NATURAL ORGANIC MATTER IN ECOSYSTEMS - A REVIEW

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Abstract: One of the most essential parameters limiting the potential use of the ecosystem (soil, water) is the content of the organic matter. The natural organic matter (NOM) is a ubiquitous component of the lithosphere and hydrosphere that constitutes one of the largest reservoirs of the carbon in the environment. Natural organic substances play several important functions in ecosystems and they are necessary for their normal functioning. Despite many years of the research and using many advanced analytical techniques, their structure has not been fully explained. The main aim of this review is to present the actual state of the knowledge about the natural organic matter and provide a comprehensive overview of the research that has explored up to date in this matter. The additional attention was focused on the relations within and between humic and fulvic acids in terrestrial and aquatic environments. Special attention is focused on the analytical methods used to analysis natural organic matter.

Key words: natural organic matter, humic acids, fulvic acids, humins, ecosystem, ecoanalytics

1. Introduction

The carbon is the most important element on the Earth, its resources are stable and limited. Therefore, there is the continual cycle of processes in which this element is involved. From the environmental point of view, the human life, the carbon cycle has an essential importance in the biochemical way in which living organisms are actively involved. It is estimated that the geochemical cycle is up to 1000 times slower than the biochemical cycle. The human has been actively participating in the cycle of the matter (including the carbon cycle) from the moment appeared on the Earth. However, as a result of the anthropogenic human activity this impact is becoming bigger and bigger and it leads to the disruption of natural biogeochemical cycles (for example increase of CO_2 in the atmosphere).

The natural organic matter is a ubiquitous element of the lithosphere and hydrosphere constituting one of the biggest reservoirs of the carbon in the environment (the estimated amount of the carbon in the organic matter reaches 5×10^{15} kg). Basic carbon cycle is presented on Fig. 1.

The natural organic substances fulfill a range of important functions in ecosystems and they are essential for their proper functioning. However, their presence is sometimes undesirable for example in the water absorbed by the water treatment plants for the nutritional needs of the population. A certain amount of the organic matter of the new quality appears in the form of the compost, sludge and waste due to the growth of the human ecological awareness by conscious actions. This matter does not appear in a fully natural way, so determination of the natural organic matter name makes the sense in the context of the processes that it undergoes for example the soil organic matter (mineralization and humification).

DOI 10.1515/nbec-2015-0002

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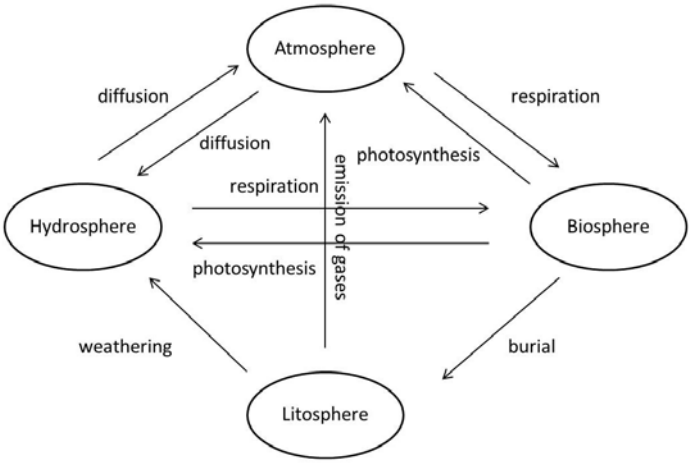

Fig. 1. Basic carbon cycle in environment (EPSTEIN, 1997).

2. Natural organic matter

The natural organic matter (NOM) is a highly heterogeneous mixture. It consist degradation products of plant and animal tissues as well as substances resulting from biochemical changes (biodegradation, resynthesis) of plant and animal origin substrates and their transitional products (NISSEN *et al*., 2001; GU *et al*., 2002). It appears in the soil as the soil organic matter (SOM), surface waters, both fresh and salt water, ground water (in the soil solution) as the dissolved organic matter (DOM), in such parts of the environment as sediments, sewage sludge, organic fertilizers, composts made on the basis of the biomass, organic waste and sewage sludge, etc. The composition of the natural organic matter, its chemical and physicochemical properties depend on the quality of the substrate and environmental conditions in which it appears.

