. Complexation	34
3.5. Bound amount determination.....	34
3.6. Thermogravimetric analysis.....	35
3.7. Differential scanning calorimetry	35
3.8. Chemical stability.....	36

4.	RESULTS AND DISCUSSION	37
4.1.	Elemental analysis.....	37
4.2.	Determination of reactive groups content	37
4.3.	Selection of initial ions concentration.....	38
4.4.	Influence of metal presence	39
4.4.1.	Influence of Co^{2+} ion	44
4.4.2.	Influence of Cu^{2+} ion	46
4.4.3.	Influence of Ni^{2+} ion.....	48
4.5.	Influence of extraction agents treatment	50
4.6.	Influence of MgCl_2 treatment.....	52
4.6.1.	Influence of MgCl_2 treatment on Co-humic complexes	52
4.6.2.	Influence of MgCl_2 treatment on Cu-humic complexes	54
4.6.3.	Influence of MgCl_2 treatment on Ni-humic complexes.....	55
4.7.	Influence of HCl treatment.....	57
4.8.	Comparison of extraction agents influence.....	59
5.	CONCLUSION	67
6.	REFERENCES	68
7.	LIST OF ABBREVIATIONS	74
8.	LIST OF APPENDICES	75

1. INTRODUCTION

Soil is from the agricultural and environmental point of view very simply defined as a part of natural environment of the Earth. In the strict sense, soil is loose top layer of the Earth's surface, consisting of rock and mineral particles mixed with decayed organic matter (humus), and capable of retaining water, providing nutrients for plants, and supporting a wide range of biotic communities. It is an open dynamic system formed by a combination of depositional, chemical, and biological processes and plays an important role in the carbon, nitrogen, and hydrologic cycles. Soil consists of solid, liquid and gaseous phase together with soil organisms, edaphon, their metabolic products and all the organic residues in different state of mineralization. Soil types vary widely from one region to another, depending on the type of bedrock they overlie and the climate in which they form.

Very important constituent of soil organic matter are humic substances (HS). The importance of humic substances in soil is due to their high adsorptive capacity. These are capable of combining with metal ions, clays, pesticides, fertilizers and different organic compounds, namely proteins, carbohydrates, vitamins, etc. Besides, these humic substances exert both direct and indirect effects on the physiological activity of plants.

Humic substances were found to influence metal transport through soil layers and control their uptake by plants via root systems. The complexation of metal ions by humic substances is extremely important in affecting the retention and mobility of metal contaminants in soils and waters. Due to their enormous chelation capacity, humic substances are capable of detoxifying lakes that are affected by metal pollution.

The increasing input of toxic heavy metals as well as organic chemicals to the agricultural production system through the use of untreated sewage, sludge, industrial effluents and chemical pesticides tends to exert a long-term impact on soil–crop environment. The question as to how soil organic matter may exert moderating influence (if any) in controlling the activity, behaviour and survival rate of these metals/chemicals is important from the viewpoint of soil ecosystem, and hence the importance of studying metal–humic interactions.

2. THEORETICAL BACKGROUND

2.1. Organic matter in soil

The term soil organic matter (SOM) refers to the whole of the organic matter in soil, including the litter, the light fraction, the microbial biomass, the water-soluble organics and the stabilized organic matter (humus).

The complete soil organic fraction is made up of living organisms and their partly undecomposed, partly decomposed, and completely transformed remains, as well as those of plants. Soil organic matter is a more specific term for the nonliving components that may be described as a heterogeneous mixture composed largely of products resulting from microbial and chemical transformations of organic debris. The transformations are known collectively as the humification process. [1]

Humus can be defined as the total of organic compounds in soil exclusive of undecayed plant and animal tissues, their partial decomposition products, and the soil biomass. Soil organic matter may be partitioned into the active (or labile) and the stable pools. The active fraction contains the comminutive nonliving plant matter (litter) that lies on the surface of the soil, the light fraction, the microbial biomass, and the non-humified substances that are not bound to the soil minerals. Litter is important for recycling of nutrients. The light fraction is incorporated in the soil but separable from it in liquids. It consists largely of plant residues in various stages of decomposition, has a rapid turnover rate, and hence provides a source of plant nutrients. [2] There is an astounding number of microorganisms in the soil environment, as many as 10^{10} per gram of soil. Microorganisms have a vital role for the turnover and transformation processes involving organic (and some inorganic) materials in the soil. Therefore, these are sources of enzymes in the soil environment, and (as well as plant root exudates) are responsible for water soluble organics in the soil solution. [1] The stable, or passive, humus pool of SOM are humification products, being resistant to some biodegradation processes. [2] Soil organic matter is one of the most complex materials in nature, containing most, if not all of the naturally occurring organic compounds. The major components are considered to be recalcitrant remains of plants and algae, including materials derived from lignins, tannins, sporopollenins, and large aliphatic molecules, such as algaenans, cutans, and suberans. [3]

In most agricultural soils, the bulk of the organic matter occurs as stable humus. The dark brown colour of soils is due to their stable humus content. Highly productive soils often have a characteristic rich odour that can be attributed to organic constituents.

Soils vary greatly in organic matter content. In undisturbed soils, the amount present is governed by the soil-forming factors of age, parent material, topography, vegetation and climate. [2]

2.1.1. Soil-forming factors influencing organic matter in soil

2.1.1.1. *The time factor*

Organic matter does not accumulate indefinitely in well-drained soils, and, with time, an equilibrium level is attained that is governed by the other soil-forming factors.

Information on the rate of organic matter accumulation during soil formation has come from studies of time sequences on mud flows, spoil banks, sand dunes and the moraines of receding glaciers. Those studies show that the rate of organic matter accumulation is rapid during the first few years, diminishes slowly and reaches equilibrium in periods of time that vary from as few as 110 years for fine-textured parent material to as many as 1500 years for sandy areas.

Although several reasons have been given for the establishment of equilibrium levels of organic matter in soil, none has proven entirely satisfactory. Included with the explanation are:

- humic substances (HS) are products that resist attack by microorganisms,
- humus is protected from decay through its interaction with mineral matter,
- a limitation of one or more essential nutrients (N, P, S) places a ceiling on the quantity of stable humus that can be synthesized.

2.1.1.2. *Influence of climate*

Climate is the most important single factor that determines the array of plant species at any given location, the quantity of plant material produced and the intensity of microbial activity in the soil; consequently, this factor plays a prominent role in determining organic matter levels.

2.1.1.3. *Vegetation*

It is well-known fact that, other factors being constant, grassland soils have substantially higher organic matter content than forest soils. Reasons given for this include:

- larger quantities of raw material for humus synthesis are produced under grass;
- humus synthesis occurs in the rhizosphere, which is more extensive under grass than under forest vegetation;
- the harsh climatic conditions under which grassland soils are formed lead to organic matter preservation.

2.1.1.4. *Parent material*

Parent material is effective mainly through its influence on texture. It has been well established that, for any given climate zone, and provided constant vegetation and topography, organic matter content depends upon textural properties. The fixation of humic substances in the form of organo-mineral complexes serves to preserve organic matter. Thus, heavy-textured soils have higher organic matter content than loamy soils, which in turn have higher content than sandy soils.

2.1.1.5. *Topography*

Topography, or relief, affects organic matter content through its influence on rainfall runoff and/or retention.

2.1.1.6. *Effect of cropping*

Marked changes are brought about in the organic matter content of the soil through the activities of man. [2]

2.1.2. Function of organic matter in soil

Organic matter contributes to plant growth through its effect on the physical, chemical and biological properties of the soil. It has a nutritional function in that it serves as a source of N, P and S for plant growth, a biological function in that it profoundly affects the activities of microflora and microfaunal organisms and a physical function in that it promotes good soil structure, thereby improving tilth, aeration, and retention of moisture. Other important functions of soil organic matter are:

- formation and the stabilization of soil aggregate structures,
- retention of plant nutrients attributable to its cation-exchange properties,
- absorption of solar radiation (thereby increasing soil temperature),
- complexation of heavy metals,
- retention of aromatic and sparingly soluble anthropogenic (synthetic) organic chemicals,
- release of soluble and colored materials in drainage waters,
- sequestration of C.

More recently, the importance of soil organic matter to considerations of environmental quality has been considered in relation to global climate changes, or the greenhouse effect. It is now accepted that human activity, both agricultural and industrial, has created an imbalance between global sinks and source of carbon, giving rise to increases in atmospheric gases. [1]

2.1.3. Components of organic matter in soil

Both chemical and physical fractionation procedures have been used in attempts to separate the various components of humus and to ascertain their location within the soil matrix. [2] Humus is now known to include a broad spectrum of organic constituents, many of which have their counterparts in biological tissues. Thus, two major types of compounds can be distinguished:

- Nonhumic substances, consisting of compounds belonging to the well-known classes of organic chemistry. Included with this group are such constituents as amino acids, carbohydrates and lipids.
- Humic substances, a series of high-molecular-weight, yellow to black substances formed by secondary synthesis reactions. They can be generally characterized as being rich in oxygen-containing functional groups, notably COOH but also phenolic and/or enolic OH, alcoholic OH and C=O of quinones.

2.2. Humic substances

2.2.1. Basic information

Humic substances are natural organic compounds arising from microbial degradation of decaying plant and animal tissues and synthetic activity of microorganisms. They are the major constituents of humus and largely influence soil quality (e.g. fertility). The most well-known and general definition, as mentioned above, describes them as a series of high-molecular-weight, yellow to black substances formed by secondary synthesis reactions.

HS are among the most widely distributed organic materials on the Earth. They are found not only in soils but also in natural waters, sewage, compost heaps, marine and lake sediments, peat bogs, carbonaceous shales, terrestrial sediments, brown coals (leonardites, lignites) and miscellaneous other deposits.

Humic substances are important in geochemistry and the environment for the following reasons:

- They may be involved in the transportation and subsequent concentration of mineral substances, such as bog ores and nodules of marine strata.
- Humic substances serve as carriers of organic xenobiotics in natural waters. Humic substances play role in reducing toxicities of certain heavy metals to aquatic organisms, including fish.
- HS act as oxidizers or reducing agents, depending on environmental conditions.
- The sorption capacity of the soil for a variety of organic and inorganic gases is strongly influenced by humus. The ability of the soil to function as a “sink” for nitrogen and sulphur oxides in the atmosphere may be in part due to reactions involving organic colloids.
- Humic-like materials in waste waters treated by biological secondary treatment processes create problems at considerable importance in many water works.

HS are not chemical individuals, but mixture of several substances with various composition and structure. [4] One of the unique characteristics of HS is their heterogeneity, which is conditioned by presence of components with several degree of humification with unequal chemical composition. [5] The composition is dependent on their origin, source and extraction method.

There are several ways of classification of HS. One of them divides these substances according to their solubility in acids and alkalis into three basic groups:

- fulvic acids (FA) – soluble under all pH conditions;
- humic acids (HA)– not soluble under acidic conditions ($\text{pH} < 2$) but soluble at basic pH values; and
- humins – insoluble at any pH value.

Humic acids count among the most important fraction of humified soil organic matter, because its participation on global carbon cycle is about 16 %, in contrast with fulvic acid and humins, which participate on global carbon cycle only by 8 %. [6]

2.2.2. Structure

From the point of view of elemental analysis, HS consist of carbon, oxygen, hydrogen and small amounts of nitrogen and occasionally phosphorus and sulfur. The C content ranges from 53.8 to 58.7 % in case of soil humic acids, for coal ones is reported slightly higher. Fulvic acids have lower C (usual range from 40.7 to 50.6 %) but higher oxygen (39.7–49.8 %) content. Briefly, elemental analyses data of humic samples originating from miscellaneous sources differ in their elementary composition and reactivity. [2]

From the chemical point of view, humic molecules are composed of aromatic and/or aliphatic moieties and with specific content of functional groups. Particularly carboxyl COOH, phenolic OH, enolic OH, quinone, hydroxyquinone, lactone, ether, and alcoholic OH groups have been reported in humic substances. Their number and position depend on the conditions of formation. Biphenolic and/or triphenolic aromatic cycles are fundamental structural units, on which $-\text{COOH}$, $-\text{O}-$, $-\text{NH}-$, $-\text{CH}_2-$, $=\text{N}-$, $-\text{S}-$ are bonded. Although, undisputable differences exist in way of their genesis, humic substances from different sources should be considered as members of the same class of chemical compounds. [7]

Concerning the structure, many models of HA and FA were suggested, but they should be considered only as models taking into account average composition.

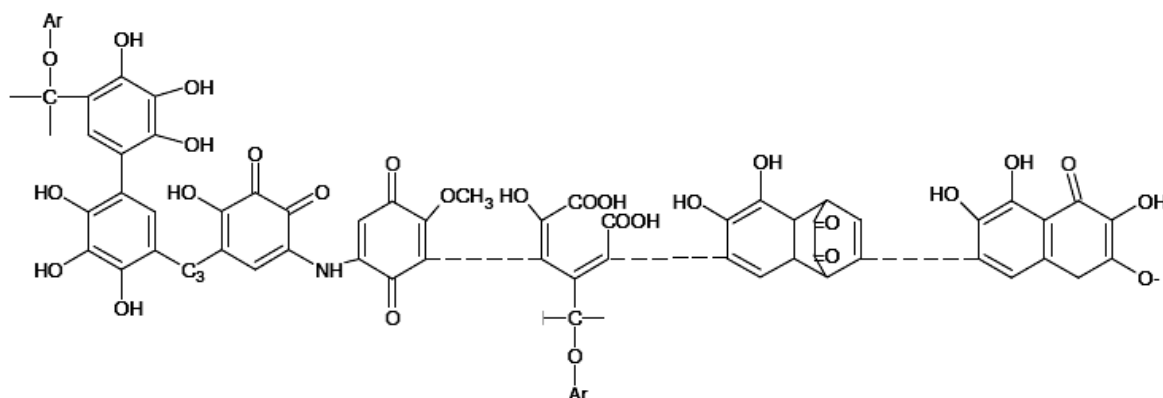
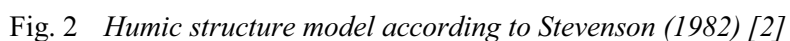


Fig. 1 Humic structure model according to Flaig (1960) [2]

One of these models was suggested in 1960 by Flaig (Fig. 1). There is a great amount of quinone units and bonded phenolic OH groups. Noticeable imperfection of this model is the assumption of very low carboxylic groups content. This drawback was suppressed in the case of Stevenson's model, in Fig. 2 one can see much more carboxylic groups in the scheme.



13

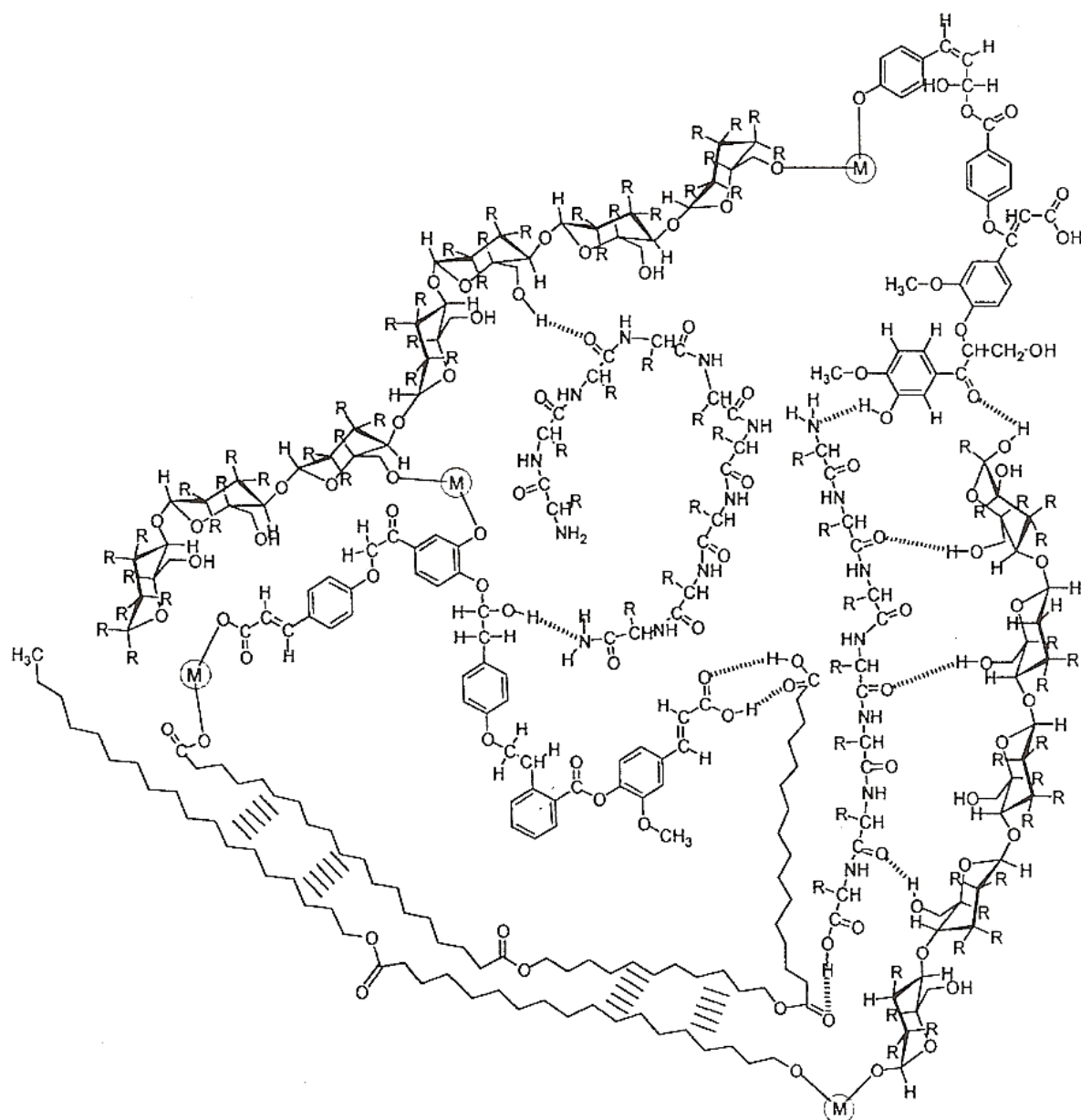


Fig. 3 Recent model of structure of humic acid according to Simpson et al. (2002) [1]

Up-to-date, there are two well-established models used for description of humic substances structural and conformational properties. Both of them have their supporters and dissenters.

So-called “polymer model” says that humic substances comprise randomly coiled macromolecules, which have distinct properties in dependence on acidity and ionic-strength of their ambience. They have elongated shapes in basic or low-ionic-strength solutions, and became coils in acidic or high-ionic-strength solutions. [2] This concept is based e.g. on the careful ultracentrifugation study of humic substances by Cameron. [8]

More recent model of humic substances is the model of supramolecular associations, in which many relatively small and chemically diverse organic molecules form clusters linked by hydrogen bonds and hydrophobic interactions. This theory is strongly supported by Piccolo in his studies. [9][10][11]

2.3. Other sources of humic acids

Up to now, soil has been mentioned as the major source of natural organic matter. But there are several more humified materials worth mentioning. The most important ones are peats and miscellaneous types of coals. They all play crucial roles in a process called *coalification*.

The so-called coalification or carbonification includes the process of diagenesis, and the metamorphic changes of the coal. The diagenesis of the coals is often referred to as “biochemical coalification”, whereas the metamorphism of coals is called “geochemical coalification”. The coalification begins with peat and ends with highly metamorphic anthracite. Graphite is formed only at pressures and temperatures prevailing during the metamorphism of rock. The diagenesis of rocks should be considered as terminated with the transformation of anthracite to graphite. Inasmuch as carbonification is a very complex process one must use different properties to measure the rank of a coal. The values of the different rank parameters change with the degree of carbonification. [12]

Most coals originate from peats or low moors with plant associations of forests or reeds. Plants usually decompose after their death, i.e. under the influence of oxygen they are converted into gaseous compounds and water. In swamps with a high water table and lack of aeration, however, the plant residues are in a reducing environment, which is conducive to the process of peat formation.

One can differentiate between humic coals and sapropelic coals. Humic coals originate from true peats, which develop through the accumulation of dead plant matter at the site of the peat forming plants. Sapropelic coals, on the other hand, are formed from organic muds, which are deposited on the floor of poorly aerated quiet-water lakes and ponds. They contain many allochthonous elements. The sapropels do not undergo peatification, but pass through a process of “saprofication”, which is characterized by putrefaction processes under anaerobic conditions. The sapropelic coals are relatively rare. Most humic coals originate from forest peats, and thus mainly from wood and bark substances, leaves and roots of swamp vegetation. In swamps with herbaceous plant associations (e.g. reed marshes), the roots of the sedges and grasses play an important role in the formation of peat. During the process of peat formation, wood, bark, leaves and roots are usually almost completely transformed into humic substances, which are characterized by relatively high oxygen content. These humic substances eventually form vitrinite. Exinite is formed from the chemically resistant and relatively hydrogen-rich plant components such as pollen and spores, leaf epidermis (cuticles), resins, and waxes. These substances are of minor importance in comparison with the humic matter. Besides, relatively carbon-rich macerals (i.e. smallest petrographical unit of coal) occur in coals such as fusinite and micrinite, which are combined under the term inertinite. These components originate from the strong aerobic decomposition of plant residues at the peat surface, with the exception of a part of the fusinite which is derived from fossil charcoal. [13]

Chemically, the parent material of coals consists mainly of cellulose, hemicelluloses and lignins, with minor amounts of proteins, sugars, pentosanes, pectines, tannins and bitumens. The bitumens comprise such substances as fats, oils, waxes, resins, sterins, sporopollenins, cutine and suberine. The inorganic components of the coal originate partially from the plants.

Most of them, however, were transported by water or air into the swamps (clay, silt, sand); or they were precipitated syngenetically or epigenetically from solutions in the peat or coal (pyrite, quartz, calcite, siderite, dolomite, etc.).

The principal characteristics of a coal are its thickness, lateral continuity, rank, maceral content and quality. Apart from rank, which is governed by burial and subsequent tectonic history, the remaining properties are determined by factors controlling the mire where the peat originally formed. These factors include type of mire, type(s) of vegetation, growth rate, degree of humification, base-level changes, and rate of clastic sediment input.

About 3 % of the of the earth's surface is covered by peat, totalling 310 million hectares. During the last years, numerous studies have attempted to understand more fully how peat-producing wetlands or mires are developed and maintained, and in particular how post-depositional factors influence the formation of coals. Peat producing wetlands can be divided into ombrogenous peatlands or mires (owing their origin to rainfall) and topogenous peatlands (owing their origin to a place and its surface/groundwater regime). A great variety of topogenous peats form when water-logging of vegetation is caused by groundwater, but ombrogenous peats are of greater extent but less varied in character. The classification of the two hydrological categories of mire lists a number of widely used terms. [14]

The resultant characteristics of coals are primarily influenced by the following factors during peat formation: type of deposition, the peat-forming plant communities, the nutrient supply, acidity, bacterial activity, temperature and redox potential. For a mire formation and peat accumulation inflow and precipitation must be in balance with outflow, evapotranspiration and retention.

The conditions necessary for peat accumulation are therefore a balance between plant production and organic decay. Both are a function of climate, plant production and organic decay; such decay of plant material within the peat profile is known as humification (as described above). The upper part of the peat profile is subject to fluctuations in the water table and is where humification is most active. The preservation of organic matter requires rapid burial or anoxic conditions, the latter being present in the waterlogged section of the peat profile. In addition, an organic-rich system will become anoxic faster than an organic-poor one as the decay process consumes oxygen. This process is influenced by higher temperatures, decay rates being fastest in hot climates. Rates of humification are also affected by the acidity of the groundwater, as high acidity suppresses microbial activity in the peat. Peat formation can be initiated by:

- terrestrialization, which is the replacement due to the setting up of a body of water (pond, lake, lagoon, interdistributary bay) by a mire;
- paludification, which is the replacement of a dry land by a mire, e.g. due to a rising groundwater table. [15]

One general system of nomenclature divides coal into four major types: anthracite coal, bituminous coal, subbituminous coal and lignite coal. Lignite is the coal that is lowest on the metamorphic scale. It may vary in colour from brown to brown-black and is often considered to be intermediate between peat and the subbituminous coals. Lignite is often

distinguished from the subbituminous coals having a lower carbon content and higher moisture content. Lignite may dry out and crumble in air and is certainly liable to spontaneous combustion. It is known to contain high portion of humic acids, usually up to 80 %. [16]

Slight oxidation of lignite deposits produces so-called leonardite, which is even richer in humic acids content (up to 90 %). These materials are traditional sources for humic acids extraction, used also by the International Humic Substances Society.

2.4. Adsorption and Langmuir isotherm

Adsorption can be described as capturing of molecules (gaseous or liquid) on solid surface. There are two main types of adsorption. In the first type the forces are of physical nature and the adsorption is relatively weak. These forces are known as van der Waals forces and this type of adsorption is named physical adsorption or physisorption. The second type is based on holding of molecules to the surface by covalent forces, it is called chemisorption. An important consequence of chemisorption is that after the surface has become covered with a single layer of adsorbed molecules, it is saturated; additional adsorption can occur only on the layer already present, and this is generally weak adsorption. Thus the chemisorption involves the formation of an unimolecular layer. [17]

During the contact of solid with solution, the solute slowly focuses on the surface. Concentration of the solute in the solution decreases, while the concentrations in solution and on the surface are in equilibrium. [18]

An equation that relates the amount of a substance attached to a surface to its concentration in the gas phase or in solution, at a fixed temperature, is known as an adsorption isotherm. The simplest model is called the Langmuir isotherm, according to its discoverer (Irving Langmuir, 1916). [17]

It was theoretically established on the basis of kinetic concept with the assumptions of:

- formation of only one molecular layer,
- equal probability of adsorption at any part of the surface, and
- independence of adsorbed molecules (they do not influence each other).

Commonly used form of this equation is as follows:

$$a = a_{\max} \frac{bc}{1 + bc}, \quad (1)$$

where a is adsorbed amount, a_{\max} is the maximal amount which can be adsorbed, b is constant of equilibrium between adsorption and desorption and c is concentration. [18]

There also exist several other types of isotherm, e.g. Freundlich, Brunauer-Emmet-Teller.

2.5. Complexes

Complex is a molecular entity formed by loose association involving two or more component molecular entities (ionic or uncharged), or the corresponding chemical species. The bonding between the components is normally weaker than in a covalent bond. [19]

Coordination compound is characterized by its composition (the central atom and ligands present), as well as the spatial arrangement of these components – its structure. Central atom and donor atoms of ligands are usually bound by donor-acceptor bonds. [20]

2.5.1. Complexes of humic substances – state of the art

Thanks to great amount of carboxylic and phenolic groups, HS are able to chelate positively charged ions. [4] Metal-HS complexation in soils is of practical and theoretical significance for several reasons. For example, chemical weathering of rocks and minerals, and related soil genesis and evolution are known to involve metal complexation reactions with HS. The bioavailability of several metal ions, especially trace elements, is strongly influenced by complexation with the soluble and insoluble fractions of HS. For instance, at pH values commonly found in soils, nutrient metal ions that would ordinarily be converted to insoluble forms may be maintained in solution by complexation with HS, thus increasing their bioavailability. On the contrary, the concentration of a toxic metal ion may be reduced to a nontoxic level through complexation to insoluble fractions of soil HS, whereas soluble HS complexes can work as metal-carrier in the transport to ground- and surface-water bodies, thereby rendering the water unfit for several uses. Further, complexation of metal ions by HS may represent a challenging problem for their analytical determination in soil samples. [21]

Humic substances contain a large number of complexing sites per molecule, and thus behave as other natural “multiligand” complexing agents like proteins and metal oxides, which are distinguished from “simple” ligands such as inorganic anions and amino acids. The principal molecular characteristics that govern the complexing ability of HS are polyelectrolyte character, hydrophilicity, and the capacity to form intermolecular associations and change molecular conformation. [22]

The major functional groups in HA and FA that can bind metal ions are O-containing groups, including carboxylic, phenolic, alcoholic and enolic hydroxyl groups as well as carbonyl functionalities of various types. Aminogroups and S- and P-containing groups are also involved in metal binding.

Several types of binding reactions can be visualized between metal ions and HS, the simplest case being the 1:1 binding. Formation can also be expected of mononuclear complexes with the central group being either the HS macromolecule or the metal ion, and of polynuclear complexes. The most common stoichiometries assessed are HS:metal = 1:1 and 2:1, often involving the formation of metal chelates. Aromatic carboxyls and phenolic groups play a prominent role in the 1:1 binding of metal ions by soil HA and FA, by forming chelates that involve two COOH groups in a phthalic-type site (Eq. (2)) and both phenolic and COOH groups in a salicylate-site (Eq. (3)). The most stable complexes involve the more strongly acidic COOH groups, whereas the least stable complexes are believed to be associated with

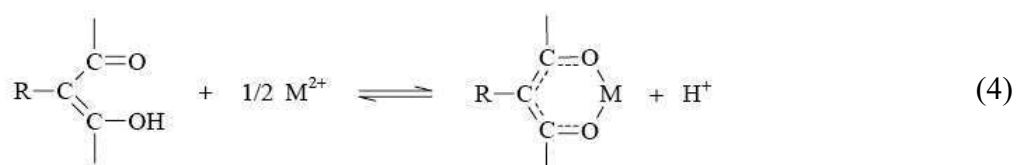
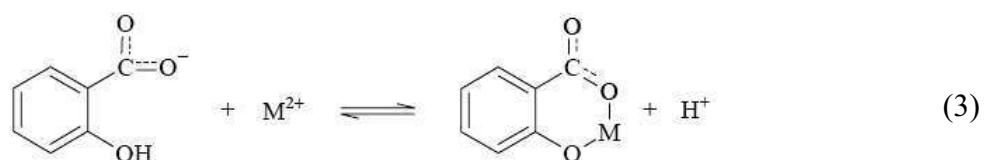
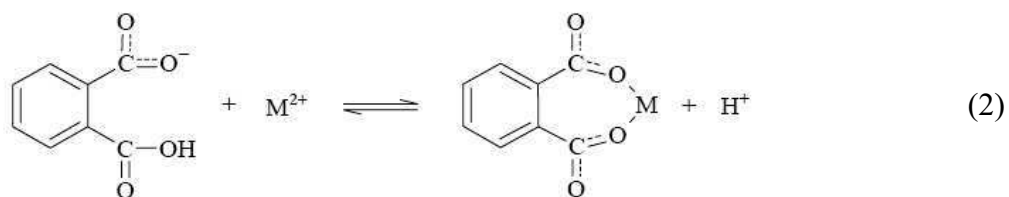
weakly acidic COOH and phenolic OH groups. Other possible combinations involve two phenolic OH, quinone, NH₂, and sulphuryl groups and conjugated ketonic structures (Eq. (4)). Non-aromatic carboxyl and hydroxyl sites also may be involved in metal ion binding by soil HA and FA. The monomeric analogues of some of these sites, e.g., pyruvic and glycolic acid (Eq. (5) and (6)), have binding constants similar to phthalic and salicylic acids.

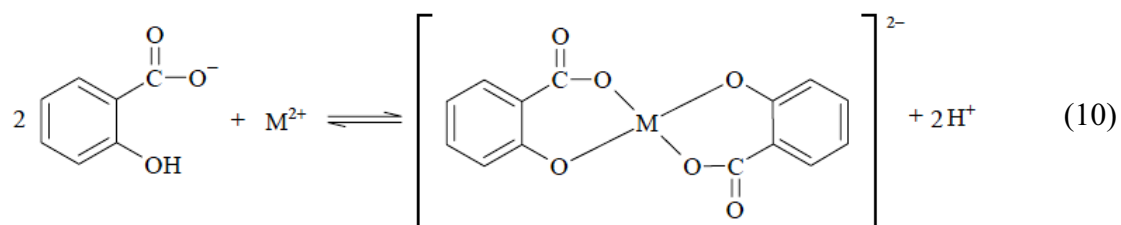
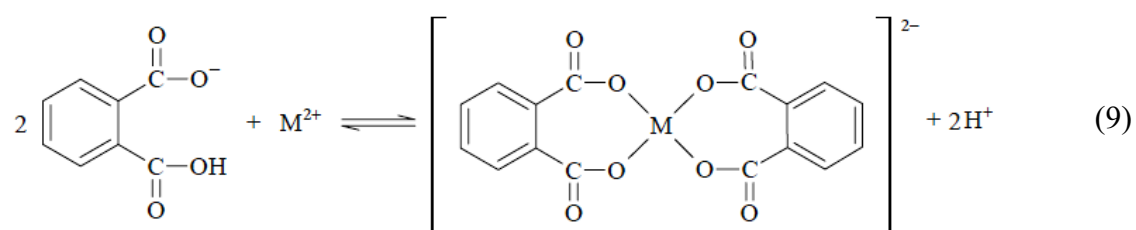
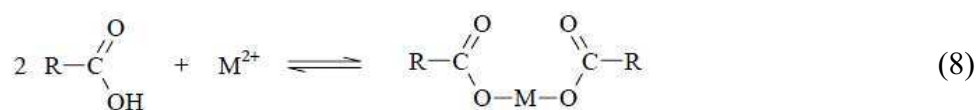
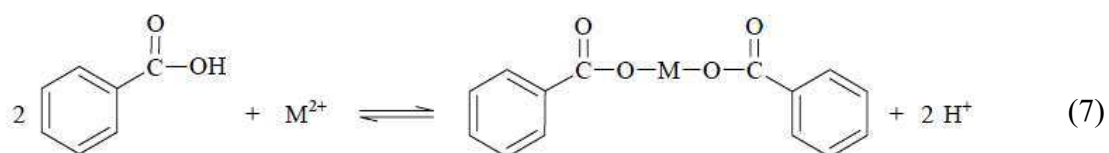
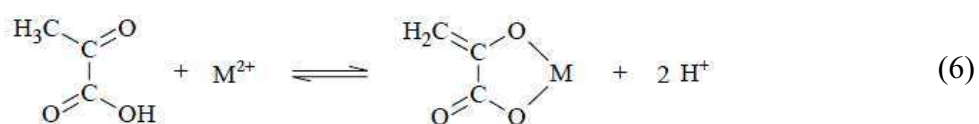
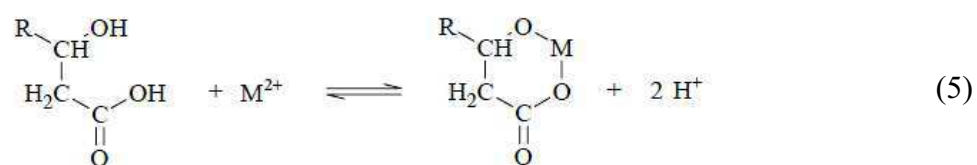
Metal ions also may coordinate with ligands belonging to two (or more) HS molecules, forming 2:1 complexes (Eq. (7) and (8)) and/or chelates (Eq. (9) and (10)), and eventually producing a chain structure (Eq. (11)) that may result in the aggregation and precipitation as the chain grows at high metal to HS ratios.

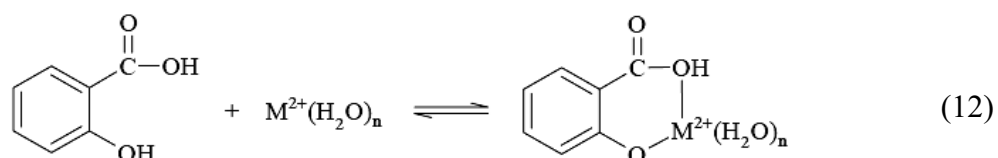
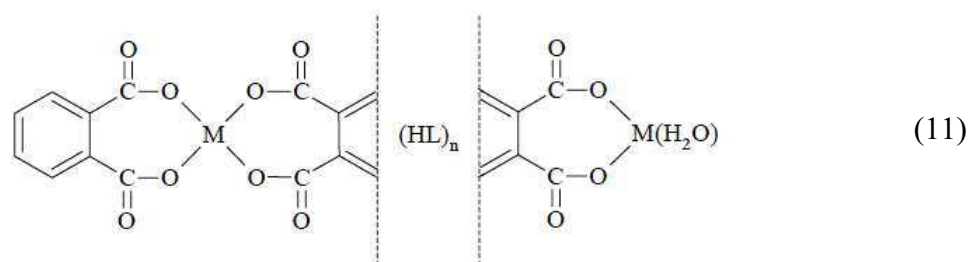
Two main types of complexes may be formed between metal ions and HS:

- inner-sphere complexes, resulting in the formation of bonds with some covalent character between the ligand atom(s) and the metal ion, both completely or partially dehydrated, and
- outer-sphere complexes that result in the electrostatic attraction between the ligand(s) and the metal ion that remains completely hydrated.

For simplicity, all reaction schemes described in Eqs (2) to (11), show formation of inner-sphere complexes, but they may represent outer-sphere HS complexes if the cation is solvated (e.g. Eq. (12)). [21]







Lishtvan et al. [23] assumed in their studies that the mechanism of metal-humic complex (MHC) formation from solutions consists of two stages, namely, micro- and macro-coordination. The micro-coordination term refers to binding of metal cations to functional groups of HAs to form coordination sites; the macro-coordination, to binding of additional amounts of cations to the developed surface of colloidal aggregates, which was formed at the first stage, to yield macromolecular complexes. The validity of this mechanism of MHC formation is based on the fact that HAs are characterized by the presence of both functional groups and supramolecular formations of colloidal nature with a charged surface. According to infrared (IR) and electron paramagnetic resonance (EPR) spectroscopy data, when copper ions interact with HAs, the ions are first bound directly with carboxyl groups. This is evident from a decrease in the intensity of the absorption bands attributed to carboxyl groups and the enhancement of the absorption of carboxyl ions in the course of MHC saturation with the metal, as well as from the disappearance of the EPR signal of bound trivalent iron ions as a result of its inner-sphere displacement by copper ions in the case of HAs of low-moor peat. An additional argument for the penetration of copper into inner-sphere complexes of HA associates is the absence of a solvation shell upon its sorption by carboxyl cationites, which are, actually, represented by HAs. Thus, the formation of copper-humic acid complexes is consistent with the mechanism based on micro- and macro-coordination stages of MHC formation.

Datta et al. [24] compared properties of natural and synthetic humic acids concerning their complexing ability. The natural and synthetic humic acids were characterized by potentiometric titrations, viscosity and surface tension measurements, as well as visible spectrometry. The stability constant of complexes formed by these humic acids with Cd^{2+} ions in aqueous phase was evaluated by the ion-exchange method. According to this study the humic acid extracted from the soil organic fraction tends to exhibit complexation tendencies for Cd^{2+} ions in a manner essentially quite similar to that exhibited by the synthetic humic acid samples. This demonstrates the usefulness of studying the properties and metal-complexation behaviour of synthetic humic acids as analogues of the natural ones.

The aim of work of Jose M. Garcia-Mina team [25] was to investigate the influence of pH and the metal:humic substances ratio on HS complexing capacity and the stability and solubility of metal–HS complexes in solution. Their results showed that HS complexing capacity significantly varied as a function of pH, thus indicating the influence of both functional group ionization and molecular conformation on this property. As was expected, total acidity affected the complexing capacity of the selected HS. The results related to stability and complexing capacity indicated the possible presence of two binding patterns, one at acid-neutral pH probably involving carboxylates, and another at alkaline pH probably involving carboxylates and phenolic groups. The relationship between these binding patterns and the strength of the binding process varied according to the complexed metal.

Kislenko's study has shown that humic acids and sodium humates are bound with double-charged transition metal cations in aqueous solutions within the pH range 3–5. The amount of transition metal bound to humic acids grows with the pH increasing from 4.0 to 4.6. The bending point in the curve of potentiometric titration of double-charged transition metal cations with humic acids and sodium humates occurs at ratios equal to 1.6, 2.0, and 1.4 mmol g⁻¹ for Cu²⁺, Co²⁺, and Ni²⁺, respectively; its position only slightly depends on the initial pH of the titrants. [26]

Identical metal ions were used also in [27]. This work is aimed on studying sorption kinetics of differently treated lignite and humic acids. The quantity of adsorbed ions was calculated on the basis of decrease of UV-VIS absorbance (measured at characteristic wavelengths) of each metal salt solution. These results were in good accordance with the Langmuir model, thus the chemical binding of metal ions to the surface was assumed. The most effective adsorption was confirmed for Cu²⁺ ions on all samples of lignite and humic acids used. The sorption capacity of humic acid was substantially higher in comparison with lignite, hence the humic acid was considered as the dominant component of lignite for the sorption of metal ions.

Martyniuk et al. [28] accomplished experiments to shed light on the course of adsorption of metal ions from single- and multi-component solutions in dynamical conditions. They have found that the highest affinity to ion-exchange centers of humic acids has the Pb ion. The two forms of humic acid (solid and gel-like) adsorbed on average of 290 mg Pb g⁻¹ HA. Metals characterized with large atomic weights are adsorbed in considerable amounts (Ag, Hg, Cu, Ba and Cd are from 100 to 150 mg Me g⁻¹). Ions of Ni, Co, Mn, Zn and Ca are adsorbed in smaller amounts (average of 70 mg Me g⁻¹), whereas relatively light ions of a high valence like Mg²⁺, V⁴⁺, Cr³⁺, Al³⁺ and Fe³⁺ are adsorbed in the smallest amounts. Different adsorption abilities of metal ions on humic acids can be illustrated by following line: Pb > Ag ~ Hg > Cd ~ Ba ~ Cu > Ni ~ Co ~ Mn ~ Zn ~ Ca > Mg ~ V ~ Al ~ Cr.

The examination of metal ion exchange properties of humic acids carried out in multi-component solutions, simulating industrial wastes, showed different behavior of the humic acids, as compared to behavior in single-component solutions. Generally, humic acids adsorbed more or at least the same amount of metal ions. Solid and gel forms of humic acids showed selective affinity to ions of Pb from multi-component solutions. On the other hand, the gel forms showed selective affinity mainly to trivalent ions Cr³⁺ and Al³⁺. In case of multi-component solutions, the smallest affinity for humic acids showed Cd, Ni, Mn, Ca and Mg

ions. The relatively large adsorption of Fe^{2+} and Fe^{3+} ions on gels of humic acids may be explained with physical adsorption of iron salts, as confirmed by EPR spectra.

Porasso et al. [29] performed potentiometric titrations for humic acid in solution in the presence of different environmentally important (heavy) metals (Ca, Cd, Cu, Ni, and Pb) at various metal concentrations by titrating with potassium hydroxide without additional salt. From proton release data obtained for the initial point in the titration, it was estimated that the interaction of the different metals with the humic acid in terms of binding strength increased in the order $\text{Ca} < \text{Cd} \sim \text{Ni} < \text{Pb} \sim \text{Cu}$.

The studies of Madronová et al. [30] were directed to properties of the ion exchange of humic acids prepared from oxidized young coals of the North-Bohemia coal field, the so-called oxyhumolites. They have tested solutions containing only one metal (Pb, Cd, Cu, Zn, Ni, Ca, Mg, Co, Mn) and also solutions containing two and more metals. The result of their experiments is that the sorption efficiency of metals is dependent on the composition of the solution and the pH. It is important that already sorbed metals are not washed out by demineralized water and remain sorbed. They can be ‘pushed-out’ by another metal in the solution that sorbs more firmly or by an acid.

Jin et al. [31] investigated properties of single and multiple metal adsorptions. The extent of reaction was $\text{Cr}^{3+} > \text{Pb}^{2+} > \text{Cu}^{2+} > \text{Ag}^+ > \text{Cd}^{2+} = \text{Co}^{2+} = \text{Li}^+$ for single metal adsorption and $\text{Cr}^{3+} > \text{Pb}^{2+} > \text{Cu}^{2+} = \text{Ag}^+ = \text{Cd}^{2+} = \text{Co}^{2+} = \text{Li}^+$ for multiple metal adsorption on humic acid. The sorption rates of all metal ions on HA seem to be very rapid. Most of the adsorption occurred within 2 minutes, but sorption did not reach equilibrium in 1 day under competitive conditions. The presence of Cr^{3+} greatly decreased the adsorption of all other metals to the extent that some were not adsorbed at all. The study demonstrates lower total sorbed amount during the multiple sorption in comparison with single metal sorption.

Hoop et al. [32] studied stability behaviour of heavy metal complexes of humic acids by voltammetry for a number of different cases, including different metal ions; various concentrations of the supporting salt covering a range of more than two orders of magnitude; different total metal concentrations of more than one order of magnitude and different charge densities of the humic acid. The general conclusion is that voltammetry is a sound experimental technique for dealing with these types of natural polyelectrolytes in solution with (trace) concentrations of heavy metal ions. On the theoretical side, the present model offers a very satisfying description of the experimental results over the whole range of the different system variables explored. This agreement has furthermore been reached by variation of only one parameter, the intrinsic free energy of binding, G_b . It is found that a value of $G_b = -12.0$ is sufficient for a very good description of all different experimental situations studied in this work. It is furthermore found that this particular value, which indicates a strong bonding interaction, is the same for the two analyzed systems: Zn-humic acid and Cd-humic acid. This implies that the strength of the polymer-metal ion interaction is very similar for these two environmentally important heavy metal ions.

The effect of the consecutive annual additions of pig slurry at rates of 0 (control), 90 and 150 $\text{m}^3 \text{ha}^{-1} \text{y}^{-1}$ over a 4-year period on the binding affinity for Cu^{2+} of soil humic acids was

investigated by Plaza et al. [33] in a field plot experiment under semiarid conditions. The appropriate treatments of pig slurry previous to its application to soil, which are able to transform organic matter into more stabilized forms by increasing its humification degree, would enhance its affinity for Cu^{2+} . This, in turn, would reduce the pollution hazards and phytotoxicity problems associated with the excessive input of copper by pig slurry into amended soils.

Fluorescence behaviour of HA and its Zn and Ni complexes were studied by Provenzano et al. [34]. According to their experiment, fluorescence spectra of HA appear to be related to the nature and origin of the sample. A strong reduction of intensity of all peaks was observed in the spectra of HA–metal complexes as compared to those of untreated HA. Ni^{2+} exhibits greater quenching ability than Zn^{2+} .

Another study devoted to complexation of humic acids was performed by Prado et al. with humic acids from peat soil fraction and Cu^{2+} and Zn^{2+} ions. After complexation and lyophilization of all samples, the infrared spectroscopy was used to observe the changes on intensity of unaltered HA and its Cu/Zn complexes. Results obtained from IR spectroscopy confirm the interaction between HA and Cu/Zn. With the application of Lingane equation, the authors determined the stability constants of produced complexes. Substantial difference between the values for Cu and Zn complexes was found. This difference is ascribed to the probability of creation of each bond. Thus, the Cu-humic complex has more than 8 orders of magnitude higher possibility to emerge. [35]

Ioselis et al. [36] prepared a series of humic acid-metal (Fe, Al) complexes and subjected them to thermogravimetric and spectroscopic studies. The activation energies of decarboxylation were found to be 6–9 kcal mol⁻¹. H₂O and CO₂ accounted for the main weight loss of HA and its complexes. No distinction could be made between organic and inorganic sources of water. Interaction of Fe^{3+} with HA at a low pH probably causes reactions, such as oxidation of phenols to quinoids, transamination, decarboxylation, etc. This is supported by IR spectroscopy and elemental analysis. The authors assume that humic acids fixed on clays might possess thermal properties similar to those of the HA oxidized by Fe^{3+} at low pH.

The investigation of Lu et al. [37] has shown that the decomposition of the metal forms of humic substances occurs at temperatures that are significantly lower than that of humic substances. These different thermal stabilities are most likely related to ligands in humic substances. To account for the lower thermal stability of the metal complexes, the interactions between the metal ion and its ligands, together with the surrounding structure need to be clarified and the physical properties of the metal ions should be considered.

It has been known that an intermolecular chelation of metal ions to organic ligands leads to a charge-density rearrangement in the course of a reaction, e.g. for the reaction $\text{AB} + \text{C} \rightarrow \text{AB} - \text{C}$, a degree of charge transfer takes place from B (acting as an electron donor) to C (acting as an electron acceptor). [38]

According to Schnitzer and Kodama [39], the thermal stability of the sodium and calcium forms of HA appears to be related to the cation radii. This is because the smaller ionic radii and higher charge of the calcium ion can abstract more electrons from the bound ligands so that the calcium-humic substance has a lower thermal stability than the sodium-humic substance.

On the other hand, according to Tan [40], aromatic carboxyl groups and adjacent phenolic OH groups can form a salicylate-like ring, or two COOH groups in close proximity can form a phthalate-like ring. Because of chelation of metal ions to the COOH and adjacent OH ligand in humic substances, a certain amount of charge transfers from oxygen to metal ions and can induce a charge-density arrangement within the functional groups, which affects the thermal decomposition behavior during pyrolysis.

Decomposition of the Mn^{2+} and Fe^{3+} forms of humic substances appears at lower temperatures than the decompositions of the Na^+ and Ca^{2+} forms. It was proposed by Schnitzer and Kodama [39] that di- and tri-valent metal ion reacts simultaneously with both, carboxylic COOH and phenolic OH groups, forming a complex or chelation structure, and thus adding a strain on the complexing or chelation structures. Such a strain could cause thermal decomposition at relatively lower temperature. While sodium ion reacts with one ligand only, there is less strain exerted on the binding structure and this may account for the relatively high thermal stability of this form. [37]

2.6. Heavy metals and their toxicity

Heavy metals are chemical elements including transient metals, some metalloids, lanthanides and actinides. There are several definitions according to different properties, such as density, atomic number, atomic weight or according to toxicity, characterizing heavy metals (see Table 1). [41]

Tab. 1 *Heavy metals definitions*

1. Definitions in terms of density (specific gravity)
Metals fall naturally into two groups: the light metals with densities below 4 and the heavy metals with densities above 7. [42]
Metal having a density greater than 4. [43][44]
Metal of high density, especially a metal having a density of 5.0 or over. [45][46][47]
Metal with a density greater than 6 g cm^{-3} . [48][49]
Metal with a density higher than 4.5 g cm^{-3} . [50]
Metal with a density above $3.5\text{--}5 \text{ g cm}^{-3}$. [17] [51]
2. Definitions in terms of atomic weight (relative atomic mass)
Metal with a high atomic weight. [52][53][54]
Metal of atomic weight greater than sodium. [55][56]
A metal such as cadmium, mercury, and lead that has a relatively high relative atomic mass. The term does not have a precise chemical meaning. [57]
Metal with a high relative atomic mass. The term is usually applied to common transition metals such as copper, lead or zinc. [58]
3. Definitions in terms of atomic number
In plant nutrition, a metal of moderate to high atomic number e.g. Cu, Zn, Ni, Pb, present in soils due to an outcrop or mine spoil, preventing growth except for a few tolerant species and ecotypes. [59]
Any metal with an atomic number beyond calcium. [60]
Any element with an atomic number greater than 20. [61]
Metal with an atomic number between 21 (scandium) and 92 (uranium). [62]
Any metal with atomic number >20 , but there is no general concurrence. [63]
4. Definitions based on other chemical properties
“Heavy metals” is the name of a range of very dense alloys used for radiation screening or balancing purpose. [64]
Intermetallic compound of iron and tin (FeSn_2) formed in tinning pots, which have become badly contaminated with iron. The compound tends to settle to the bottom of the pot as solid crystals and can be removed with a perforated ladle. [65]
Lead, zinc, and alkaline earth metals that react with fatty acids to form soaps. “Heavy metal soaps” are used in lubricating greases, paint dryers, and fungicides. [66]
Any of the metals that react readily with dithizone ($\text{C}_6\text{H}_5\text{N}$), e.g., zinc, copper, lead, etc. [67]
5. Definitions without a clear basis besides toxicity
Metallic elements of relatively high molecular weight. [68]
Element commonly used in industry and generically toxic to animals and to aerobic and anaerobic processes, but not every one is neither dense nor entirely metallic. Includes As, Cd, Cr, Cu, Pb, Hg, Ni, Se, Zn. [69]
Outdated generic term referring to lead, cadmium, mercury, and some other elements, which generally are relatively toxic in nature; recently, the term toxic elements has been used. The term also sometimes refers to compounds containing these elements. [70]

Heavy metals are stored in living organisms (plants and animals) and after their death, the metals become nutrition for other animals (up to humans), for which a greater concentration of heavy metals in their body is mortal. [71]

Metals in the environment migrate in geochemical and biological cycles. In different moment they can get out of these cycles to cumulate very often in for example soils and living organisms. The mobility of toxic elements in soils is strongly influenced by soil acidity, meaning that the mobility of most elements increases with decreasing pH. The increase of mobility, depending on the pH value is different for each element.