Three main fractions were identified that are part of the natural organic matter:

- high molecular chemical substances with recognized structure that could be classified in one of the structural groups in the organic chemistry such as polysaccharides and proteins,
- chemical compounds with well-explored chemical structure such as amino acids, carbohydrates and other low molecular organic compounds,
- humic substances, the final product of the transformation of plant and animal residues.

This definition is very general although it is very illustrative. However, six main fractions of the organic matter: fats, waxes, oils, resins, hemicellulose, cellulose, protein and lignin-humus complexes can be identified (WAKSMAN, 1936).

A lot of misunderstanding and confusion has accumulated in relation to the definition of the soil organic matter (SOM). This term should be used in the context of the whole soil organic substrate including living organisms. The soil organic matter is the amount of dead organic compounds (mostly plant) occurring in the soil beginning from fresh, plant and animal residues that are not decomposed to amorphous products of the decomposition and re-synthesis excluding living organisms. In the scientific literature it is common to use the term of the SOM in terms of the soil organic substance or humic substances (also known as humus). The individual fractions of the soil organic matter according to GONET (2003) can be presented on Fig. 2.

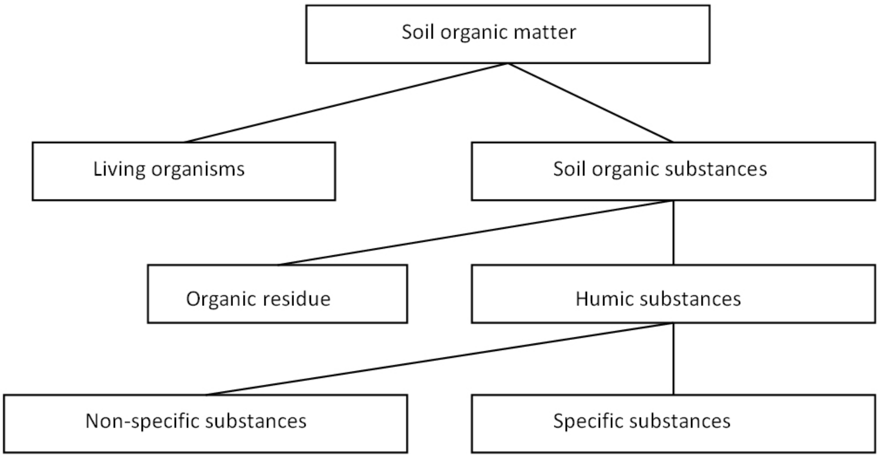

Fig. 2. Partition of the soil organic matter (GONET, 2003).

Both the qualitative and quantitative composition of the natural organic matter depends mostly on the chemical composition of its source – a substrate. Some differences in the chemical structure of the natural organic matter substrates (LYNCH, 1986; EPSTEIN, 1997; KHIL'KO *et al*., 2011) were identified (within the same substrate e.g grass or straw).

The composition of the natural organic matter also depends on environmental factors and it may be subject to considerable changes during the time. Therefore the criterion for defining the main fractions of the natural organic matter may be the susceptibility to the microbiological decomposition of organic substances (EPSTEIN, 1997). Three groups of substances and organic compounds were identified according to their susceptibility to the decomposition (biodegradation):

- substances that are easily decomposed in the microbiological way (polysaccharides (starch, glycogen, pectin)) fatty acids and glycerol, lipids and fats, amino acids and proteins, nucleic acids,
- difficult degradable substances (hemicellulose, cellulose, chitin),
- substances resistant to the decomposition (lignin cellulose).