The mobility of toxic elements in soil is also influenced by the amount and quality of organic matter. Organic compounds with relatively low molecular weight, small proportion of the aromatic core and low degree of condensation (fulvic acids) form chelates and organic complexes with hazardous elements. Such complexes are often highly soluble and increase the mobility of risk elements. In contrast, organic acids with a predominance of aromatic cores of large molecular weight with a high degree of condensation (humic acid) immobilize the hazardous elements in soil. The mobility of these elements has a significant influence on their migration in the soil profile and on their bioavailability. [72]

2.6.1. Cobalt

Cobalt is essential to all animals, including humans. It is a key constituent of cobalamin, also known as vitamin B₁₂. A deficiency of cobalt leads to anemia, a lethal disorder. Anemia secondary to cobalt deficiency is very rare, though, because trace amounts of cobalt are available in most diets. The minimum presence of cobalt in soils markedly improves the health of grazing animals, and an uptake of 0.2 mg kg⁻¹ a day is recommended.

Cobalt poisoning can occur only if the organism is exposed to large quantities of cobalt. After long-term exposure to dust, respiratory problems like asthma or more permanent problems such as pulmonary fibrosis were observed. Permanent contact with skin may cause irritation and skin rash, which slowly disappears. Dietary intake of large quantities of cobalt at once is very rare and probably not too dangerous. It can cause nausea and vomiting, long-term intake is then known to cause thyroid problems, neurological problems and an increase in blood density.

2.6.2. Copper

Copper is in very low content also an essential element for all living organisms for many reasons. On the other hand, salts of copper are irritative for skin and eyes. Consumption of greater amount of copper causes emesis, diarrhea and salivation excess. Chronicle accumulation in human body is caused by genetic disorder, disability of copper secretion by bile, named Wilson's disease. The main sites of copper accumulation are liver and brain.

Copper is only slightly toxic for animals and plants, but is very toxic for lower organisms of fungus character, bacteria and lower mushrooms.

There are quite strict limits for copper concentration due to its toxicity to water organisms, including fish. Concentration about 1 mg l⁻¹ and greater gives a distasteful pinching taste to water.

2.6.3. Nickel

In various compounds like chloride, nitrate, phosphate or sulfate, nickel exhibits distinctive toxic effect on human organism. Especially dust originating from fabrication of nickel or nickel plated components can be reason of lung or neck and nose mucosa cancer creation. Skin contact with nickel compounds can lead to grave dermatitis, which can fade in chronicle form of eczema. Intoxication with nickel results in failure of myocardium, kidneys and central nervous system. Nickel is toxic also to animals and mostly to plants. [71]

2.7. Sequential extraction of heavy metal from soil and SOM

Sequential extraction simulates various environmental conditions to which the soil compounds can be subjected. [73]

Several theories describing fractionation of metals bound on different soil fractions have been introduced in history. One of the most well-known metal-extraction procedures is according to Tessier [73]. The whole metal content is divided into 5 different fractions, following the strength of the extraction agent (and thus the strength of metal-soil bond), as can be seen in Table 2.

Tab. 2 *Fractions following various environmental conditions*

step	ions fraction	extraction agent
1	exchangeable	MgCl ₂
2	bound to soils carbonates	NaOAc
3	bound to iron and manganese oxides	NH ₂ OH–HCl in HOAc
4	bound to organic matter	H ₂ O ₂ in HNO ₃ then NH ₄ OAc in HNO ₃
5	residual	HF and HClO ₄

Another heavy metal sequential extraction procedure according to McGrath and Cegarra [74], has the following steps described in Table 3.

Tab. 3 *Fractionation steps of metal sequential extraction following McGrath and Cegarra*

step	ions fraction	extraction agent
1	in soil solution in exchangeable forms	CaCl ₂
2	associated with organic matter	NaOH
3	bound to soils carbonates	Na ₂ H ₂ EDTA
4	residual	aqua regia

The sorption of most metals by HA greatly increases with increasing pH of the solution. [75] Consequently, with a decrease of pH one can reach the extraction of metals from HA. The extraction can be further supported by additional decrease of pH. Hseu at al. used HCl as extraction agent for studying the Cu and Zn extractability in dependence on addition of CaO, which reduces the bioavailability of these two metals. [76]

A series of extractants were applied by Siqueira et al. [77] to investigate different ability to release metal ions, which were previously bound onto humic acids. With results of this experiment the authors proved that 0.5M HCl and 1M HNO₃ lead to 100% extraction of Cu²⁺ ions from humic acids, while the MgCl₂ solution tend to extraction success of Cu²⁺ ions about 60–70 % in dependence on pH value.

2.8. Thermal analysis (TA)

2.8.1. Generalities

The term thermal analysis is frequently used to describe analytical experimental techniques which investigate the behavior of a sample as a function of temperature.

The advantages of TA over other analytical methods can be summarized as follows:

- the sample can be studied over a wide temperature range using various temperature programmes,
- almost any physical form of sample (solid, liquid or gel) can be accommodated using a variety of sample vessels or attachments,
- a small amount of sample is required, typically (cca 0.1–50 mg),
- the atmosphere in the vicinity of the sample can be standardized,
- the time required to complete an experiment ranges from several minutes to several hours,
- TA instruments are reasonably priced. [78]

Temperature program can be described by linear dependence:

$$T = T_0 + \beta t, \quad (13)$$

where T is temperature at time t , T_0 is the initial measuring temperature and β is the temperature coefficient. When $\beta > 0$, the sample is heated, when $\beta < 0$, we speak of linear cooling and when $\beta = 0$, the measurement is under isothermal conditions. [79]

TA data are indirect and must be collated with results from other measurements (for example NMR, FTIR, X-ray diffractometry, and so on) before the molecular processes responsible for the observed behavior can be elucidated. The recorded data are influenced by experimental parameters, such as the sample dimension and mass, the heating/cooling rate, the nature and composition of the atmosphere in the region of the sample and the thermal and mechanical history of the sample.

2.8.2. Thermogravimetric Analysis (TGA)

Thermogravimetry is the branch of TA which examines the mass change of a sample as a function of temperature in the scanning mode or as a function of time in the isothermal mode. TGA is used to study processes with decrease/increase of mass, e.g. various types of sorption, sublimation, vaporization, oxidation and primarily for determination the decomposition and thermal stability of materials under a variety of conditions and to examine the kinetics of the physico-chemical processes occurring in the sample. The mass change characteristics of a material are strongly dependent on the experimental conditions employed. Factors such as sample mass, volume and physical form, the shape and the nature of the sample holder, the nature and pressure of the atmosphere in the sample chamber and the scanning rate, all have important influences on the characteristics of the recorded thermogravimetric curve.

Thermogravimetric curves are normally plotted with the mass change (Δm) expressed as a percentage on the vertical axis and temperature (T) or time (t) on the horizontal axis. [78]

The most frequent use of TGA is in determining the material thermal stability, relative volatility and also process kinetics. Coupling of TGA and DTA enables some other characteristics to be measured and assessed, for example temperatures of first and second order phase transition, namely changes of crystal modification, melting, sublimation, boiling or glass transition. [80]

2.8.2.1. *Factors affecting thermogravimetric measurements*

The size and shape of crucible can appreciably influence the course of the TG curve. If the volatility of a substance is followed under isothermal conditions, the rate of volatilization depends on the shape of the crucible, as this determines the surface area of the sample. The size of the crucible and the amount of the sample play a major role as they affect the method of heating and also the rate of diffusion of gases liberated from the sample. In view of this, it appears that the best results are obtained with crucibles in the shape of a small shallow dish permitting homogeneous heating of the sample over a relatively large surface area. It is important to take into account other properties of the investigated substance such as decrepitation, or foaming, during heating. If any of these occurs the crucible described is unsuitable and cylindrical or conical or closed crucible should be used. The shallow crucible is also unsuitable in cases where a gas stream is used, as aerodynamic effects cause complications.

Another effect that can influence the course of measurements is the construction of the crucible. The effect of the material is negligible if Pt, Au or Al crucibles are used.

Sample properties influence mostly the course of measurements. One of the most important parameters is its quantity which influence the time required for the reaction to take place throughout the whole volume of the sample.

Thermogravimetry requires heating and weighing of the sample simultaneously. This means there must be no contact between the sample and the furnace wall. Thus the problem of heating the sample is primarily one of heat transfer. This is controlled by a number of factors, e.g. the properties, size and nature of the sample, and the enthalpy change of any reactions undergone by the sample. A temperature gradient is formed between the sample and the furnace wall, which introduces errors into measurement of the sample temperature and determination of the range of temperature reactions being investigated. A temperature gradient may also be formed inside the sample. The temperature difference between the furnace wall and the sample is affected primarily by sample properties, the enthalpy change of any reactions it undergoes, and the rate of temperature increase.

The atmosphere in the reaction space of the furnace is also very important factor influencing the measurements. If the sample is dried or decomposed in air, the atmosphere in the neighborhood of the crucible is continually changing owing to liberation of gaseous products or reaction of the sample with the original atmosphere.

The question of the sensitivity of the weighing mechanism and the recording system is closely related to the amount of sample used. The mass of the sample may exert an appreciable influence on the course of the reactions being followed. Often, in order to follow the reaction under required conditions, as low a sample weight as possible is taken. Great attention must always be devoted to long-term stability of the thermobalance, periodic calibration of weight ranges, quality and strength of the record, etc. These factors will introduce errors into the TG measurement mainly in prolonged experiment and at high sensitivities of measurement.[81]

2.8.3. Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry observes the enthalpy changes. [81]

DSC measures the rate of heat flow and compares its difference between the sample and the reference material.

The measurement of sample and reference is carried out in two crucibles and the most common range is on order of ones to tens of milligrams. A substance, which doesn't undergo any thermal changes in the temperature range of the measurement, serves as a reference. Most frequently an empty crucible is used. [79]

2.8.3.1. *Heat-Flux DSC*

It concerns measurement of temperature difference between the sample and reference as a function of temperature or time, under controlled temperature conditions. The temperature difference is proportional to the change in the heat flux (energy input per unit time).

The thermocouples are attached to the base of the sample and reference holders. A second series of thermocouples measures the temperature of the furnace and of the heat-sensitive plate. The variation in heat flux causes an incremental temperature difference to be measured between the heat-sensitive plate and the furnace. The heat capacity of the heat-sensitive plate as a function of temperature by adiabatic calorimetry during the manufacturing process, allowing an estimate of the enthalpy of transition to be made from the incremental temperature fluctuation.

2.8.3.2. *Power Compensation DSC*

The sample and reference holders are individually equipped with a resistance sensor, which measures the temperature of the base of the holder, and a resistance heater. If a temperature difference is detected between the sample and reference, due to a phase change in the sample, energy is supplied until the temperature difference is less than a threshold value, typically < 0.01 K. The energy input per unit time is recorded as a function of temperature or time. A simplified consideration of the thermal properties of this configuration shows that the energy input is proportional to the heat capacity of the sample. [78]

2.8.3.3. *Advantages and drawbacks of DSC*

Both types of DSC have their advantages and drawbacks but the final result is the same, they both provide same information.

The benefit of heat-flux DSC is the possibility of measuring larger-volume samples. This type has very high sensitivity and the maximal temperature may reach values over 900 °C. The disadvantage is low heating and cooling rate when using older types of instruments. Principal advantage of power compensation DSC is no need of calibration, because the heat is obtained directly from electric energy supplied to the sample or reference (calibration is still needed to convert this energy into effective units). High scanning rate can be obtained. The disadvantage of such arrangement is the necessity of extremely high sensitive electronic system and his susceptibility to fluctuating environment to avoid compensating effects, which are not caused by the sample. [82]

2.9. Induced coupled plasma - mass spectrometry (ICP-MS)

Mass spectrometry with inductively coupled plasma is an ultra-trace analytical method used to determine the amount of trace elements in the sample. This technique allows analysis of almost all elements from lithium to uranium.

The main advantages of ICP-MS analysis are fast acquisition of data and very low detection limits in the order of ng l^{-1} , for some elements even of pg l^{-1} . ICP-MS is used when very high sensitivity analysis is required. ICP-MS also provides practical advantage in the possibility to rapidly and accurately analyze a relatively large number of elements in one sample. It is used to control the quality of water, food and pharmaceuticals.

The disadvantage is the high purchase and operation cost as well as mastering and maintenance of the instrument itself. Another disadvantage is the limit for solutions of high salt concentration (seawater, blood ...) that can block the entry of cones and change energy conditions in the plasma. [83]

3. EXPERIMENTAL PART

3.1. Extraction of humic acid

Humic acid was extracted from South-Moravian leonardite-type lignite from the mine Mír in Mikulčice, near Hodonín, Czech Republic. For our purposes, the extraction procedure following the recommendations of the International Humic Substances Society (IHSS) was slightly modified. Briefly, 150 g of previously air-dried and 0.2–0.3 mm sieved lignite was mixed with 1.5 l of 0.5M NaOH and 0.1M Na₄P₂O₇ solution and stirred for 2 hours at slightly elevated temperature (50 °C). After separation of phases by centrifugation (ROTINA 46R, 4000 RPM, 15 minutes), the supernatant was treated with concentrated HCl until the pH value 1–2 was reached in order to precipitate the HA. Then, approximately 40 ml of HF was added and the final solution was diluted with distilled water to 1.5 l and shaken overnight to remove the residual ashes. After that the sample was centrifuged (same conditions as described above), rinsed with distilled water, centrifuged again, dialyzed (Spectra/Por[®] dialysis tubes, 1000 Da cut-off) against distilled water until chloride-free (AgNO₃ test). Final solution was freeze-dried (Labconco FreeZone).

3.2. Characterization of humic acid

Elemental analysis was accomplished at the Institute of Rock Structure and Mechanics, The Academy of Science of The Czech Republic. The used device was Carlo Erba CHNSO microanalyser.

3.3. Determination of acidic groups content

The carboxylic acid groups content was determined by conductance titration according to Riggle and von Wandruszka [84]. 100 mg of HA was dissolved in 100 ml 0.01M NaOH and stirred overnight. This solution was automatically titrated (Schott TitroLine alpha plus) with 0.05M HCl. 0.05 ml of HCl solution was added every 30 seconds. During the retitration, values of conductance (WTW inoLab Cond 730) and pH (Mettler Toledo SevenMulti) were recorded before each HCl addition.

3.4. Complexation

100 mg of HA was mixed with 5 ml of metal ions solutions (Ni²⁺, Cu²⁺, Co²⁺) of several concentrations (0.05, 0.1, 0.2 M) and mechanically stirred for 48 hours. After that the samples were centrifuged (same conditions as described above), rinsed with 5 ml of distilled water to remove the mobile fraction of metal ions, which is not bound to HA, and centrifuged again. Rinsing and centrifuging was repeated two times (rinsing with 10 ml of H₂O in total).

3.5. Bound amount determination

Bound amount of each metal ion from solutions of 3 initial concentrations (0.05, 0.1 and 0.2 M) was determined by ICP-MS. Accurate concentration was measured using Thermo X-Series ICP-MS, connected to the PC, where measured data were collected. Approximately 20 ml of each solution was filled into the dosing tubes and placed on autosampler. The conditions of measurement are listed in Table 4. Prior to the ICP-MS analysis the metal ions solutions were diluted 10 000 times.

Tab. 4 ICP-MS operating conditions

parameter	value
forward power	1.3 kW
reflected power	< 5 W
intermediate gas flow rate	0.7 l min ⁻¹
outer gas flow rate	13 l min ⁻¹
injector gas flow rate	0.87 l min ⁻¹
solution delivery rate	0.8 ml min ⁻¹
nebulizer	Meinhard concentric
Scott-type cooled double-pass spray chamber	5 °C
expansion stage	2.2 mbar
intermediate stage	1×10 ⁻⁴ mbar
analyzer stage	1.6×10 ⁻⁴ mbar
internal standard (¹⁵⁵ In)	50 µg l ⁻¹

These values were compared with acidic group content and for each concentration the saturation degree was calculated. From the dependence of percentage of saturation on initial metal ions solutions concentration, the concentration corresponding to 100% saturation was determined. From these results arises the possibility of using the Langmuir isotherm and the necessity to prepare samples with higher initial ions concentration to reach 100% saturation. Thus, 0.5M solutions were used in order to prepare new series of complexes (procedure described above) and for following experiments the concentrations (c_0) 0.05, 0.2 and 0.5 M were used.

3.6. Thermogravimetric analysis

Thermogravimetric analyses of all samples, including unaltered HA, were performed. For this purpose TA Instruments TGA Q 5000 IR was used, connected to the PC, where experimental data were collected. The furnace was calibrated using Curie temperatures of nickel and alumel (358.2 and 152.6 °C, respectively). Samples were carefully homogenized in an agate mortar. Approximately 2–3 mg of each sample was loaded into an open aluminum crucible. The air (reaction gas) flow rate was set at 50 ml per minute, nitrogen (purge gas) flow rate being 20 ml per minute. The heating rate was 10 °C per minute. The measurement was carried out from room temperature to 600 °C. Obtained results were evaluated by means of Universal Analysis 2000 software.

3.7. Differential scanning calorimetry

Calorimetric analyses of all samples, including unaltered HA, were performed employing Shimadzu DSC-60. The instrument is connected through TA-60WS to the computer. The furnace was calibrated using fusion temperatures of indium, tin, lead and zinc (156.60, 231.93, 327.46 and 419.53 °C, respectively). 0–1 mg of homogenized sample was placed into an open aluminum crucible. The air flow rate was 25 ml per minute. Samples were heated (10 °C min⁻¹) from room temperature to 600 °C. Obtained results were evaluated by means of TA-60 software.

3.8. Chemical stability

The solid residues of all samples were divided into two 50 mg parts. The first one was mixed with 10 ml of 1M MgCl_2 , the second part with 10 ml of 1M HCl , and mechanically stirred for 48 hours in order to determine their chemical stabilities. After that the samples were centrifuged (same conditions as described above), rinsed with 5 ml of distilled water and centrifuged again. Rinsing and centrifuging was repeated two times (rinsing with 10 ml of H_2O in total). The amount of metal ions loosened from the complexed HA structure into the MgCl_2 and HCl solutions was determined by ICP-MS as described above.

Thermogravimetric and calorimetric analyses of all samples were performed as described above.

4. RESULTS AND DISCUSSION

4.1. Elemental analysis

Proportional content of fundamental biological elements in dry ash-free sample of humic acid is listed in Table 5.

Tab. 5 *Percentage of biogenic elements*

C	H	N	S	O
atomic %				
37.12	47.22	3.10	0.10	12.46

4.2. Determination of reactive groups content

Obtained specific conductance was plotted against volume of added HCl to solution of HA previously amended to pH approx. 10 as reported in Fig. 4. As it has been stated in [84], the conductance decrease at the beginning of dependency corresponds to neutralization of excessive OH^- ions. After a local minimum, an increase of conductivity was observed. This region (as indicated in Fig. 4) matches to ion exchange of carboxylic groups present in the humic acid from Na^+ to the H^+ form and in this study it was used for the acidity assessment. Final part, where the conductivity increases rapidly corresponds to the accumulation of Cl^- and especially H^+ ions in the solution. Accordingly, the total carboxylic acidity was determined to 7.06 mmols of binding sites per gram of humic acid.

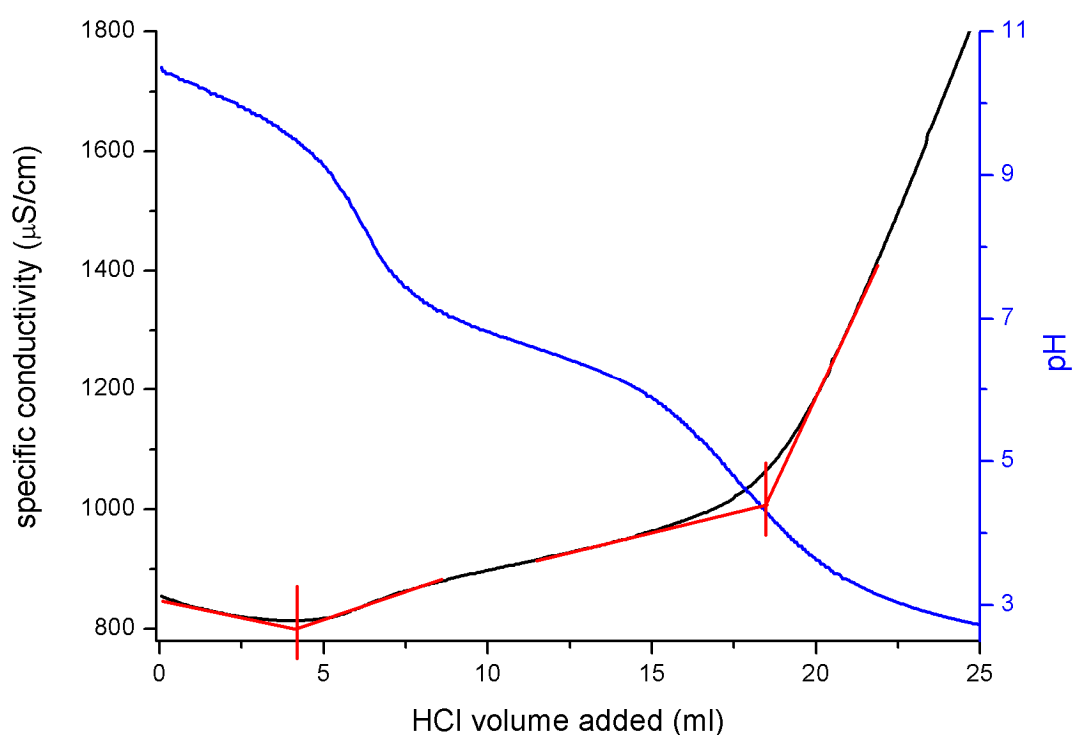


Fig. 4 *Specific conductivity and pH during retitration of HA solution*

4.3. Selection of initial ions concentration

Adsorbed amount of each ion from the first set of concentration range ($c_0 = 0.05, 0.1, 0.2$ M) was determined by ICP-MS and obtained values are listed in Table 6. The highest of these concentrations corresponds to 36% saturation of humic acid binding sites.

Tab. 6 *Adsorbed amount of each metal ion with the conversion to percentage of HA acidic groups*

c_0 (M)	adsorbed amount					
	Co^{2+}		Cu^{2+}		Ni^{2+}	
	(mmol g ⁻¹ HA)	%	(mmol g ⁻¹ HA)	%	(mmol g ⁻¹ HA)	%
0.05	0.39	11.20	0.36	10.24	0.40	11.45
0.1	0.78	21.99	0.88	25.02	0.93	26.41
0.2	1.28	36.22	1.28	36.24	1.29	36.40
0.5	3.62	102.61	3.51	99.54	3.52	99.58

The data were fitted with Langmuir isotherm, which parameters are listed in Table 7, and extrapolated. In order to reach 100% saturation, 0.5M solutions of each ion must be used, which is highlighted in the last row in Table 6.

Tab. 7 *Langmuir isotherm parameters*

Co^{2+}		Cu^{2+}		Ni^{2+}	
a_{\max} (mmol g ⁻¹)	b (dm ³ mol ⁻¹)	a_{\max} (mmol g ⁻¹)	b (dm ³ mol ⁻¹)	a_{\max} (mmol g ⁻¹)	b (dm ³ mol ⁻¹)
93.44	0.11	10.16	1.25	16.33	0.67

Maximal adsorbed amount according to Lagmuir isotherm contains all types of sorptions including adsorption on different binding sites (not only COOH) and physical sorption. As stated in [85], at lower initial metal solution concentration, the metal ions are first bound to acidic functional groups, then to other binding sites and then the eventual physisorption asserts. Therefore, the values of maximal bound amount a_{\max} are higher than the amount adsorbed in the cases of 100% saturation (as summarized in Table 6).

4.4. Influence of metal presence

As a main step, the influence of various metal ions bound in the HA structure on thermoanalytical behaviour was tested.

Tab. 8 *Heat transfer information obtained from DSC*

sample	heat released during thermo-oxidative degradation		
	after complexation	after MgCl ₂ extraction (kJ g ⁻¹)	after HCl extraction
HA	10.45	12.32	8.23
HA + 0.05M Co	13.65	12.33	5.94
HA + 0.2M Co	13.42	11.35	6.08
HA + 0.5M Co	12.28	11.82	9.52
HA + 0.05M Cu	12.99	13.95	7.48
HA + 0.2M Cu	16.45	17.03	4.63
HA + 0.5M Cu	14.12	16.72	7.53
HA + 0.05M Ni	14.01	9.63	4.08
HA + 0.2M Ni	13.12	13.85	6.31
HA + 0.5M Ni	19.91	13.40	8.10

Amount of heat released during thermo-oxidative degradation of HA and its complexes before and after MgCl₂ and HCl extraction is given in Table 8. The most interesting information is considerable decrease of heat release during the degradation of samples after HCl extraction.

Table 9 summarizes properties of samples obtained from TGA. The most interesting information lies within the ash content before extractions. Its values are highest in the case of Cu-humic complexes. After MgCl₂ treatment, samples exert only slight increase of ash content, whereas after HCl extraction the ash content of all samples has greatly decreased. Concerning the moisture content, one can say that there is no substantial difference among all samples, which is most probably due to very similar adsorbed amount of each metal ion. This can be connected with atomic masses of the metals, which are very much alike.

Tab. 9 *Samples properties obtained from TGA*

sample	total weight loss			ash content			moisture content		
	after complexation	after MgCl ₂ extr. (%)	after HCl extr.	after complexation	after MgCl ₂ extr. (%)	after HCl extr.	after complexation	after MgCl ₂ extr. (%)	after HCl extr.
HA	97.84	96.61	99.59	2.16	3.39	0.41	7.7	7.79	5.85
HA + 0.05M Co	97.72	96.82	99.72	2.28	3.18	0.28	6.44	8.26	5.92
HA + 0.2M Co	97.83	96.43	99.52	2.17	3.57	0.48	6.77	8.07	6.03
HA + 0.5M Co	97.18	96.53	99.67	2.82	3.47	0.33	8.67	8.49	5.94
HA + 0.05M Cu	95.67	95.49	99.65	4.33	4.51	0.35	6.86	8.36	6.28
HA + 0.2M Cu	94.88	95.38	99.58	5.12	4.62	0.42	7.07	8.71	6.16
HA + 0.5M Cu	95.08	93.64	99.53	4.92	6.36	0.47	6.69	8.67	5.98
HA + 0.05M Ni	97.93	96.17	99.67	2.07	3.83	0.33	6.74	8.37	6.21
HA + 0.2M Ni	97.76	96.34	99.61	2.24	3.66	0.39	7.35	8.51	6.12
HA + 0.5M Ni	96.75	96.43	99.86	3.25	3.57	0.14	6.76	8.37	6.08

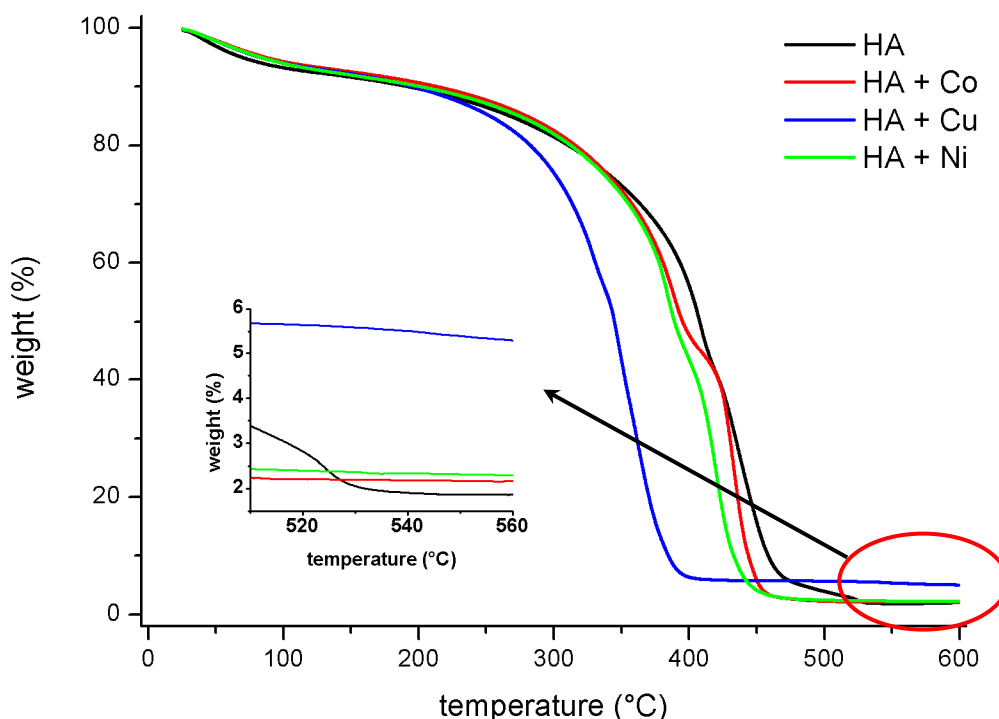


Fig. 5 Comparison of TGA records of unaltered HA and metal-humic complexes; $c_0 = 0.2\text{ M}$

In Fig. 5, one can see thermogravimetric degradation curves of native HA and its metal complexes prepared from 0.2M solutions. The degradation has several steps, which is better observable in Fig. 6. In the zoomed view in Fig. 5 the ash content differences are visible; the complexes have greater ash content than the native HA, since there are more thermo-stable inorganic parts. None of studied metal ions type proved thermo-oxidative stabilization effect on HA. The most interesting is Cu-humic curve, which has very different shape from the others. The organic part of Cu-humic complex is completely burned at temperature about 400 °C but there remains the largest amount of residues (see the inset graph). This can mean that Cu ions interrupt the intermolecular forces linking the individual parts of HA structure. The dissociation of these bonds may destabilize the integrated HA structure by fragmentation to smaller individual parts, which are not linked together. These are, understandably, less resistant to high-temperature oxidative atmosphere.

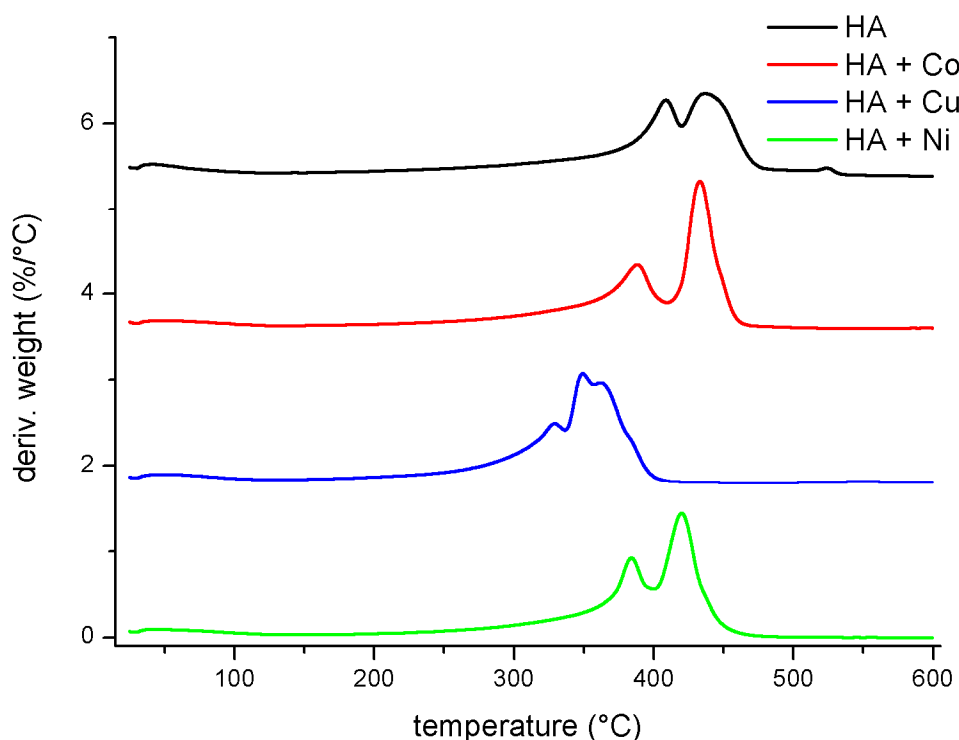


Fig. 6 Comparison of DTG curves of unaltered HA and metal-humic complexes; $c_0 = 0.2\text{ M}$

Fig. 6 presents derivative thermogravimetric curves (of native HA and its complexes), which have the meaning of weight loss velocity with increasing temperature. The onset of the first peak is linked with the beginning of degradation, the lowest temperature of the degradation start is visible within the Cu-humic curve, this sample has the lowest thermo-oxidative stability.

The lower stability of all metal humic complexes, as indicated by the onset temperature on DTG can be caused by re-aggregation of humic quaternary structure. Possibly, the system of H-bonds stabilizing humic molecules is corrupted by the presence of metal ions, aliphatic chains are not protected any longer and undergo easier oxidation. Moreover, the presence of Cu^{2+} in an organic molecule is associated with free radical generation which accelerates the degradation process of HA. This can be supposed also for other ions of transition elements such as Ni^{2+} and Co^{2+} .

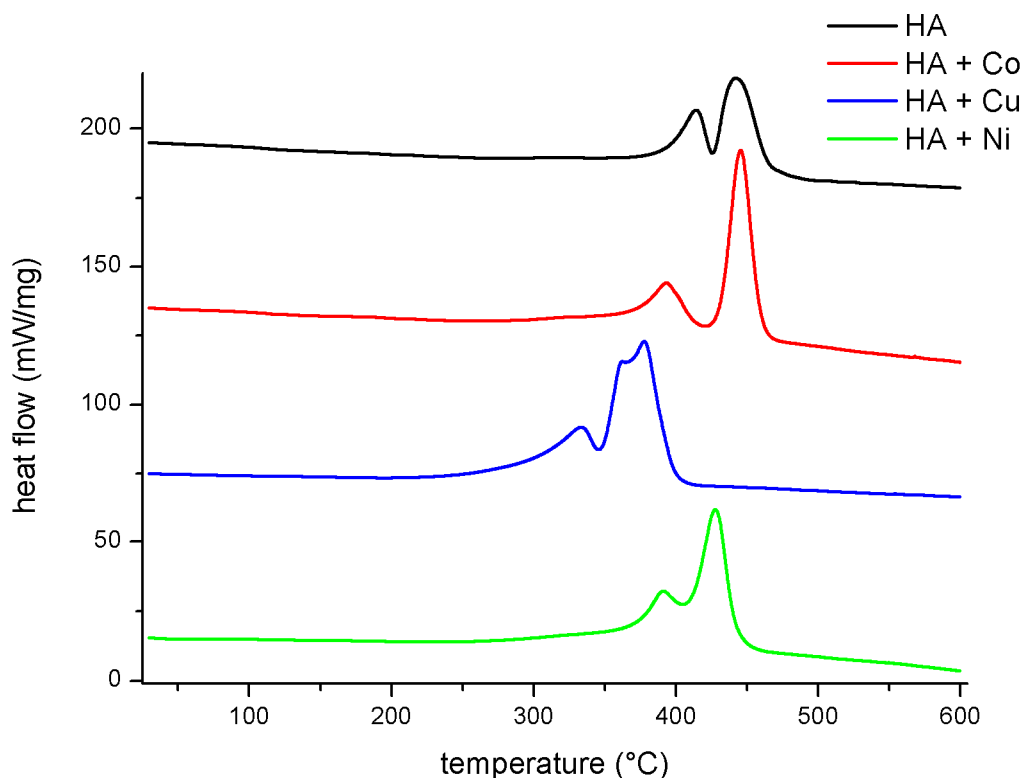


Fig. 7 Comparison of DSC curves of unaltered HA and metal-humic complexes; $c_0 = 0.2\text{ M}$

DSC records of untreated HA and its complexes are presented in Fig. 7, where the enthalpic changes during linear temperature increase are shown. All DSC records show several exothermic peaks. Exothermic peaks reflect the processes of degradation and disclose the difference among tested samples. All samples show more than one clear exothermic peak. It is noteworthy that all of the exothermic processes of HA complexes begin at lower temperatures than that of unaltered HA. The degradation process of complex of HA with Cu starts at the lowest temperature.

The comparison of the pattern of DTG and DSC curves brings the information that during heating in the temperature range up to 600 °C, there is no thermally tinged process connected to loss of weight, because the curve of weight loss rate has very similar shape to the calorimetric curve. For this reason it is not necessary to describe the other DSC records any further. These are presented as an Appendix 3.

4.4.1. Influence of Co^{2+} ion

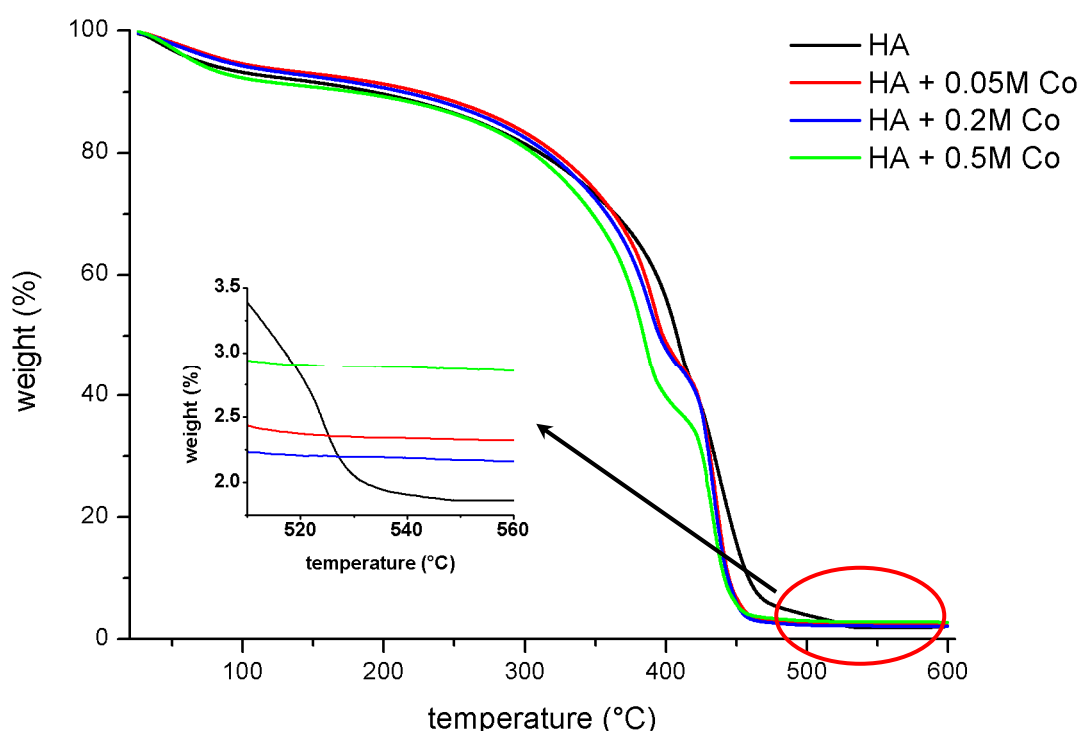


Fig. 8 Comparison of TGA records of differently concentrated Co-humic complexes

In Fig. 8, one can see thermogravimetric degradation curves of native HA and its Co^{2+} complexes. All Co-humic samples are completely degraded at only slightly lower temperature than unaltered HA, which does not mean substantial influence of Co^{2+} on HA thermo-oxidative stability. The inset graph might be perceived as ash content indicator, which tells that there is no direct relation between ash content and concentration of Co^{2+} solution. On the other hand, the highest ash content is present in the sample with the highest concentration of used Co^{2+} solution. Thus, there is noticeable difference between sample prepared from 0.5M CoCl_2 and those prepared from lower concentrated solutions. The difference between samples prepared from the solutions of lower concentration is insignificant.

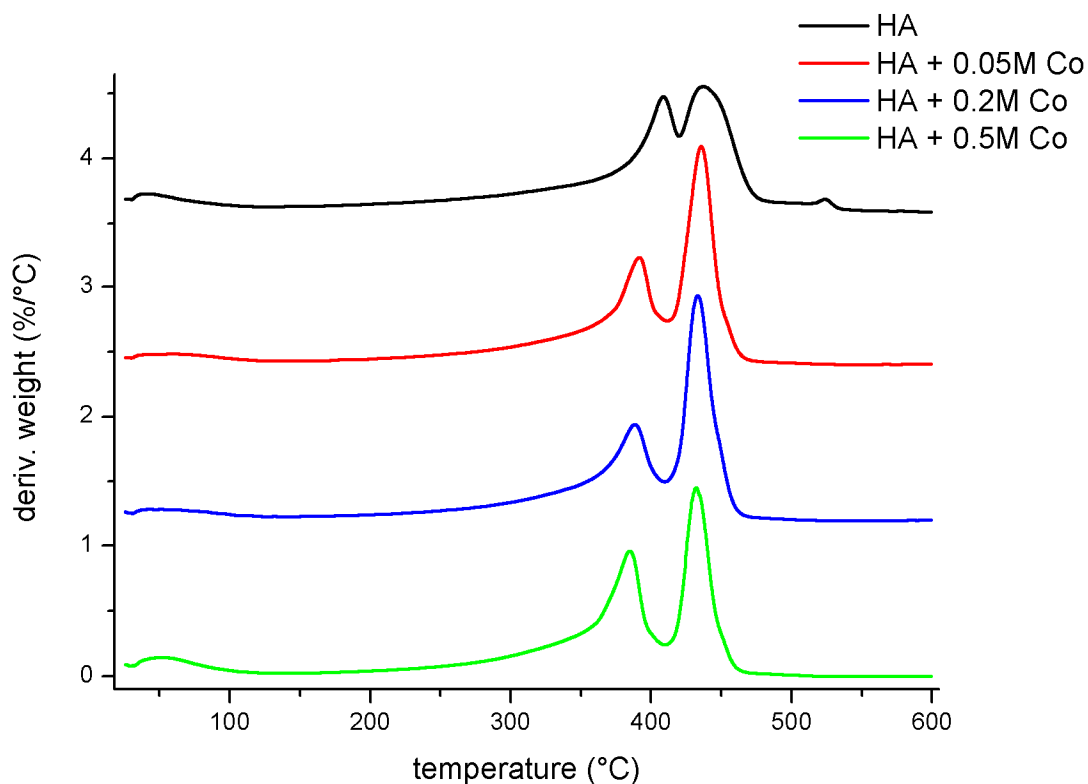


Fig. 9 Comparison of DTG curves of differently concentrated Co-humic complexes

There is a presentation of DTG curves of unaltered HA and its Co-complexes in Fig. 9. The records did not show any significant difference between temperatures of degradation start. From the shapes of the curves the two-step degradation is evident. The difference between first and second peaks heights decreases with increasing concentration of metal ion solution.

4.4.2. Influence of Cu^{2+} ion

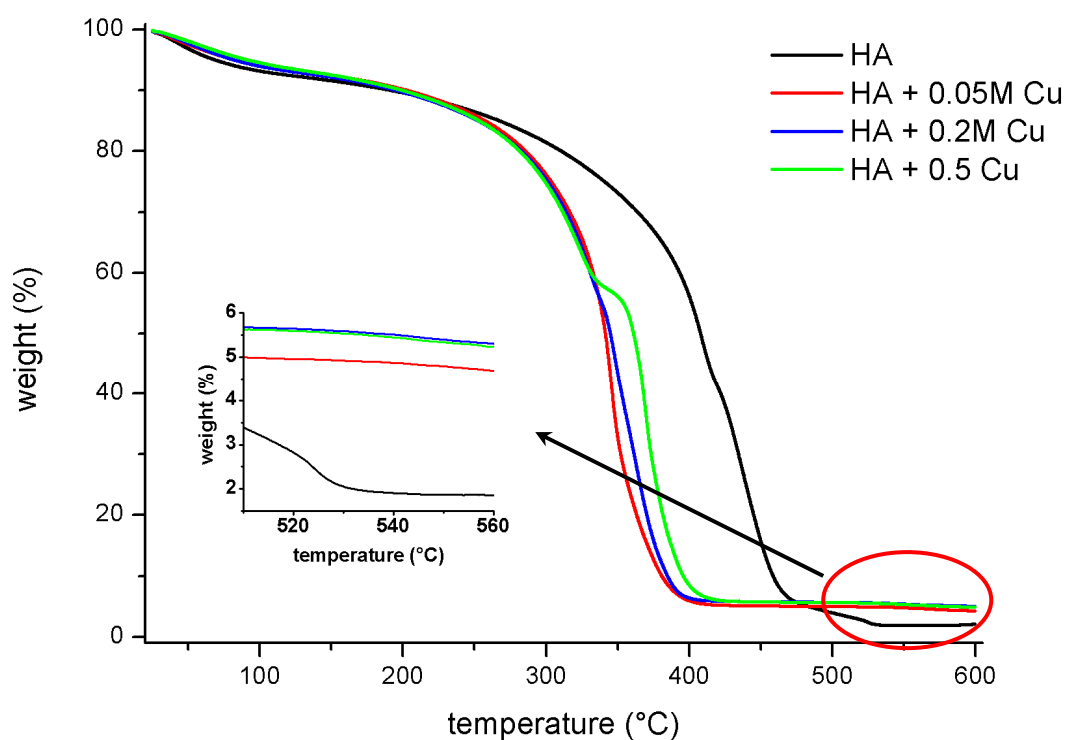


Fig. 10 Comparison of TGA records of differently concentrated Cu-humic complexes

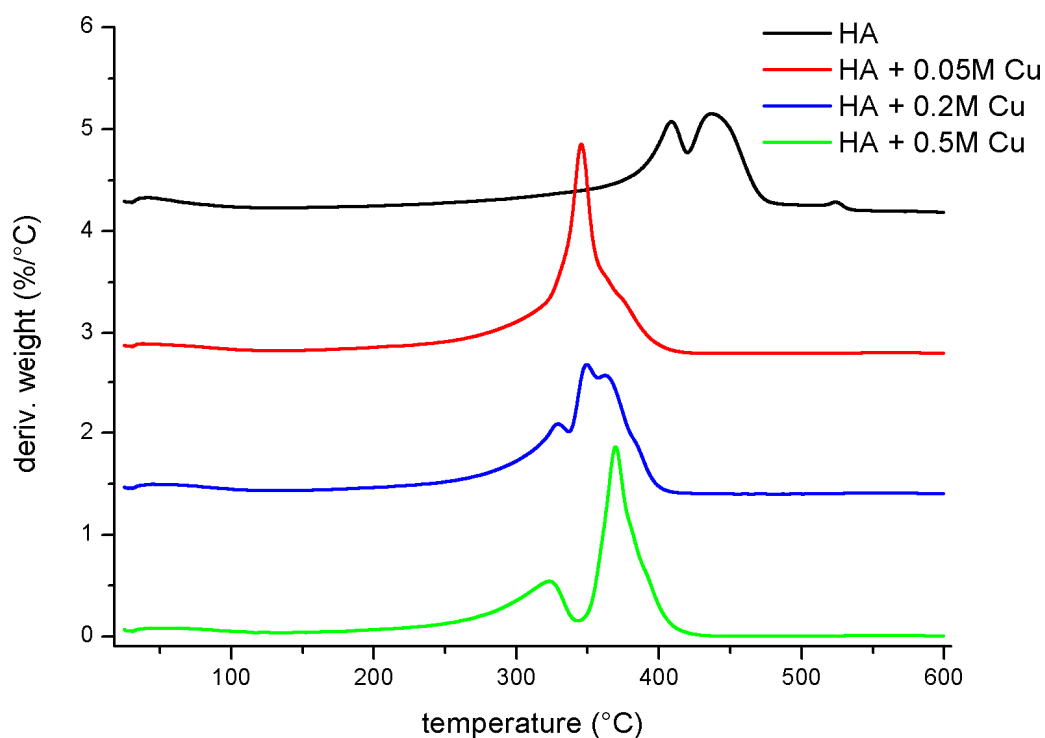


Fig. 11 Comparison of DTG curves of differently concentrated Cu-humic complexes

Fig. 10 is the representation of weight loss during degradation in the temperature range from 30 to 600 °C. There is one perfectly obvious result concerning the duration of degradation. Whereas the untreated HA is completely degraded at the temperature about 550 °C, the other samples with incorporated metal ions do not show any weight changes from the temperature about 400 °C above. From the zoomed view one can interpret that there are much more ash residues after burning the Cu-humic complexes in comparison with the native HA.

DTG records in Fig. 11 demonstrate the start of degradation of Cu-humic complexes at lower temperature as compared with native HA. The shapes of curves are various, although there are displayed complexes of the same metal ion. This fact can imply that Cu ions can bind to several binding sites of HA and influence different parts of HA, i.e. different functional groups. This assertion is in accordance with results published in [28]. The authors mention that Cu is one of few metals, whose complexes with humic acid involve phenolic OH groups. Another study [86] dealing with this problem results in ascertainment that the increase of pH corresponding to formation of free H^+ ions (during adsorption of Cu^{2+} on HA) does not proceed with the same rate as the decrease of Cu^{2+} ions from the solution. Hence, the adsorption process is not based only on bonds with acidic functional groups, the aromatic structures and eventual physical sorption are also involved. Moreover, this effect depends on initial concentration of metal solution, which can explain the differences among our samples.

One can see an interesting phenomenon on the DTG curve shape of the sample prepared from 0.5M $CuCl_2$ at about 350 °C. Whilst the two samples with lower initial concentration have almost the same degradation pattern at this temperature, the above mentioned one proves some kind of structural resistance at this temperature area. That can be explained as an occupation of functional groups of some of the HA structural units, which normally, without excessive Cu^{2+} treatment, degrade successively, being linked with the others parts of HA structure. The presence of the Cu^{2+} ion disturbs this gradual degradation sequence chain and fractionates it into more steps. Similar situation, although on minor scale occurs in Fig. 11 in the case of sample prepared from 0.2M $CuCl_2$.

4.4.3. Influence of Ni^{2+} ion

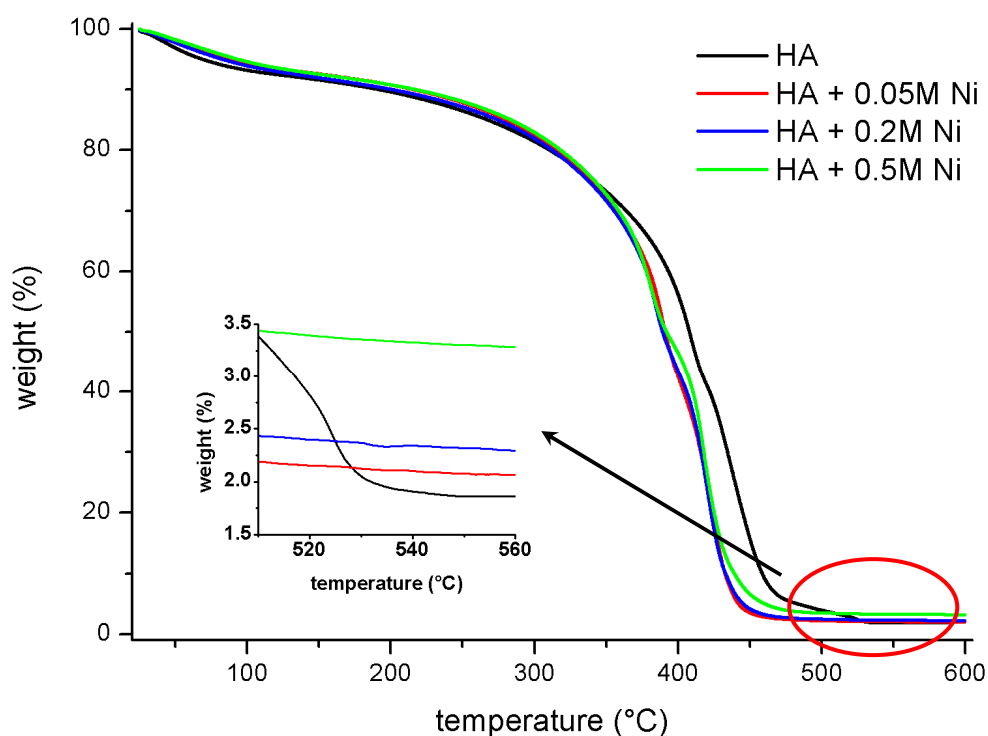


Fig. 12 Comparison of TGA records of differently concentrated Ni-humic complexes

In Fig. 12 the thermogravimetric curves of HA and its Ni complexes are presented. All Ni-humic samples are completely degraded at only slightly lower temperature than untreated HA, which signifies poor influence of Ni^{2+} on HA thermo-oxidative stability. The inset graph has the function of ash content indicator. The amount of residues increases with the concentration of metal ion solution, from which one can infer that HA is more able to complex from higher concentrated solution, because higher content of ash means higher amount of metal (and thus inorganic residues).