In the case of organic composts, seven main groups of chemical substances included in the compost matter should be listed: carbohydrates and sugars; proteins; fats; hemicellulose; cellulose; lignin and mineral matter.

The substances belonging to the first three groups are decomposed relatively fast , as a source of easily digestible nutritious elements for the flora and fauna of i.e. the soil environment. The hemicellulose, cellulose and lignin decompose slower. The last group is the mineral matter of the compost that is not biodegradable (EPSTEIN, 1997).

The organic matter of composts and fertilizers make a highly amorphous mixture of the raw organic waste, the products of their decomposition and re-synthesis. During the composting process, the organic matter was divided into three fractions:

- soluble in water (Dissolved Organic Matter - DOM),
- undergoing the acid hydrolysis,
- non-hydrolysis.

The first group consists of water-soluble carbohydrates, proteins, fulvic acids. The second group consists of hemicellulose, cellulose and fats. The non-hydrolysis in the acid environment fraction includes complexes of lignin, humic acids and humin.

3. Humic substances in the terrestrial environment

Humic substances are one of the main fractions of the natural organic matter that have a significant ecological role in the natural environment (STEVENSON, 1994). Humic substances are the most common organic substances in the world. Because of its changeability they are highly resistant to the biodegradation, they are found in almost all components of the natural terrestrial and marine environment. They constitute 85-90% of the general amount of the organic matter in the soil, regulating a range of its fundamental physicochemical properties mainly connected with the fertility (EPSTEIN, 1997; SUN *et al.*, 2006).

Humic substances, otherwise known as peculiar humus substances (in relation to the soil organic matter) are a complex of brown and yellow organic substances that can be extracted from alkaline solutions, neutral salts or organic solvents (KONONOWA, 1968). These substances are formed in the biochemical processes of the decomposition and re-synthesis of the organic material of different origins with the help of microorganisms. Moreover, they have a strong heterogeneous nature, they are a mixture of polymolecular and polydispersive copolymers with the unknown chemical structure (EPSTEIN, 1997; JONES *et al.*, 1998). The definition of humic substances includes their division into three main fractions based on their behavior in the acid-alkaline environment (KONONOWA, 1968; STEVENSON, 1994):

- humic acids - the fraction of humic substances, insoluble in water in the acid conditions below pH 2, but they are soluble at the higher pH values,
- fulvic acids - the fraction of soluble humic substances in the whole pH range,
- humins - the fraction of humic substances insoluble in water in the whole pH range (Fig. 3).

Creon and apocreon acids were distinguished as subgroups of the fulvic acids fraction, hymatomelanic acid regarded as the initial product in the biosynthesis of humic acids, ulmic acids and ulmins - substances belonging to the same faction as humic acids and humins (KONONOWA, 1968). In the literature we can find the division according to which the fraction of humic substances consists of two subgroups: brown humic acids and gray humic acids. Due to the fact that the differentiation of humic substances is based on the fractionation, it should be treated as a test which means that the status of humic substances may be quite different in the home environment (SCHIAVON *et al.*, 2010; QI *et al.*, 2012).

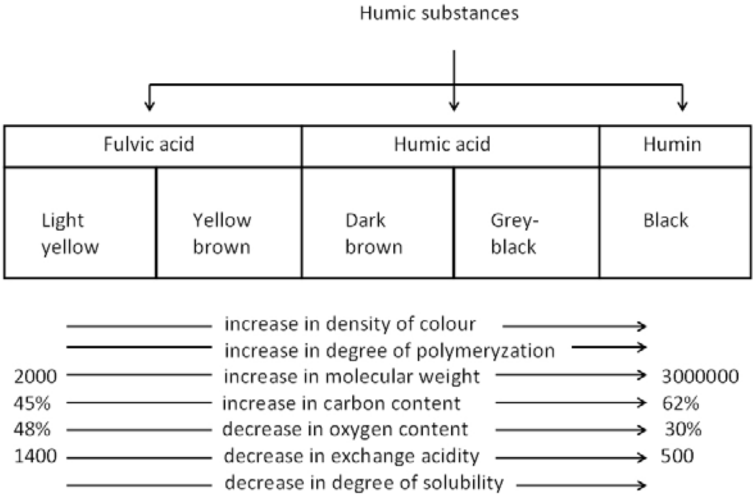

Fig. 3. Divide of the humic substances (KONONOWA, 1968).

4. Natural organic matter in the aqueous environment

The term of the dissolved organic matter (Dissolved Organic Matter - DOM) or the dissolved organic carbon (Dissolved Organic Carbon - DOC) concerns the fraction of the organic matter (organic carbon) separated from a soil sample (or other solid material) by the centrifugation (GONET, 2003). These terms are also widely used in the studies of natural organic substances found in surface waters (rivers, lakes and sea), groundwater and the fraction of the organic matter of solid samples (sediment, soil) extracted with water (CHEFTEZ *et al*., 1998). The method of the concentration and fractionation on adsorption gels and ion-exchange resin is used in the analysis of the natural organic substances dissolved in water (KOSOBUCKI, 2011). The methods using processes of freezing, the precipitation, the solvent extraction, the reverse osmosis and the ultrafiltration of the water sample for the isolation of organic substances can be found in the literature (ZBYTNIEWSKI *et al*., 2005).

The ddissolved organic matter can be separated into two main groups: hydrophobic and hydrophilic, each of them can be further divided into three next fractions (LEENHER, 1981): hydrophobic basic (HoB), hydrophobic acid (HoA), hydrophobic neutral (HoN), hydrophilic base (HiB), hydrophilic acid (HiA) and hydrophilic neutral (HiN).

The composition of individual fractions of the dissolved organic matter was confirmed after connecting the extraction and/or the fractionation techniques of the dissolved organic matter and the ^{13}C NMR technique (LEENHER, 1981; CHEFTEZ *et al*. 1998). HoB fraction is the carbon in the aliphatic combinations, hydrocarbons and amines, HoA consists of tannins, polyphenols and organic complexes of humic substances, HoN contains highly nonpolar connections like humins. The hydrophilic fraction named HiB consists of protein substances, peptides, amino sugars, HiA has highly oxidized organic compounds like humic substances of the low atomic mass, HiN is polysaccharides and oligosaccharides of the plant and microbiological origin (CHEFTEZ *et al*., 1998).

A group of substances of the dissolved organic matter of the crucial, ecological importance for the aquatic ecosystem is the fraction of humic substances like in the case of the terrestrial environment (SIYANITSA *et al*., 2007; VEPRIKOVA *et al*., 2007). Although the natural organic matter in the marine environment may be both the autochthonous and alochthonous origin, it is assumed that most of the humic substances dissolved in fresh surface water come from the basin (KOSOBUCKI, 2011). However, significant differences in the properties of humic substances from the sea water and from the soil were indicated - 95% humic acids are synthesized in the sea (KLUCAKOVA *et al*., 2012).

5. Carbon changes in natural environment

The natural organic matter in the environment changes constantly in the quantity and quality way. In the course of biochemical transformations, the organic matter reduces in the environment. Easily absorbed by the microfauna substances such as polysaccharides, fatty acids, lipids and fats, amino acids and proteins, nucleic acids are a source of the carbon and energy for organisms. Humic substances are formed from the remaining part as a result of the product re-synthesis of the transformation of plant and animal residues (HSU *et al*., 1999). The first direction of the transformations is the mineralization. It is connected with simple inorganic compounds such as CO_2, H_2O, NH_3 and ions SO_4^{2-}, NO_3^-, HPO_4^{2-}. The second direction, which is a synthesis of humic substances, is the humification (EPSTEIN, 1997).