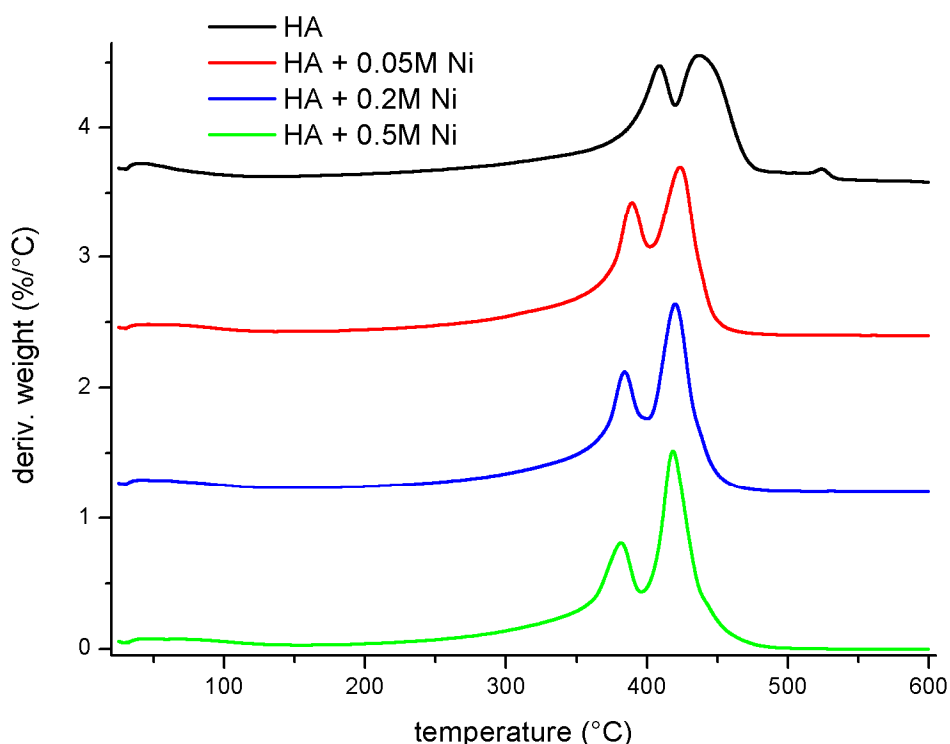


Fig. 13 Comparison of DTG curves of differently concentrated Ni-humic complexes

DTG curves in Fig. 13 present velocity of weight changes of native HA and Ni-humic complexes prepared from differently concentrated Ni^{2+} ion solutions. There is no significant difference between temperatures of degradation start. From the shapes of the curves the two-step degradation is evident. The difference between first and second peak height increases with the concentration of metal ion solution. The course of degradation of untreated HA is in comparison with Ni-humic complex very similar, which signifies very low influence of Ni^{2+} on the thermo-oxidative behaviour of HA. This may be caused by binding of Ni^{2+} only on peripheral and/or terminal functional groups in the aliphatic parts of HA.

Comparing Fig. 9 with Fig. 13, one may find that the influence of both metals is very similar, this can be due to similar atomic mass and thus similar structural and binding properties.

4.5. Influence of extraction agents treatment

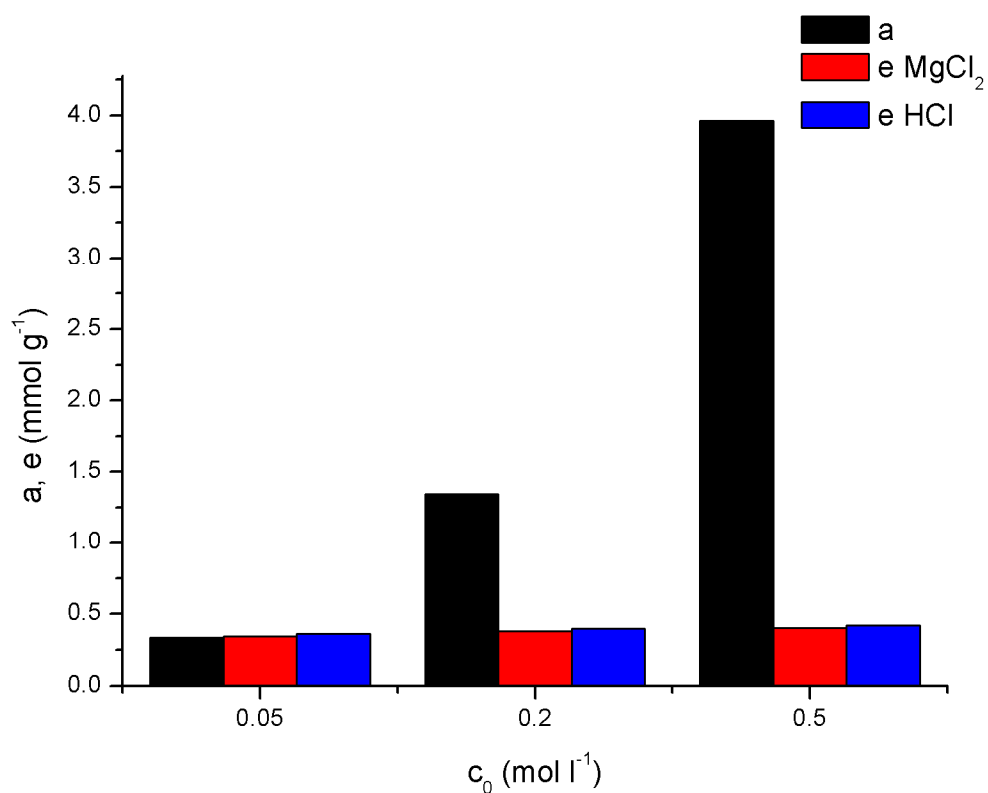


Fig. 14 Comparison of bound amount of Co^{2+} and loosened amount of Co^{2+} after extractions

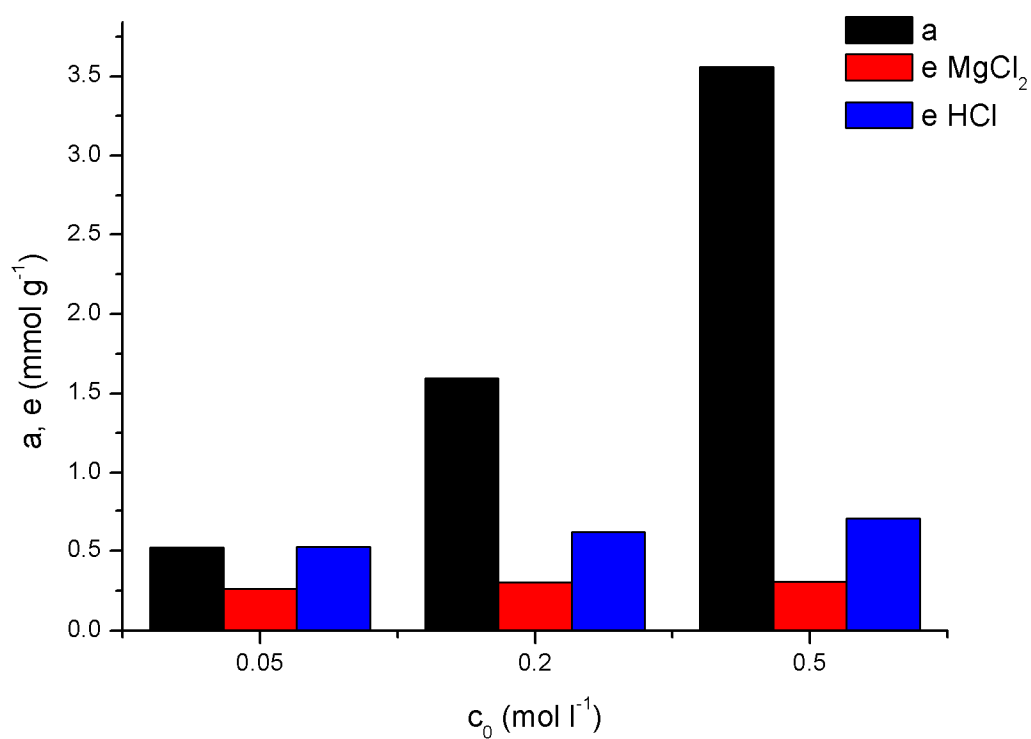


Fig. 15 Comparison of bound amount of Cu^{2+} and loosened amount of Cu^{2+} after extractions

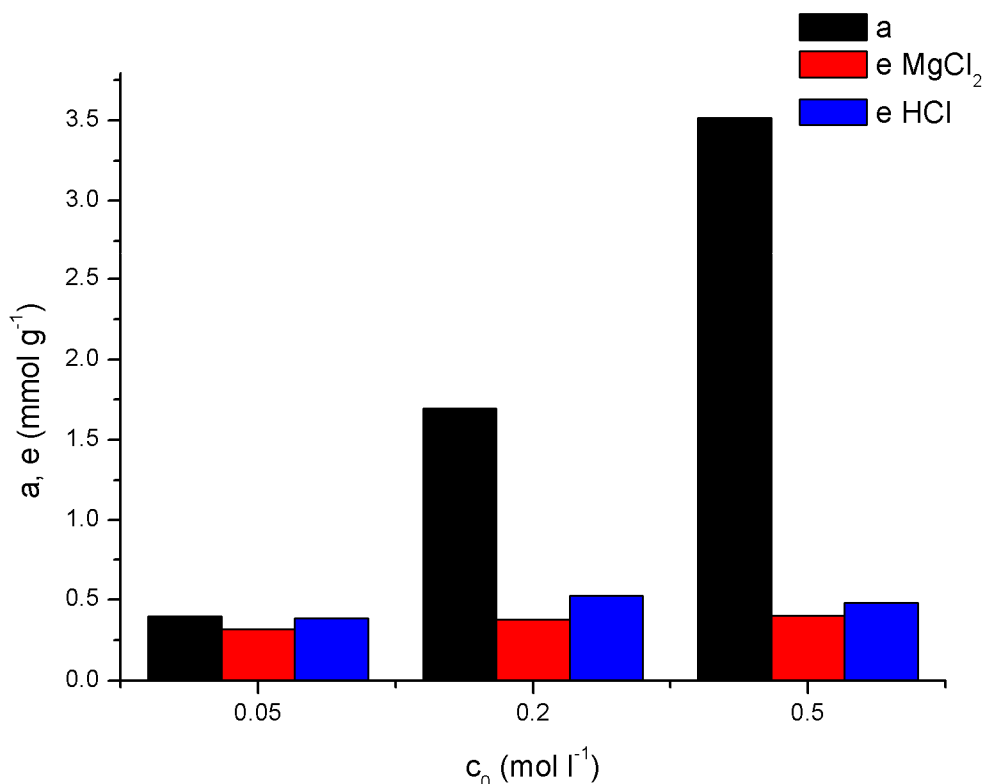


Fig. 16 Comparison of bound amount of Ni^{2+} and loosened amount of Ni^{2+} after extractions

The bound amount of each metal (a) determined from Langmuir isotherm was compared with the loosened amount of each metal (e) after extraction with MgCl_2 and HCl obtained from ICP-MS as reported in Fig. 14, Fig. 15 and Fig. 16. There is no relation between the initial concentration and the amount extracted by extraction agents. The extracted amount remains the same without relation to initial concentration. This can be interpreted as some kind of extraction capacity of the agents. HCl shows higher ability to extract the metals from HA as seen in Fig. 14, Fig. 15 and Fig. 16. The most relevant difference between each extraction agent treatment is in the case of copper ions. HCl (being very effective agent for extracting metal ions) is able to extract twice as much Cu^{2+} ions as in the case of MgCl_2 , which is in good accordance with [77]. Similar experiments were also investigated in [87]. The strength of Cu-humic bonds was elucidated using three differently strong extraction agents and our results are confirmed by their findings.

4.6. Influence of MgCl_2 treatment

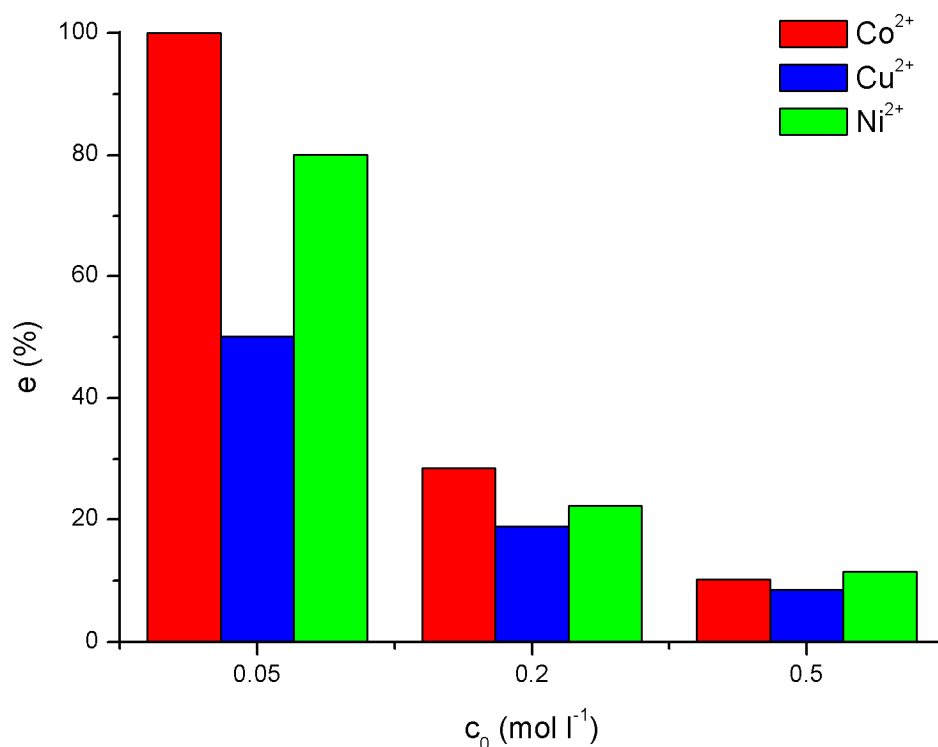


Fig. 17 Amount of each ions loosened after MgCl_2 treatment for each initial concentration c_0

Fig. 17 shows the percentage of amount of each ion extracted by MgCl_2 (per adsorbed amount) in dependence on initial concentration of metal ions. In this representation the low value of extracted Cu^{2+} ions is appreciable. MgCl_2 exhibits the highest ability to extract the cobalt ions. The dependence of increased extracted amount on initial concentration is indisputable.

4.6.1. Influence of MgCl_2 treatment on Co-humic complexes

The two-step thermo-oxidative degradation course is saved, whereas there are differences between each concentration of Co^{2+} ions, visible in Fig. 18 and Fig. 19. Sample with the greatest initial concentration of Co^{2+} ions has the lowest degradation start temperature. Not a single sample is completely degraded at 600 °C, there is still obvious decrease of the curves at this temperature.

The shapes of curves presented in Fig. 19 demonstrate, that after MgCl_2 treatment there is no clear difference between HA and its Co complexes with different concentration concerning their thermo-oxidative behaviour.

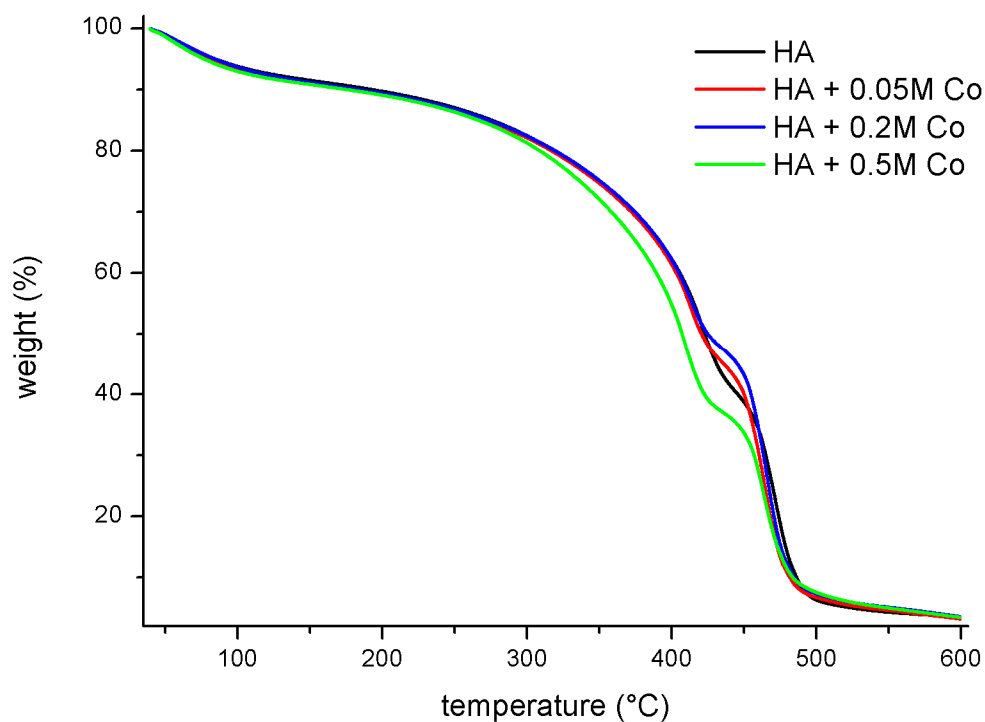


Fig. 18 Comparison of TGA records of differently concentrated Co-humic complexes after MgCl_2 extraction

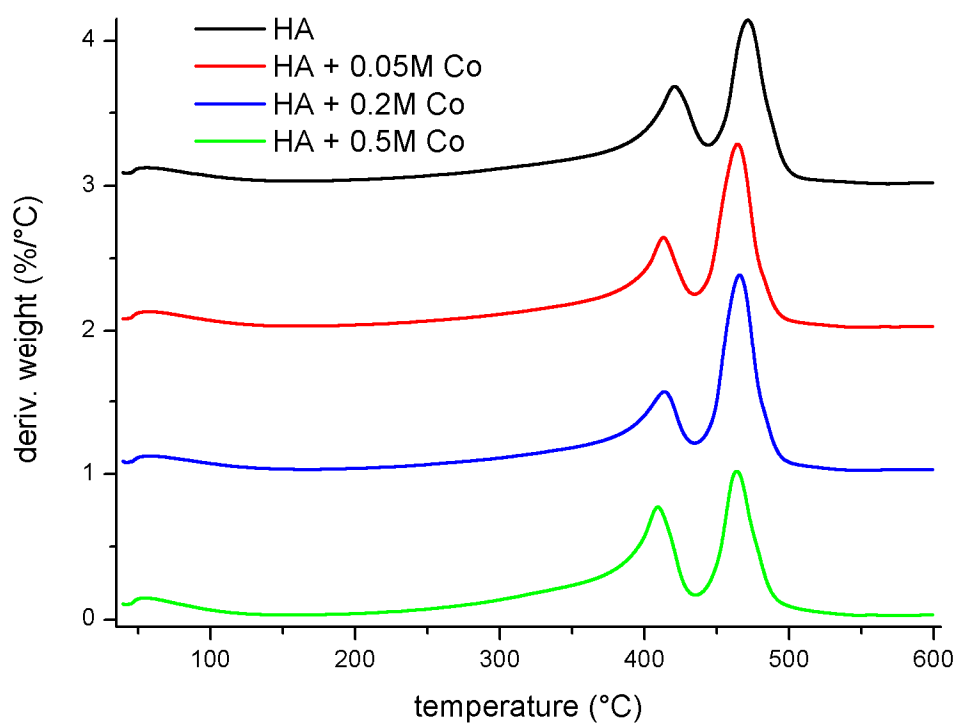


Fig. 19 Comparison of DTG curves of differently concentrated Co-humic complexes after MgCl_2 extraction

4.6.2. Influence of MgCl_2 treatment on Cu-humic complexes

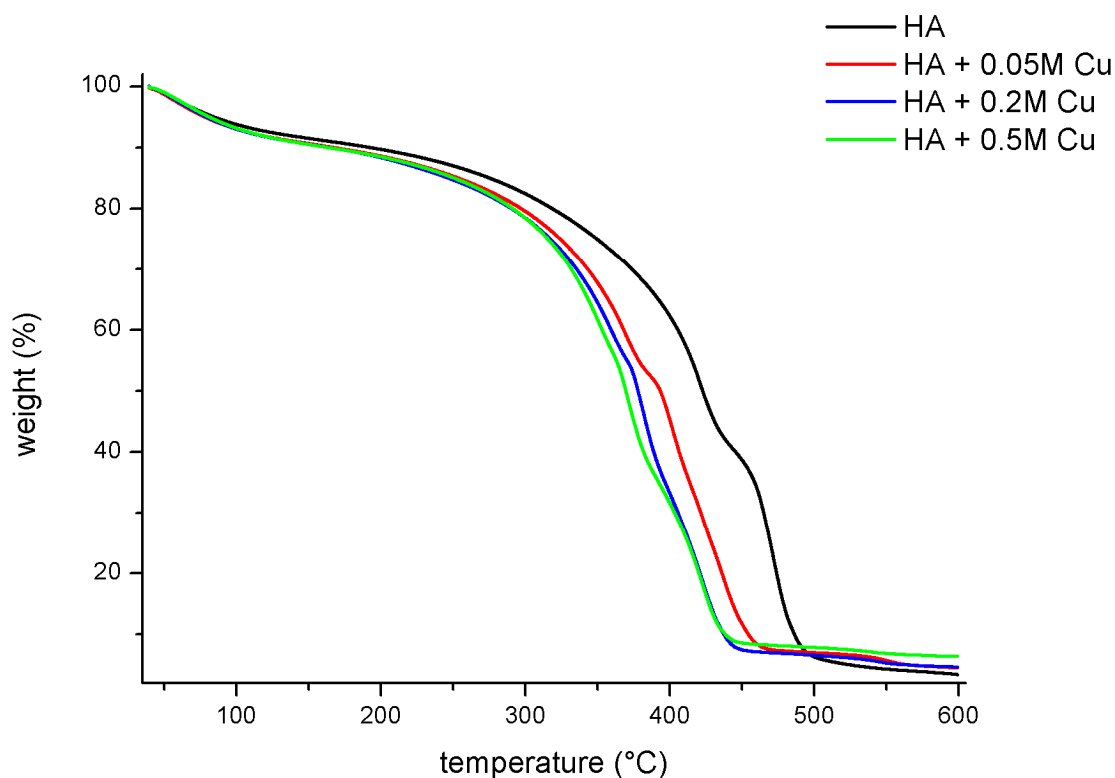


Fig. 20 Comparison of TGA records of differently concentrated Cu-humic complexes after MgCl_2 extraction

The intervention of MgCl_2 to the Cu-humic complex structure is reflected in the changes of thermo-oxidative behaviour of these samples. The ending degradation temperature is higher than in the case of samples before MgCl_2 extraction (cf. Fig. 20 and Fig. 10). The same trend has the starting degradation temperature observable in Fig. 21 and Fig. 11. This fact indicates a decrease of amount of Cu^{2+} bound on HA after extraction, thus an increasing amount of Cu^{2+} extracted by MgCl_2 with increasing concentration of complexing solution. Sample of humic acid with no incorporated metal has the highest starting degradation temperature, see Chapter 4.3, Fig. 5.

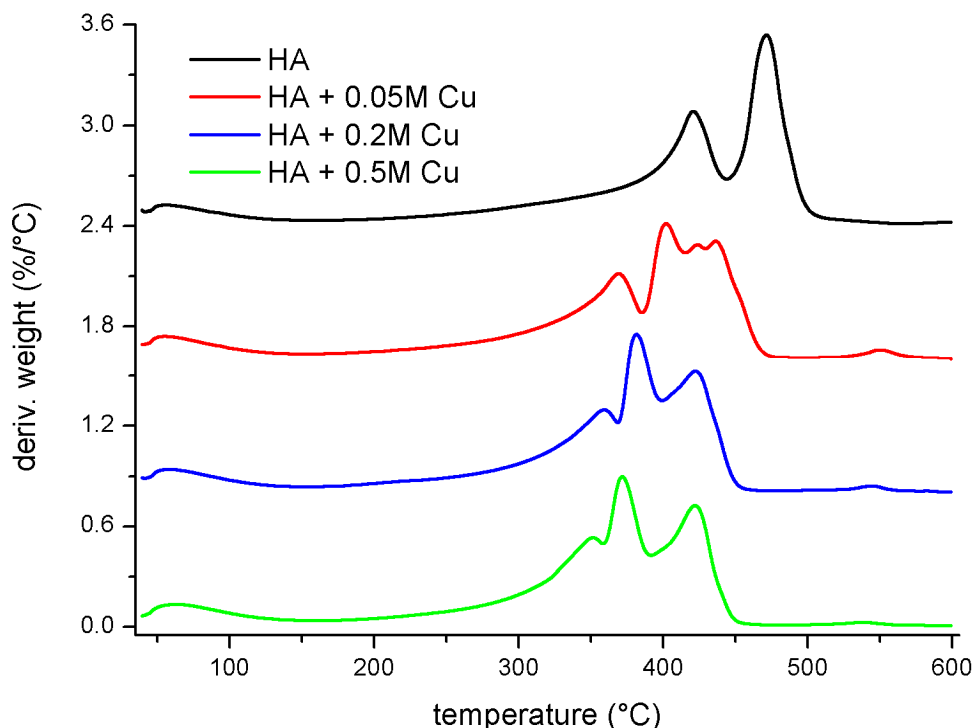


Fig. 21 Comparison of DTG curves of differently concentrated Cu-humic complexes after $MgCl_2$ extraction

Fig. 21 presents derivative thermogravimetric records of differently saturated Cu-humic complexes after $MgCl_2$ extraction. Shapes of the curves reflect the multi-step degradation, which can be interpreted as disruption of intermolecular forces between individual parts of HA structure, thus the creation of several independent units.

The structure of humic acid may relax without the presence of intermolecular forces. HA does not degrade as one aggregate but as a number of individual parts.

4.6.3. Influence of $MgCl_2$ treatment on Ni-humic complexes

Fig. 22 and Fig. 23 are used to describe the influence of $MgCl_2$ on differently saturated Ni-humic complexes as well as on unaltered HA. Comparing with Fig. 18 and Fig. 19 one can observe very similar shape of the curves, which reflects similar influence of $MgCl_2$ on Co- and Ni-humic complexes. The treatment with $MgCl_2$ results in moderation of degradation velocity of the most stable part of HA (second peak in Fig. 23), which would probably continue even at temperatures above 600 °C (slow but continual decrease of weight from approx. 500 °C observable in Fig. 22).

All metal ions incorporated into the humic structure exhibit the same behaviour after $MgCl_2$ treatment at temperature above 500 °C (see Fig. 18, Fig. 20 and Fig. 22). This trend can be explained by Mg^{2+} occupation of the most stable parts of HA structure, which decelerate their degradation.

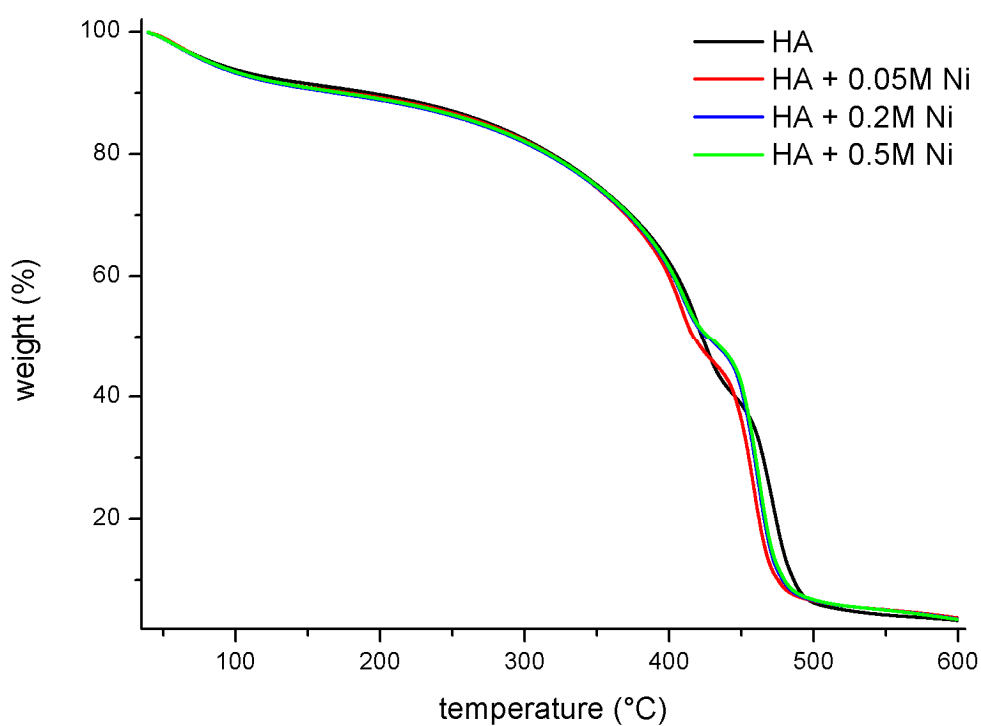


Fig. 22 Comparison of TGA records of differently concentrated Ni-humic complexes after MgCl_2 extraction

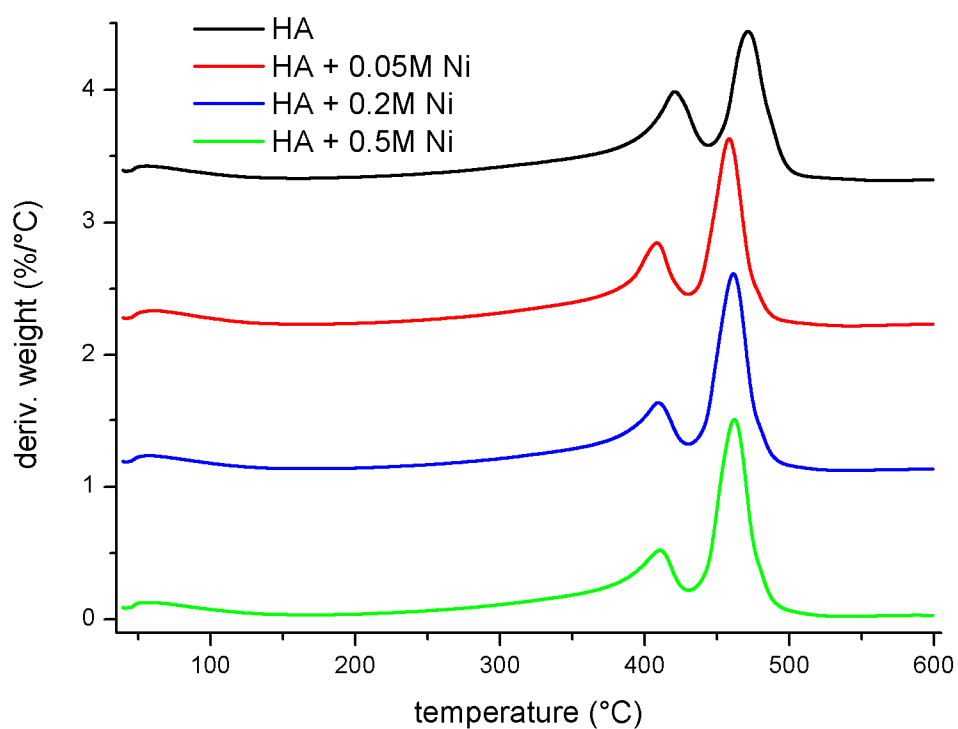


Fig. 23 Comparison of DTG curves of differently concentrated Ni-humic complexes after MgCl_2 extraction

4.7. Influence of HCl treatment

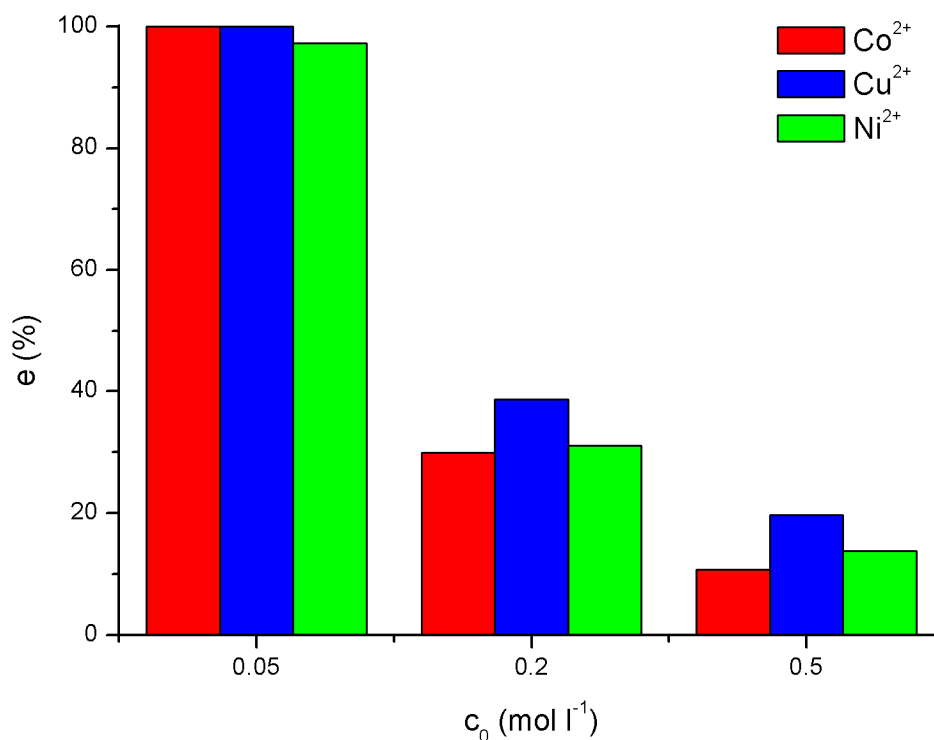


Fig. 24 Amount of each ions loosened after HCl treatment for each initial concentration c_0

The percentage of amount of each ion extracted by HCl from adsorbed amount is displayed in Fig. 24 in dependence on initial concentration of metal ions. Comparing Fig. 24 and Fig. 17 it is possible to reveal an interesting behaviour of Cu²⁺ ions. While HCl extracts preferentially Cu²⁺, during and after the MgCl₂ treatment the Cu²⁺ ions seem to be more resistant than Co²⁺ and Ni²⁺ ions. There is also trend of relation between increasing extracted amount and initial concentration.

Unlike MgCl₂, HCl is much stronger extraction agent, its influence on the samples is generally more intensive than that of MgCl₂. TGA records of HCl treated samples of Co-humic complexes with different initial concentration are presented in Fig. 25. HCl has changed all of presented samples including unaltered HA. There is no significant difference between each initial concentration (c_0), which is clearly visible in Fig. 26, where the derivative thermogravimetric records are presented. The same situation is demonstrated in Appendix 1 and 2 for the samples with Cu- and Ni-humic complexes. Concerning the degradation end point, the unaltered HA one is slightly pushed to higher temperature. Hydrochloric acid greatly influences the whole structure of HA itself and its complexes regardless of bound metal ion, especially concerning the extraction of inorganic residues, which, prior to the extraction itself, caused higher ash content. It has a profound positive effect on thermo-oxidative stability. The two-step degradation has been changed to one-step degradation, which indicates more gradual process. The structure of HA might convert to more closed aggregate without any peripheral parts, which are more susceptible to degradation at lower temperature.

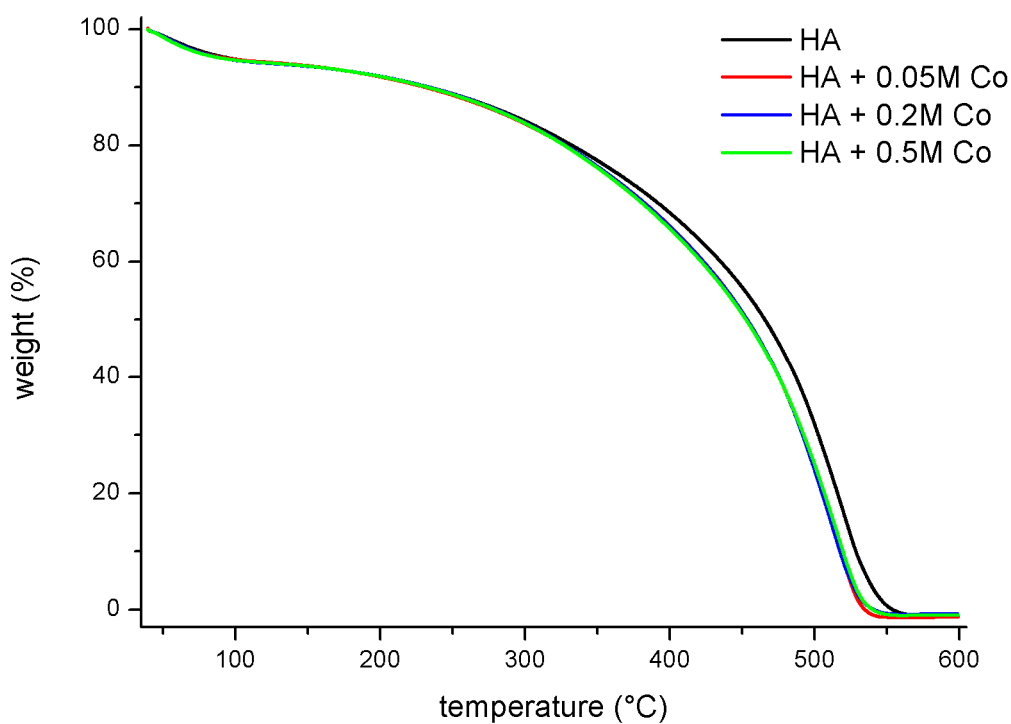


Fig. 25 Comparison of TGA records of differently concentrated Co-humic complexes after HCl extraction

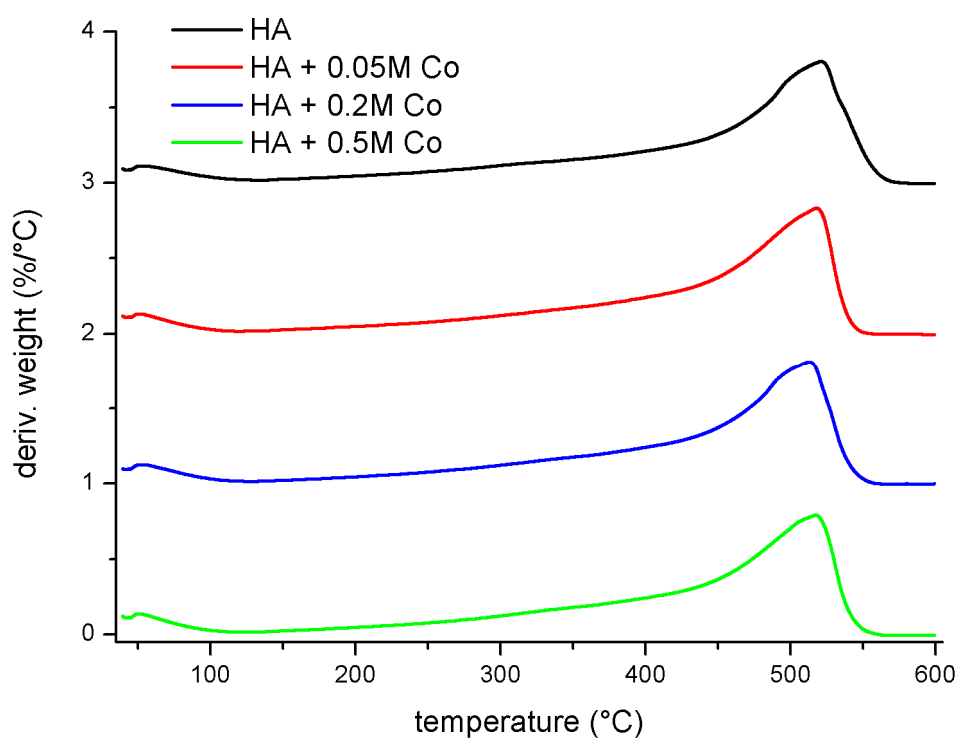


Fig. 26 Comparison of DTG curves of differently concentrated Co-humic complexes after HCl extraction

4.8. Comparison of extraction agents influence

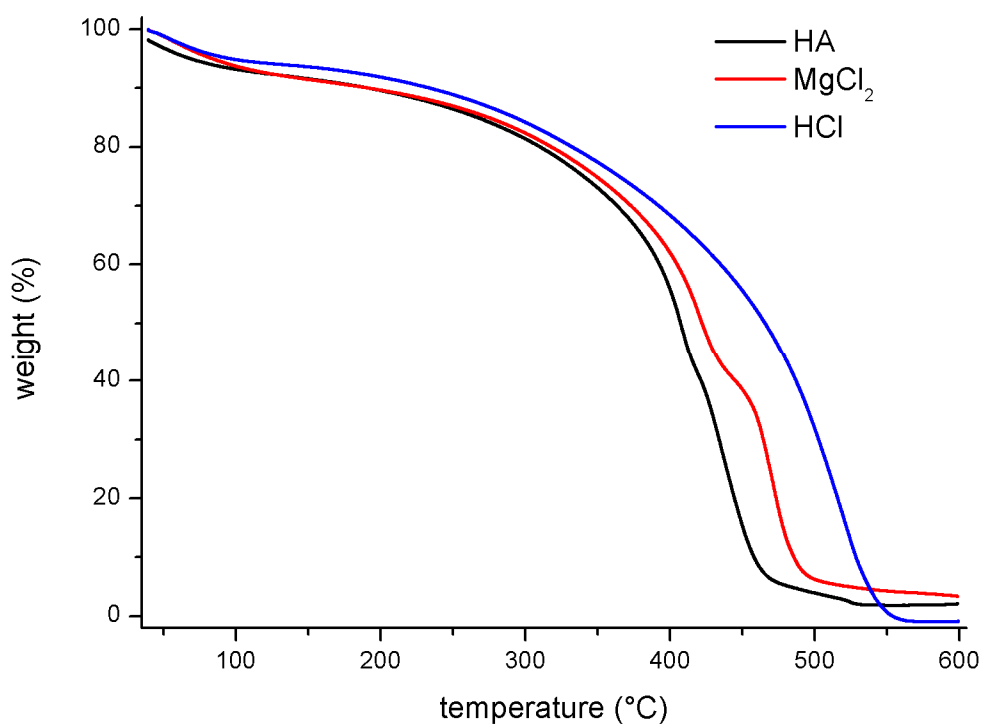


Fig. 27 Comparison of TGA records of differently treated HA

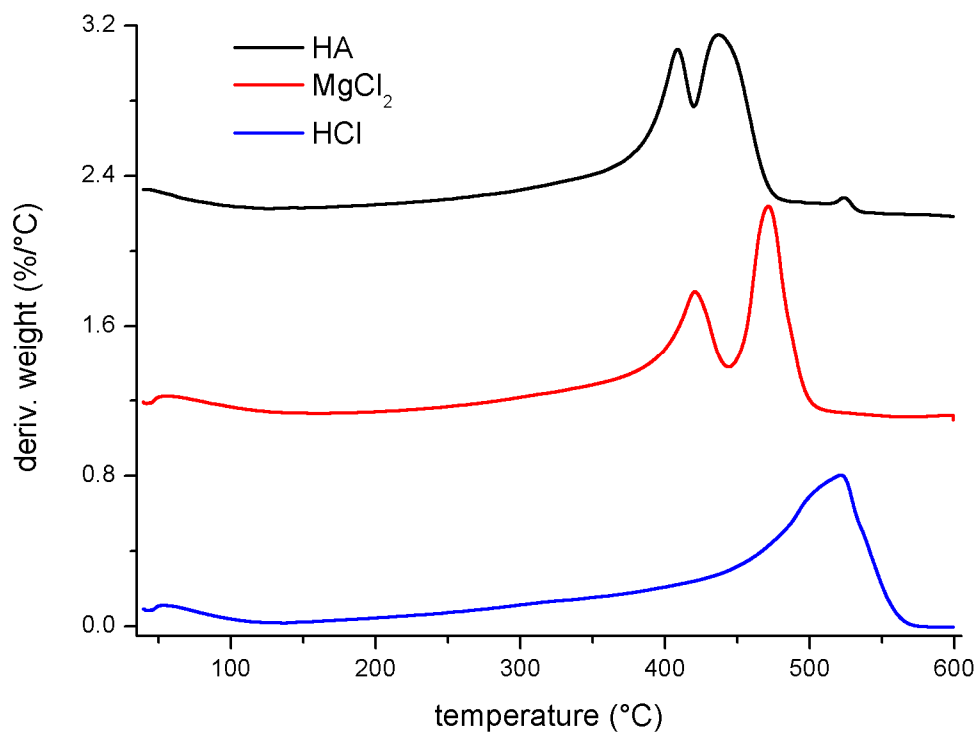


Fig. 28 Comparison of DTG curves of differently treated HA

Both extraction agents tend to stabilize HA, i.e. the main degradation step as well as the end of the degradation phase occurs at higher temperature, in case of HCl the first degradation peak is more-or-less congruent or disappears completely. After MgCl_2 treatment there is larger amount of ash (as reported in Fig. 27), which is caused by presence of metal-containing residues (there still persist strongly bound ions) and eventual magnesium interactions. On the contrary, samples after HCl treatment do not show any (metal) residues in their structure at all.

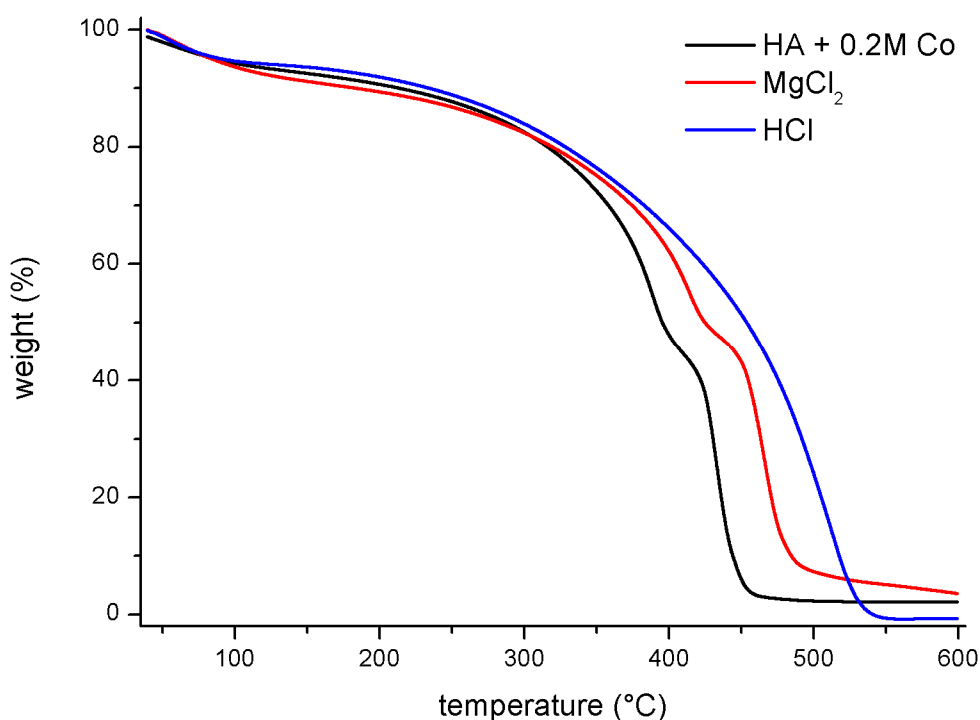


Fig. 29 Comparison of TGA records of differently treated Co-humic complex

The great influence of both agents is clearly visible in Fig. 29. In the case of MgCl_2 the basic shape of the curve remains the same, but the degradation temperature is pushed to higher values. The curve representing the record of sample treated by HCl has a very gradual course. The temperature where the degradation is completed is various; for Co-humic complex without extraction treatment it is about 460 °C, in the case of sample treated by MgCl_2 the situation is more difficult. Degradation of the major step is finished at about 500 °C, but it is visible that not even 600 °C is temperature high enough for complete degradation. There is almost no ash after degradation of sample treated with HCl in comparison with the others, where the metal (and possibly some other) residues are present after the degradation of the organic matter of HA.

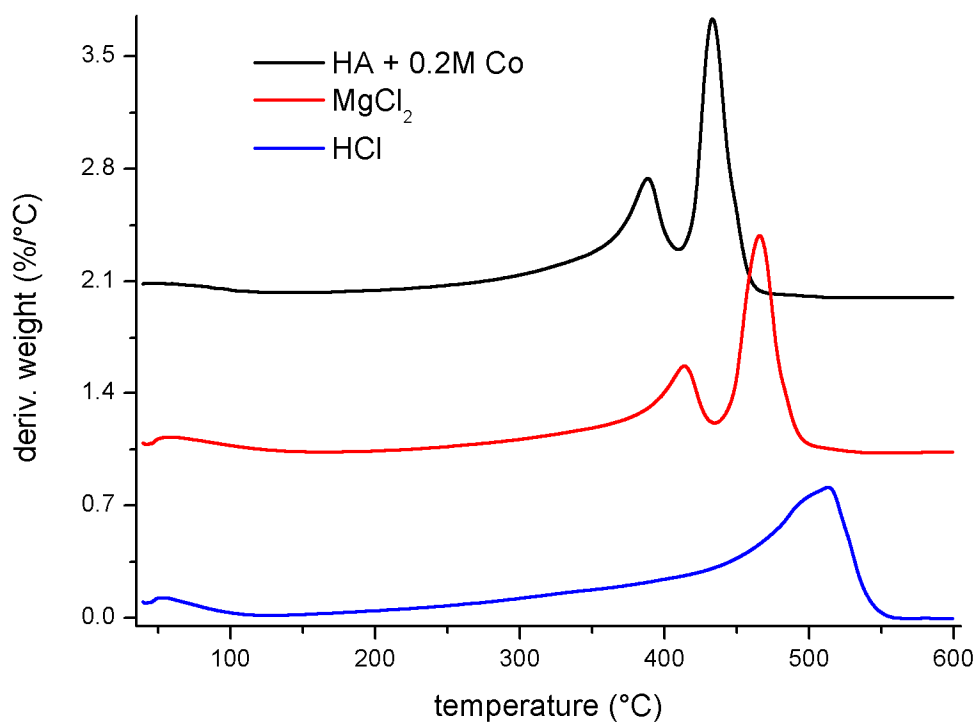


Fig. 30 Comparison of DTG curves of differently treated HA Co-humic complex

Fig. 30 represents derivation of records in Fig. 29. There is more visible the various beginning temperature of degradation. Whereas the degradation of the sample before extraction and after extraction with MgCl_2 is two-step, the last curve displays only one-step degradation, which corresponds to intervention of HCl to the structure of the complex and extraction of inorganic residues from HA structure.

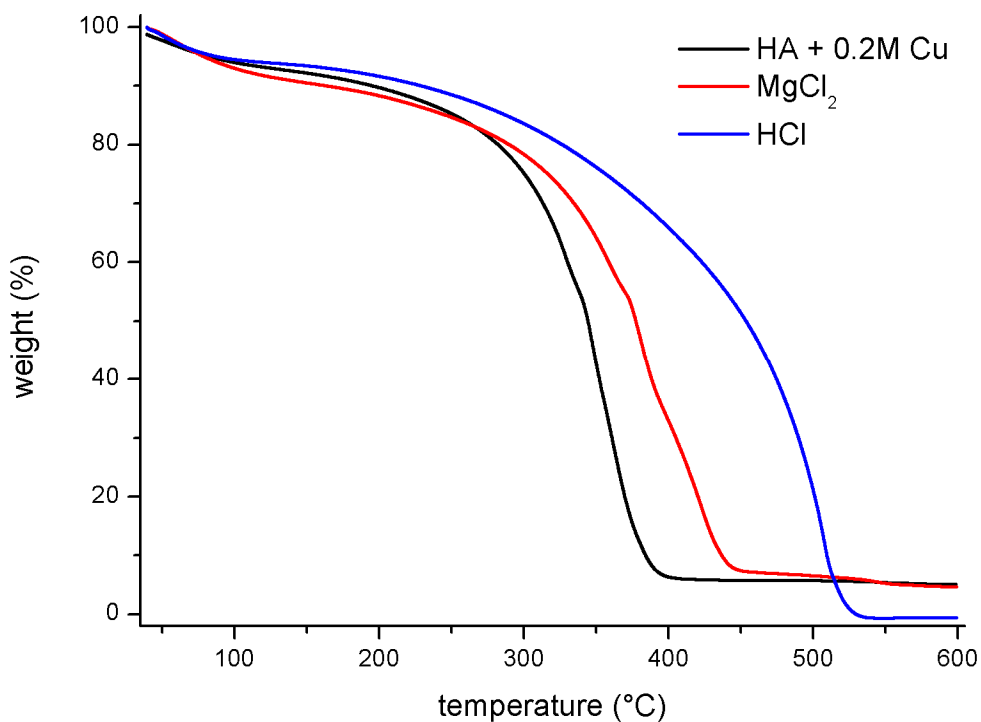


Fig. 31 Comparison of TGA records of differently treated Cu-humic complex

This record (Fig. 31) displays the same basic characteristics as in the case of Co-humic complex. There is more visible difference in the ash content between presented samples and also the difference among the ending degradation temperatures is greater. Sample without extraction agents treatment is completely degraded at about 400 °C, the sample with MgCl₂ treatment at about 450 °C (but not really completely as described above) and the sample with HCl treatment at about 530 °C.

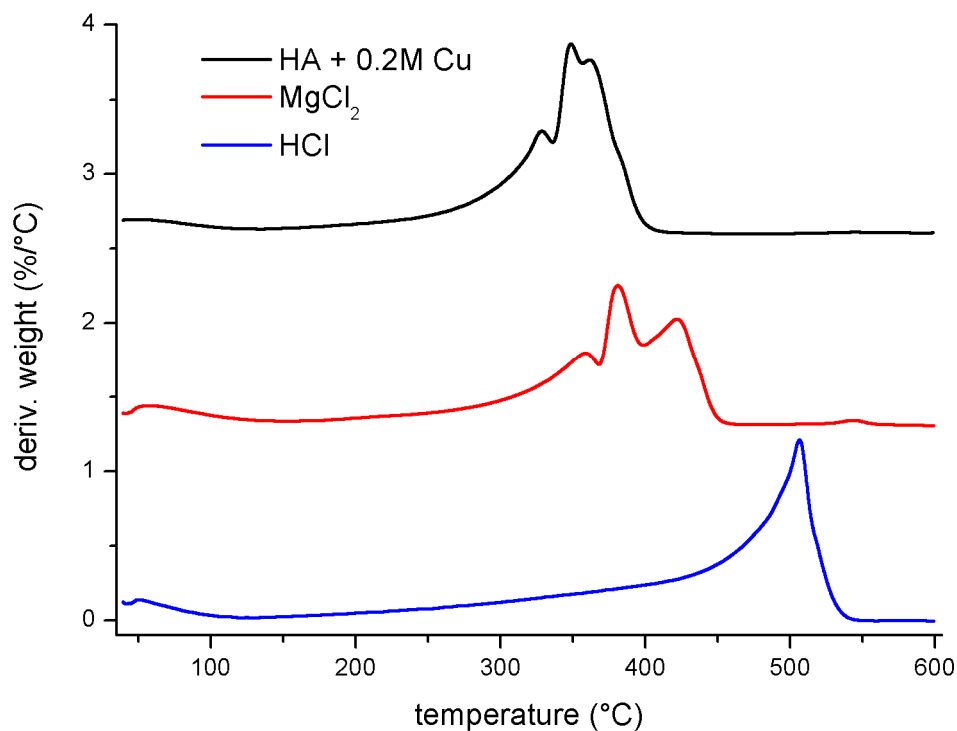


Fig. 32 Comparison of DTG curves of differently treated HA Cu-humic complex

Three-step degradation of Cu-humic complex and its residue after MgCl_2 extraction in the contrast with one step degradation of Cu-humic complex after HCl treatment is plotted in Fig. 32. In comparison with the records of Co-humic samples, there is more conspicuous influence of MgCl_2 on the Cu-humic complex structure. The shape of the curve is different concerning the temperature and proportion of peaks.

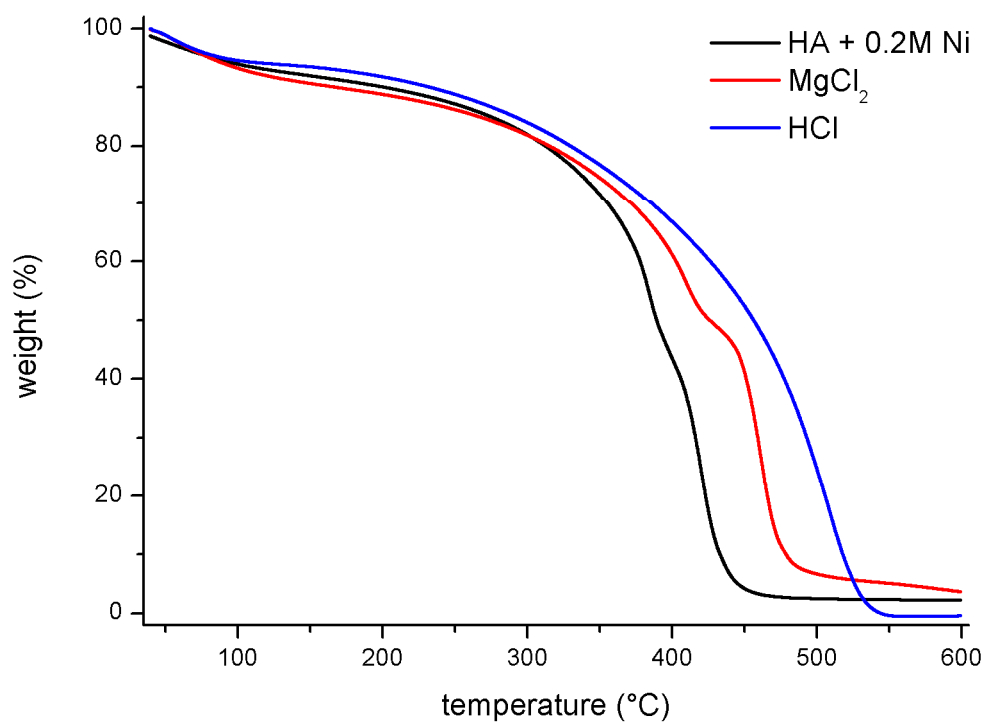


Fig. 33 *Comparison of TGA records of differently treated Ni-humic complex*

The effect of incorporation of nickel to the humic structure on thermo-oxidative behaviour is very similar to that of cobalt (cf. Fig. 33 and Fig. 29). There are the same differences between curve shapes, ash content, and degradation end point. As well as HCl mostly changes the course of degradation of previous samples, it also changes the degradation of Ni-humic complex. Instead of two-step degradation one can see gradual, smooth, one-step degradation.

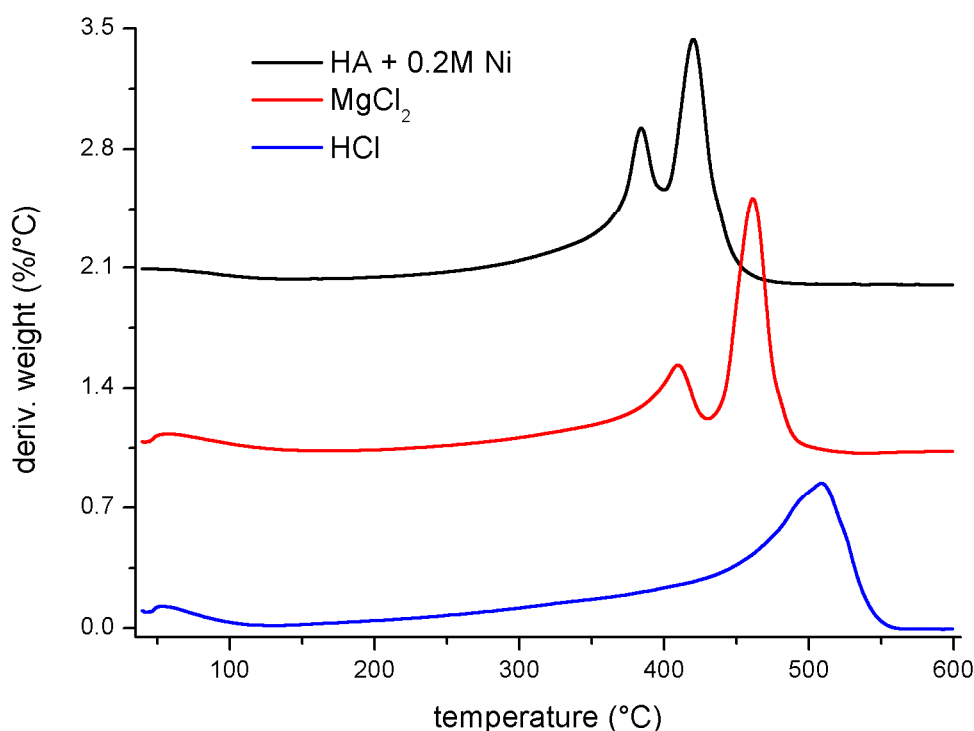


Fig. 34 Comparison of DTG curves of differently treated HA Ni-humic complex

Fig. 34 is supplying information about degradation of Ni-humic complex before and after metal extraction. There is clearly visible the shape of each curve. While the shape stays almost preserved after MgCl_2 treatment, after HCl extraction the basic structural character of the sample is notably changed.

There is not observable any significant difference in the thermo-oxidative stability between differently saturated samples of single metal-humic complex, except of Cu-humic samples. Concerning the chemical stability, the percentage of adsorbed amount extractable from complexes is dependent on the adsorbed amount, thus on initial concentration of metal ion solution. In the case of Co-humic sample prepared from 0.05M solution, already the MgCl_2 extraction was completely successful. Lower amount (80 %) was extracted by MgCl_2 from Ni-humic sample with $c_0 = 0.05$ M and only 50 % was extracted from equally concentrated Cu-humic complex. On the contrary, 100 % of adsorbed ions from all samples with the lowest initial concentration was extracted by HCl. Hydrochloric acid is very strong extraction agent. According to [77] and [87], HCl is able to extract all metal ions, thus cancel all bonds between HA and metal ions (especially Cu^{2+}). Also during our experiments HCl extracted the highest amount of metal ions from the metal-complexed humic acid, although in the case of samples prepared from solutions with initial concentration c_0 0.2 and 0.5 M, the extraction was not completely successful (see Fig. 17). Concerning the fact that the initial sample masses for thermogravimetric analysis are very low (in the order of miligram units), the amount of present metals after the extraction procedure and following thermo-oxidative degradation is below the threshold of the measuring device. Moreover, the decrease of ash content after HCl treatment is observable not only at the complexed HA samples but also at the unaltered HA

(see Table 7). This is most likely linked with the extraction of some inorganic residues present in the HA structure and leads to distortion of the results of metal-complex samples. Furthermore, the amount of HCl was most probably not sufficient enough to extract all metal ions from samples prepared from more concentrated solutions. Some properties of HA structure were changed after HCl treatment (see Chapter 4.7.), most probably due to extraction of inorganic residues and other impurities from HA. Intervention to HA structure is reflected in the shape of degradation records, the degradation step has become very slow and gradual. For all the above mentioned reasons, drawing conclusions about the metal complexation and extraction procedure from the ash content can be misleading. Therefore, this work is aimed to investigate the course of the thermo-oxidative degradation of studied samples, not to interpret the numerical results of these analyses.

5. CONCLUSION

Metal-humic complex samples from characterized humic acid and differently concentrated solutions of Co^{2+} , Cu^{2+} and Ni^{2+} were prepared and subjected to extraction by two different extraction agents (MgCl_2 , HCl). All samples before and after extractions were investigated by methods of thermal analysis (TGA, DSC). The results of this work suggest that the incorporation of metal ions influences the thermo-oxidative stability of humic acid and generally its thermo-oxidative behaviour. All metal ions bound to HA structure implicate the decrease of starting and ending temperature of HA thermo-oxidative degradation. The most substantial changes in the degradation process of humic acid (observed by means of thermogravimetric analysis) were produced by the presence of Cu^{2+} in HA structure. On the contrary, thermo-analytical records of samples with Ni^{2+} and Co^{2+} ions exert very comparable course. Extraction of all studied metal ions was completely successful only by HCl for samples prepared from 0.05M solutions of metal ions. Beside the extraction of metal ions, HCl also influences the structure of HA, which was confirmed by the shape of TA curves. In future work, the effectiveness of the extraction agents in dependence on its amount will be investigated.

6. REFERENCES

- [1] Clapp, C. E., Hayes, M. H. B., Simpson, A. J., Kingery, W. L.: Chemistry of soil organic matter. In: *Chemical processes in soils*. (Tabatabai, M. A., and Sparks, D. L., eds.), Soil Science Society of America, SSSA Book Series, Medison, 2005, pp. 1–150.
- [2] Stevenson, F. J.: *Humus chemistry: Genesis, Composition, Reactions*. 2nd ed. New York: Wiley, 1994, 512 p.
- [3] Derenne, S., Largeau, C.: A review of some important families of mocomolecules: Composition, origine, and fate in soils and sediments. *Soil Science*, 2001, vol. 166, pp. 833–847.
- [4] Pitter, P.: *Hydrochemie*. 4th ed. Praha: VŠCHT Praha, 2009, 568 s.
- [5] Kang, K-H., Shin, H. S., Park, H.: Characterization of humic substances present in landfill leaches with different landfill ages and its implication. *Water Research*, 2002, vol. 36, pp. 4023–4032.
- [6] Skokanová, M., Dercová, K.: Humínové kyseliny původ a struktúra. *Chemické listy*, 2008, roč. 102, s. 262–268.
- [7] MacCarthy, P.: The Principles of humic substances: An Introduction to the first principle. In: *Humic substances: Structures, Models and Functions*. (Ghabbour, E. A., Davies, G., eds.), Royal Society of Chemistry, Cambridge, 2001, pp. 19–30.
- [8] Cameron, R. S., Thornton, B. K., Swift, R. S., Posner, A. M.: Molecular weight and shape of humic acid from sedimentation and diffusion measurements on fractionated extracts. *Journal of Soil Science*, 1972, vol. 23, pp. 394–408.
- [9] Piccolo, A. The supramolecular structure of humic substances. *Soil Science*, 2001, vol. 166, pp. 810–832.
- [10] Piccolo, A., Conte, P., Cozzolino, A., Spaccini, R.: Molecular sizes and association forces of humic substances in solution. In *Humic Substances and Chemical Contaminants*. (Clapp, C. E., Hayes, M. H. B., Senesi, N., Bloom, P. R., Jardine, P. M., eds.), Soil Science Society of America, Madison, 2001, pp. 89–118.
- [11] Piccolo, A., Spiteller, M.: Electrospray ionization mass spectrometry of terrestrial humic substances and their size fractions. *Analytical Bioanalytical Chemistry*, 2003, vol. 377, pp. 1047–1059.
- [12] Larsen, G., Chilingar, G.V.: *Diagenesis in sediments, volume 8: Developments in Sedimentology*. Amsterdam: Elsevier, 1967, 551 p.
- [13] Thomas, L.: *Coal geology*. Chichester: John Wiley & Sons Ltd., 2002, 384 p.
- [14] Moore, E.S.: *Coal: its properties, analysis, classification, geology, extraction, uses and distribution*. 2nd ed. New York: John Wiley & Sons Ltd., 1940, 473 p.

- [15] Speight, J.G.: *The chemistry and technology of coal*. 2nd ed. New York: Marcel Dekker, 1994, 642 p.
- [16] Smith, K.L., Smooth, L.D., Fletcher, T.H., Pugmire, R.J.: *The structure and reaction processes of coal*. New York: Plenum Press, 1994, 471 p.
- [17] Laidler, K. J., Meiser, J. H., Sanctuary, B. C.: *Physical chemistry*. 4th ed. Houghton Mifflin Company: Boston, 2003, 1060 p.
- [18] Bartovská, L., Šišková, M.: *Fyzikální chemie povrchů a koloidních soustav*. Praha: VŠCHT Praha, 1999, 205 s.
- [19] McNaught, A. D., Wilkinson, A.: *Compendium of Chemical Terminology*. 2nd ed. International Union of Pure and Applied Chemistry: Blackwell Science, 1997, 464 p.
- [20] Krätsmár-Šmorgovič, J.: *Všeobecná a anorganická chémia*. Martin, Osveta, 1994, 399 s.
- [21] Senesi, N., Loffredo, E.: Metal Ion Complexation by Soil Humic Substances. In: *Chemical processes in soils*. (Tabatabai, M. A., and Sparks, D. L., eds.), Soil Science Society of America, SSSA Book Series, Madison, 2005, pp. 563–617.
- [22] Buffle, J.: *Complexation reactions in aquatic systems. An analytical approach*, Chichester, Ellis Horwood, 1988.
- [23] Lishtvan, I. I., Ducharchik, V. M., Kovrik, S. I., Smychnik, T. P., Strigutskii, V. P.: Peculiarities of the Formation of Supramolecular Structures of Copper– and Lead–Humic Acid Complexes in Aqueous Solutions. *Colloid Journal*, 2005, vol. 67, pp. 741–745.
- [24] Datta, A., Sanyal, S. K., Saha, S.: A study of natural and synthetic humic acids and their complexing ability towards cadmium. *Plant and Soil*, 2001, vol. 235, pp. 115–125.
- [25] Garcia-Mina, J. M.: Stability, solubility and maximum metal binding capacity in metal–humic complexes involving humic substances extracted from peat and organic compost. *Organic Geochemistry*, 2006, vol. 37, pp. 1960–1972.
- [26] Kislenko, V. N., Oliinyk, L. P.: Binding of Copper(II), Cobalt(II), and Nickel (II) Cations with Humic Acids and Their Sodium Salts in Aqueous Media. *Organic Synthesis and Industrial Organic Chemistry*, 2003, vol. 76, pp. 1962–1964.
- [27] Klučáková, M., Omelka, L.: Sorption of Metal Ions on Lignite and Humic Acids. *Chemical Papers*, 2004, vol. 58, pp. 170–175.
- [28] Martyniuk, H., Wieckowska, J.: Adsorption of metal ions on humic acids extracted from brown coals. *Fuel Processing Technology*, 2003, vol. 84, pp. 23–26.

- [29] Porasso, R. D., Benegas, J. C., van den Hoop, M. A. G. T., Paoletti, S.: Analysis of Trace Metal Humic Acid Interactions Using Counterion Condensation Theory. *Environmental Science & Technology*, 2002, vol. 36, pp. 3815–3821.
- [30] Madronová, L., Kozler, J., Čežíková, J., Novák, J., Janoš, P.: Humic acids from coal of the North-Bohemian coal fields III. Metal-binding properties of humic acids – measurements in a column arrangement. *Reactive & Functional Polymers*, 2001, vol. 47, pp. 119–123.
- [31] Jin, X., Bailey, G. W., Yu, Y. S., Lynch, A. T.: Kinetics of Single and Multiple Metal Ion Sorption Processes on Humic Substances. *Soil Science*, 1996, vol. 161, pp. 509–520.
- [32] van den Hoop, M. A. G. T., Porasso, R. D., Benegas, J. C.: Complexation of heavy metals by humic acids: analysis of voltammetric data by polyelectrolyte theory. *Colloids and Surfaces*, 2002, vol. 203, pp. 105–116.
- [33] Plaza, C., Senesi, N., Carcía-Gil, J. C., Polo, A.: Copper(II) complexation by humic and fulvic acids from pig slurry and amended and non-amended soils. *Chemosphere*, 2005, vol. 61, pp. 711–716.
- [34] Provenzano, M. R., D’Orazio, V., Jerzykiewicz, M., Senesi, N.: Fluorescence behaviour of Zn and Ni complexes of humic acids from different sources. *Chemosphere*, 2004, vol. 55, pp. 885–892.
- [35] Prado, A. G. S., Torres, J. D., Martins, P. C., Pertusatti, J., Bolzon, L. B., Faria, E. A.: Studies on copper(II)- and zinc(II)-mixed ligand complexes of humic acid. *Journal of Hazardous Materials*, 2006, vol. 136, pp. 585–588.
- [36] Ioselis, P., Ikan, R., Frenkel, M.: Thermal degradation of metal-complexed humic substances. *Physics and Chemistry of the Earth*, 1980, vol. 12, pp. 567–577.
- [37] Lu, X. Q., Vassallo, A. M., Johnson, W. D.: Thermal stability of humic substances and their metal forms: an investigation using FTIR emission spectroscopy. *Journal of Analytical and Applied Pyrolysis*, 1997, vol. 43, pp. 103–114.
- [38] Beraldin, M. T., Vauthier, E., Fliszar, S.: Charge distributions and chemical effects. XXVI. Relationships between nuclear magnetic resonance shifts and atomic charges for ¹⁷O nuclei in ethers and carbonyl compounds. *Canadian Journal of Chemistry*, 1982, vol. 60, pp. 106–110.
- [39] Schnitzer, M., Kodama, H.: Differential thermal analysis of metal-fulvic acid salts and complexes. *Geoderma*, 1972, vol. 7, pp. 93–103.
- [40] Tan, K. H.: Formation of metal-humic acid complexes by titration and their characterization by differential thermal analysis and infrared spectroscopy. *Soil Biology and Biochemistry*, 1977, vol. 10, pp. 123–129.

- [41] Duffus, J. H.: "Heavy metals" – A meaningless term? *Pure and Applied Chemistry*, 2002, vol. 74, pp. 793–807.
- [42] Bjerrum, N.: *Bjerrum's Inorganic Chemistry*. 3rd Danish ed., Heinemann, London, 1936.
- [43] Van Nostrand: *International Encyclopaedia of Chemical Science*. Van Nostrand, New Jersey, 1964.
- [44] Grant, R., Grant, C.: *Grant and Hackh's Chemical Dictionary*. McGraw-Hill, New York, 1987.
- [45] Brewer, M., Scott, T: *Concise Encyclopaedia of Biochemistry*. Walter de Gruyter, Berlin, 1983.
- [46] Parker, S. P.: *McGraw-Hill Dictionary of Scientific and Technical Terms*. 4th ed., McGraw-Hill, New York, 1989.
- [47] Morris, C.: *Academic Press Dictionary of Science and Technology*. Academic Press, San Diego, 1992.
- [48] Davies, B. E.: *Hydrobiologia*. 49, 213, 1987.
- [49] Thornton, I.: *Metals in the Global Environment – Facts and Misconceptions*. ICME, Ottawa, 1995.
- [50] Streit, B.: *Lexikon der Ökotoxikologie*, VCH, Weinheim, 1994.
- [51] Falbe, J., Regitz, M.: (Eds.) *Roempp Chemie Lexikon*, Georg Thieme, Weinheim, 1996.
- [52] Porteous, A.: *Dictionary of Environmental Science and Technology*, 2nd ed., Wiley, Chichester, 1996.
- [53] Harrison, P., Waites, G.: *The Cassell Dictionary of Chemistry*, Cassell, London, 1998.
- [54] Lawrence, E., Jackson, A. R. W., Jackson, J. M.: (Eds.). *Longman Dictionary of Environmental Science*, Addison Wesley Longman, Harlow, 1998.
- [55] Bennet H.: (Ed.). *Concise Chemical and Technical Dictionary*, 4th enlarged ed., Edward Arnold, London, 1986.
- [56] Lewis Sr., R. J.: (Ed.). *Hawley's Condensed Chemical Dictionary*, 12th ed., Van Nostrand Reinhold, New York, 1993.
- [57] Hunt, A.: *Dictionary of Chemistry*, Fitzroy Dearborn, London, 1999.
- [58] *Oxford Dictionary of Science*, 4th ed., Oxford University Press, Oxford (1999).
- [59] Walker, P. M. B.: (Ed.). *Chambers Science and Technology Dictionary*, Chambers Cambridge, Cambridge, 1988.

- [60] Venugopal, B., Luckey, T. D.: "Toxicology of nonradio-active heavy metals and their salts", in *Heavy Metal Toxicity, Safety and Hormology*, T. D. Luckey, B. Venugopal, D. Hutcheson (Eds.), George Thieme, Stuttgart 1975.
- [61] Hale, W. G., Margham, J. P.: (Eds.). *Collins Dictionary of Biology*, Collins, Glasgow, 1988.
- [62] Lyman, W. J.: "Transport and transformation processes", in *Fundamentals of Aquatic Toxicology*, G. M. Rand (Ed.), Taylor & Francis, Washington DC, 1995.
- [63] Phipps, D. A.: "Chemistry and biochemistry of trace metals in biological systems". In: *Effect of Heavy Metal Pollution on Plants*, N. W. Lepp (Ed.), Applied Science Publishers, Barking, 1981.
- [64] Birchon, D.: *Dictionary of Metallurgy*, Newnes, London, 1965.
- [65] Merriman, A. D.: *A Concise Encyclopaedia of Metallurgy*, MacDonald and Evans, London, 1965.
- [66] Hampel, C. A., Hawley, G. G.: *Glossary of Chemical Terms*, Van Nostrand Reinhold, New York, 1976.
- [67] Bates, R. L., Jackson, J. A.: (Eds.). *Glossary of Geology*, 3rd ed., American Geological Institute, Alexandria, VA, 1987.
- [68] Stevenson, L. H., Wyman, B.: *The Facts on File Dictionary of Environmental Science*, Facts on File, New York, 1991.
- [69] Scott, J. S., Smith, P. G.: *Dictionary of Waste and Water Treatment*, Butterworths, London, 1981.
- [70] Hodgson, E., Mailman, R. B., Chambers, J. E.: (Eds.). *Macmillan Dictionary of Toxicology*, Macmillan, London, 1988.
- [71] Kafka, Z., Punčochářová, J.: Těžké kovy v přírodě a jejich toxicita. *Chemické listy*, 2002, roč. 96, s. 611–617.
- [72] Podlešáková, E.: Kontaminace půd, půdně hygienické limity a principy asanace postižených půd. In: *Kultivace a rekultivace půd*. Výzkumný ústav meliorací a ochrany půdy Praha, Praha, 1994, 198 s.
- [73] Tessier, A., Campbell, P. G. C., Bisson, M.: Sequential Extraction Procedure for the Speciation of Particulate Trace Metals. *Analytical chemistry*, 1979, vol. 51, pp. 844–851.
- [74] McGrath, S. P., Cegarra, J.: Chemical extractability of heavy metals during and after long-term applications of sewage sludge to soil. *European Journal of Soil Science*, 1992, vol. 43, pp. 313–321.

- [75] Kabata-Pendias, A.: Behavioural properties of trace metals in soils. *Applied Geochemistry*, 1993, vol. 8, pp. 3–9.
- [76] Hseu, Zeng Yei: Effects of chemical amendments on the HCl-extractability and the sequential extraction of Cu and Zn in a contaminated rice-growing soil. *Food Science and Agricultural Chemistry*, 2003, vol. 5, pp. 66–71.
- [77] Siqueria, R. N. B.: Extracao selectiva de cadmio, chumbo, cobre e zinco pre-adsorvidos po acidos humicos. *Quimica nova*, 1989, vol. 12, pp. 9–13.
- [78] Hatakeyama, T., Quinn, F. X.: *Thermal analysis: fundamentals and applications to polymer science*. 2nd ed. Chichester, John Wiley & Sons Ltd., 1999, 180 p.
- [79] Kolman, L., Šimon, P.: Diferenčná kompenzačná kalorimetria a jej využitie pri štúdiu materialov. *Ropa, uhlie, plyn a petrochémia*, 2000, roč. 42, s. 36–38.
- [80] Okuliar, J., Daučík, P., Šimon, P.: Termogravimetria a jej využitie na štúdium materiálov. *Ropa, uhlie, plyn a petrochémia*, 2002, roč. 44, s. 39–42.
- [81] Blažek, A.: *Thermal analysis*. Van Nostrand Reinhold Company Ltd., 1973, 286 p.
- [82] Ramachandran, V., S., Paroli, R., M., Beaudoin, J., J., Delgado, A., H.: *Handbook of thermal analysis of construction materials*. New York, 2002, 691 p.
- [83] Němcová, I., Čermáková, L., Rychlovský, P.: *Spektrometrické analytické metody I*. 2. vyd., Praha, Karolinum. 2004, 166 s.
- [84] Riggle, J., von Wandruszka, R.: Conductometric characterization of dissolved humic materials. *Talanta*, 2002, vol. 57, pp. 519–526.
- [85] Klučáková, M., Pekař, M.: New Model for Equilibrium Sorption of Metal Ions on Solid Humic Acids. *Colloids and Surfaces, A: Physiochemical and Engineering Aspects*, 2006, vol. 286, pp. 126–133.
- [86] Klučáková, M.: Kinetika sorpcie kovových iontů na huminové kyseliny. *Pracovní setkání fyzikálních chemiků a elektrochemiků*, 2005, roč. 5, s. 35–35.
- [87] Kalina, M.: *Využití průtokové coulometrie ve studiu transportu kovových iontů v huminových gelech*. Brno: Vysoké učení technické v Brně, Fakulta chemická, 2010. 69 s. Vedoucí diplomové práce doc. Ing. Martina Klučáková, Ph.D.

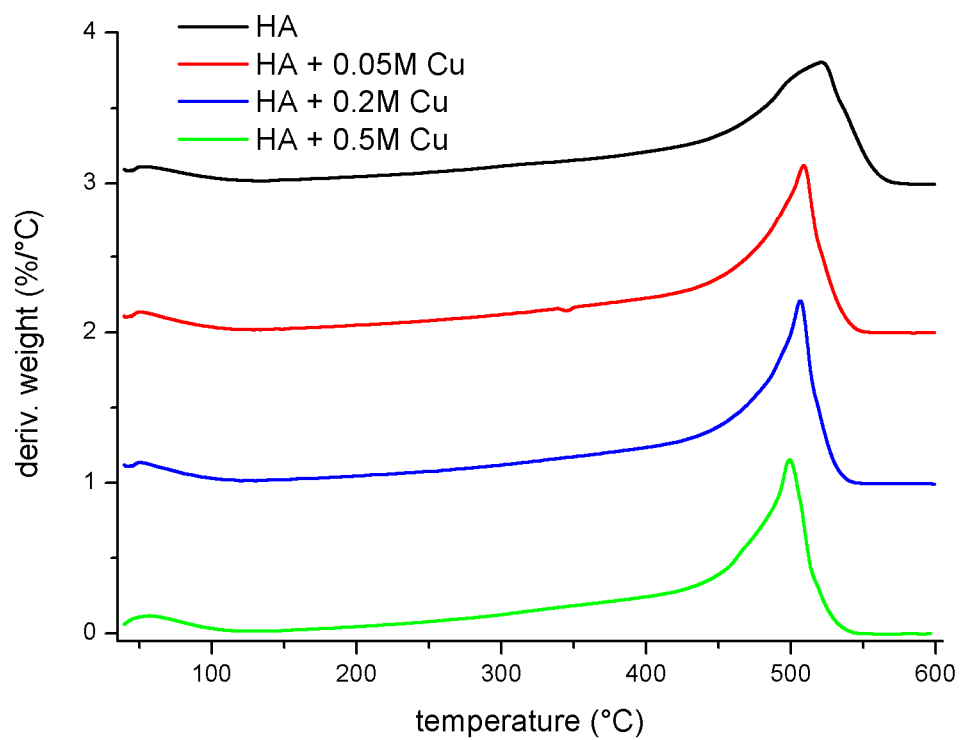
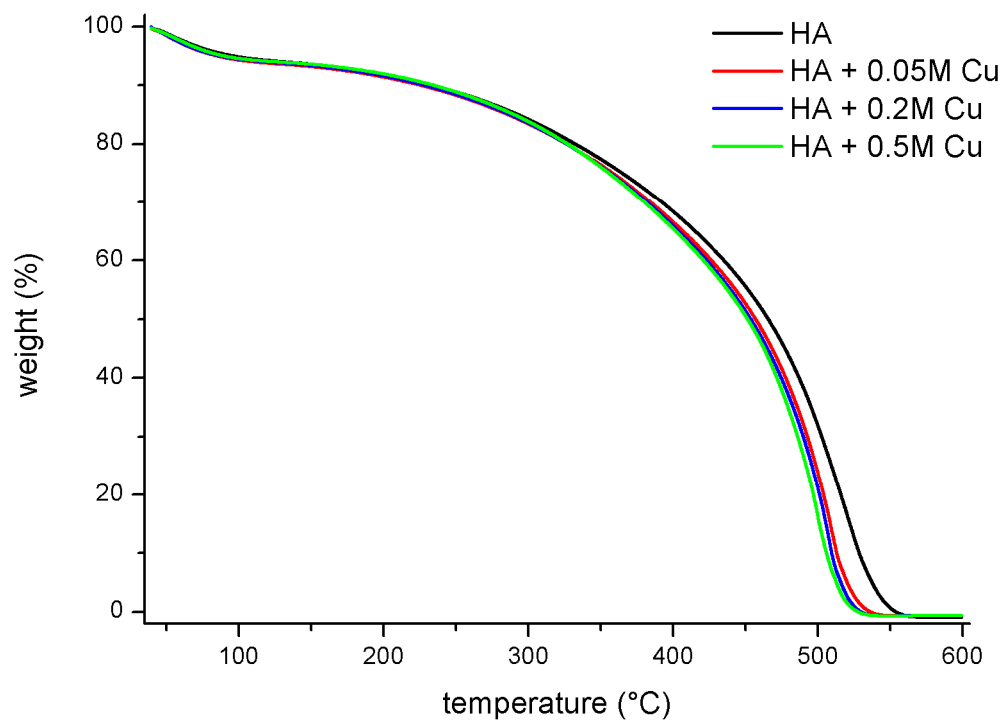
7. LIST OF ABBREVIATIONS

DTA	differential thermal analysis
DSC	differential scanning calorimetry
FA	fulvic acid
FTIR	Fourier transform infrared (spectroscopy)
HA	humic acid
HS	humic substances
ICP-MS	Induced coupled plasma – mass spectroscopy
IHSS	International Humic Substances Society
NMR	nuclear magnetic resonance
RPM	rotations per minute
SOM	soil organic matter
TA	thermal analysis
TGA	thermogravimetric analysis

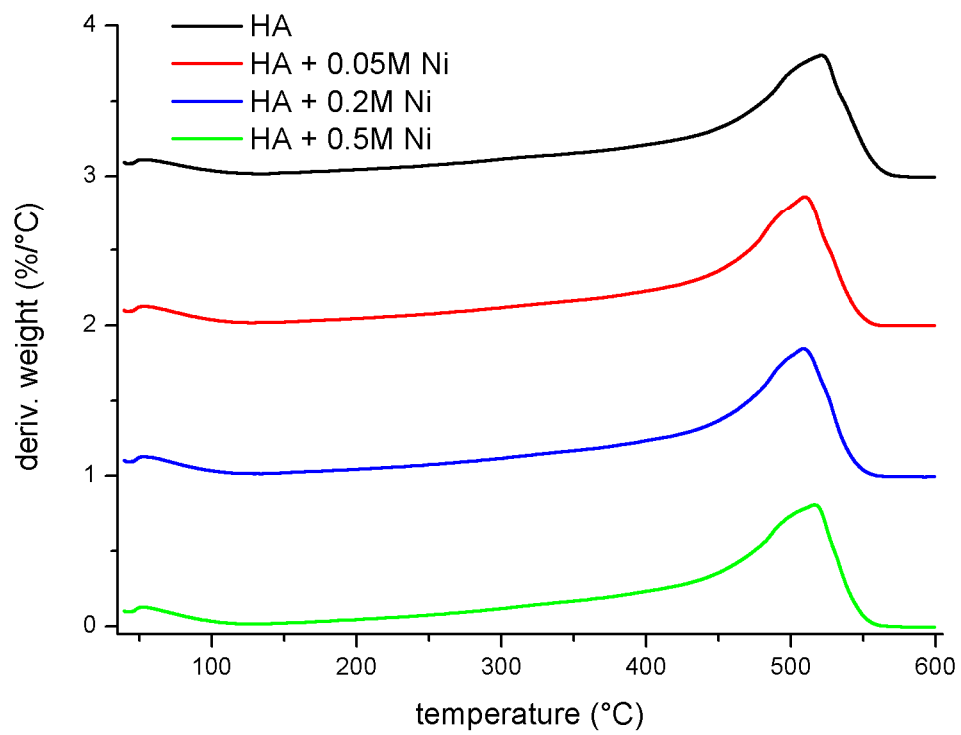
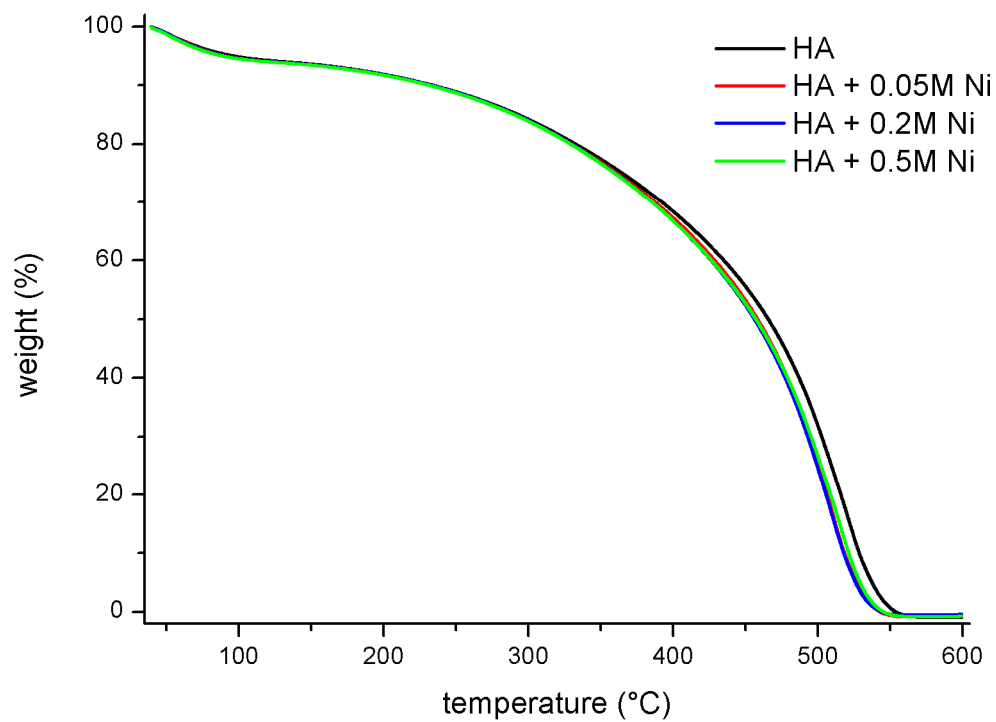
8. LIST OF APPENDICES

APPENDIX 1	TGA and DTG records of Cu-humic samples treated by HCl
APPENDIX 2	TGA and DTG records of Ni-humic samples treated by HCl
APPENDIX 3	DSC records of differently saturated complexes of each metal
APPENDIX 4	DSC records of all samples after HCl treatment
APPENDIX 5	DSC records of all samples after MgCl ₂ treatment
APPENDIX 6	DSC records of each comparison of HCl and MgCl ₂ treatment

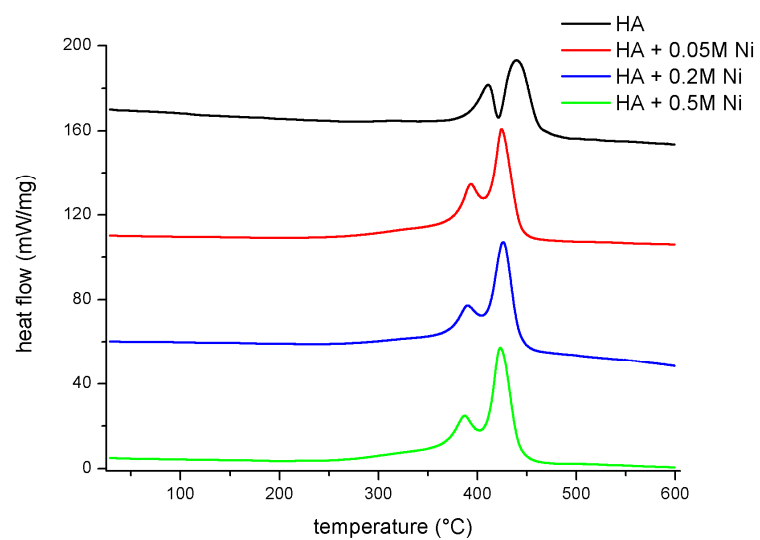
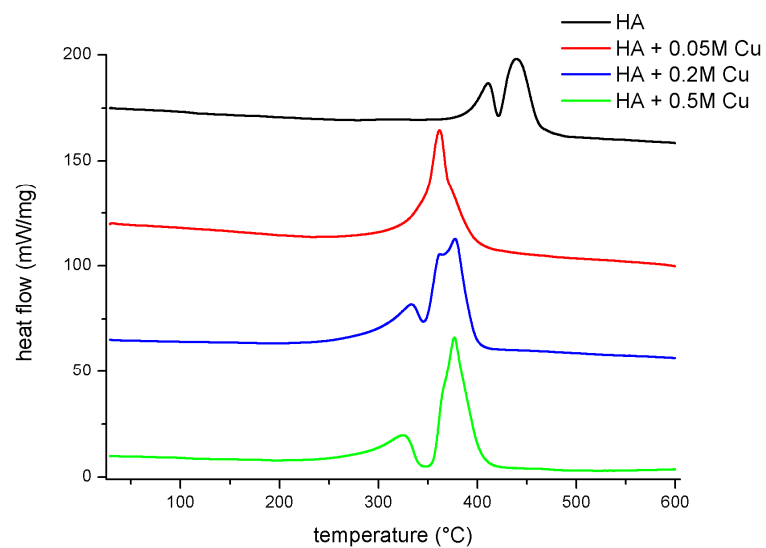
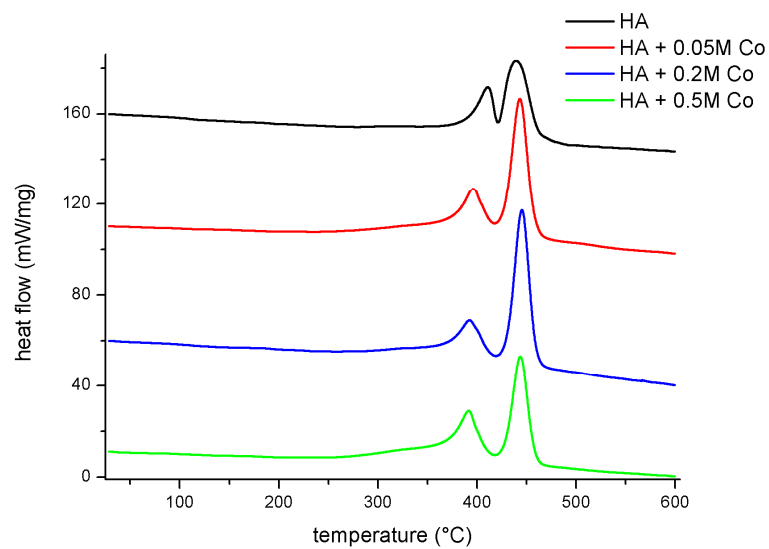
APPENDIX 1



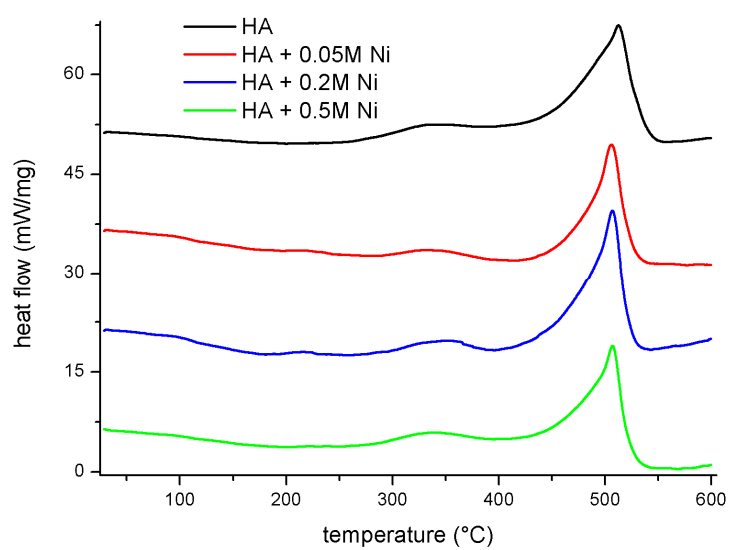
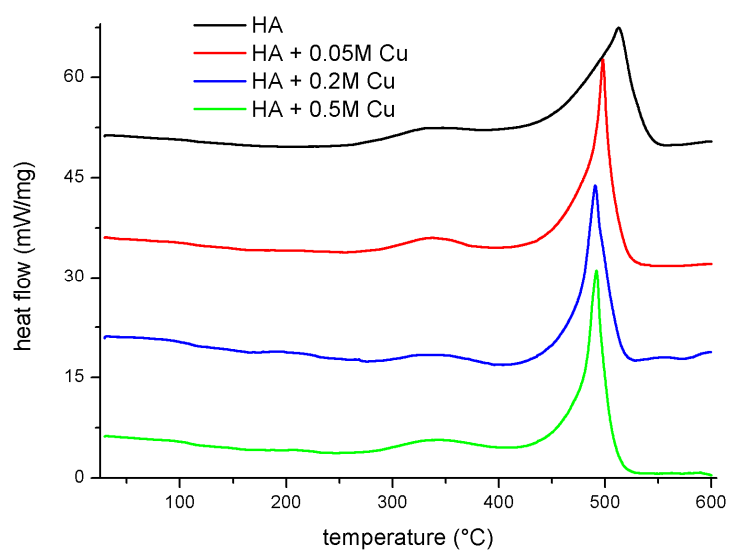
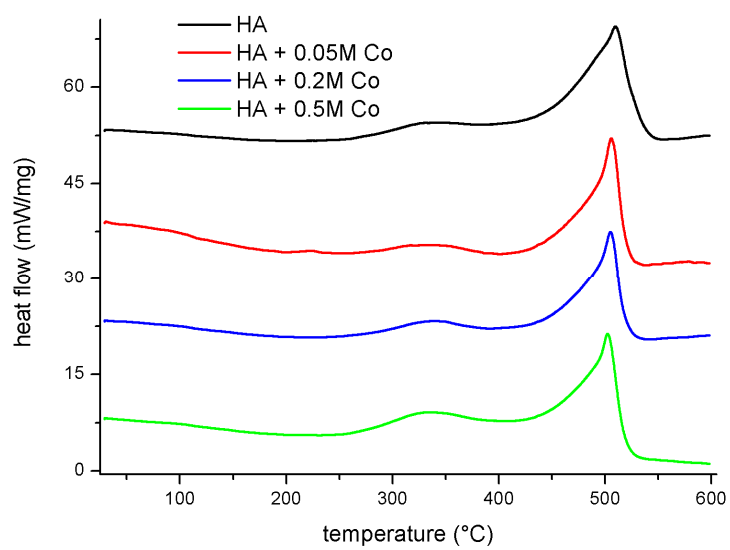
APPENDIX 2



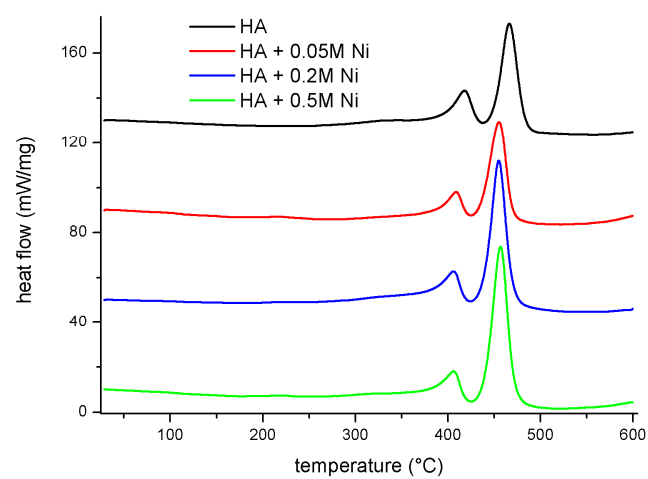
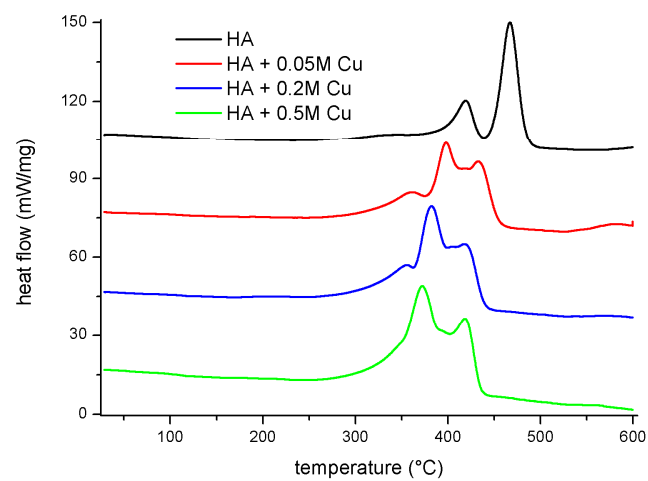
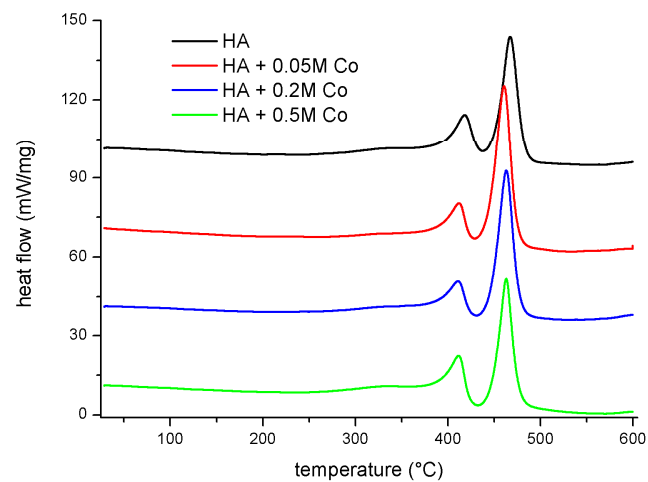
APPENDIX 3



APPENDIX 4



APPENDIX 5



APPENDIX 6