The changes that associate with the mineralization, transformation and decomposition of the organic matter in three main stages:
-the initial phase - includes various processes occurring in the dead parts of plants, mainly the oxidation and hydrolysis. The biggest changes are in aromatic compounds and proteins,
- the phase of the mechanical decomposition – grinding of the organic material occurs here under the influence of the soil microfauna and macrofauna activity and the inclusion of particulate residues to the soil mass,
- the phase of the microbiological decomposition – the organic material completely without the tissue structure undergoes the enzymatic decomposition becoming the energy resource and building resource of soil microorganisms.

The microflora and microfauna preferentially decompose plant residues due to the availability of the organic matter contained in them. Water-soluble carbohydrates are decomposed into the basic components: the starch and protein which resorbed by the organism are partly used to build their bodies. Next, fats and waxes are decomposed, the cellulose and hemicellulose, and lignin that can be biodegradable under the influence of the proper groups of microorganisms, mainly fungi and actinomycetes (EPSTEIN, 1997). These phenomena occur with different intensity within the time and space, they are dependent on internal factors of the environment in which the organic matter is present as one of its components (for example the soil type) and external factors such as the atmospheric phenomena, climate, human activity. The greatest dynamics of these changes can be observed in systems where there is a fresh substrate, for example during maturing of the compost. A lot of studies show a multidirectional course of processes of the mineralization, but basically it can be noticed that the ratio of the organic substances susceptible to the microbiological decomposition (polysaccharides, fatty acids, lipids and fats, amino acids, nucleic acids) to substances resistant to the decomposition (cellulose, chitin, lignincellulose) increases (ZBYTNIEWSKI *et al*., 2005; KOSOBUCKI *et al*., 2006) and is very much dependent on the tested substrate. The fraction of the organic matter soluble in water has the particular importance for the environment, living organisms and for the transport of pollutants. Generally, it can be noticed that the amount of the carbon of this fraction decreases when the process of the transformation of the organic matter progresses (LEITA *et al*., 1991; INBAR *et al*., 1993). Some specific maximum of the carbon content was observed in the extract at the beginning of the transformation of various organic materials (HARVEY *et al*., 1983; ZBYTNIEWSKI *et al*., 2002). The amount of the carbon in the fraction undergoing the acid hydrolysis in the relation to the carbon of the non-hydrolysis fraction decreases during the process of composting (PARE *et al*., 1998).

In the case of the dynamics of changes of the fraction of the organic matter susceptible to the oxidation under the influence of dilute solutions of oxidants observed that the systematic manuring the soil increases the part of this fraction compared to the organic matter of the soils that are not fertilized or fertilized with mineral fertilizers. Cultivated soils are richer in the fraction of the organic matter that is susceptible to the oxidation than the forest soils (SZOMBATHOVA, 1999). Agrotechnical activities in the long term reduce the amount of the matter of the oxidizable fraction in soils (CONETH *et al*., 1999).

6. Humification – formation and transformation of the humic substances

Humic substances are formed as a result of biochemical transformations of the natural organic matter, plant residues and animal residues (POLIKRETI and CHRISTOFIDES, 2009). The humification process proceeds parallel to the processes associated with the decomposition of the chemical connections and the mineralization (FOURTI *et al*., 2010). It is preceded by three mentioned earlier phases of the decomposition of the organic material. The humification proceeds essentially in two stages: - the decomposition of the organic substrate to some simpler elements,
- more or less complicated synthesis and re-synthesis of these simpler substances that cause the appearance of humic substances (SAVEL'EVA *et al*., 2010).

Despite the various methods, the researchers claim that the course of these processes depends on the organic substrate, type and environmental conditions (KOSOBUCKI *et al*., 2011). The main source of the organic matter in soils is the plant substrate in which lignin, polypeptides and polysaccharides dominate (INBAR *et al*., 1989). Thus, it is commonly believed that the formation of humic substances from the residues of plant organisms (and animal residues) can occur according to four different mechanisms (Fig. 4) (STEVENSON, 1994).

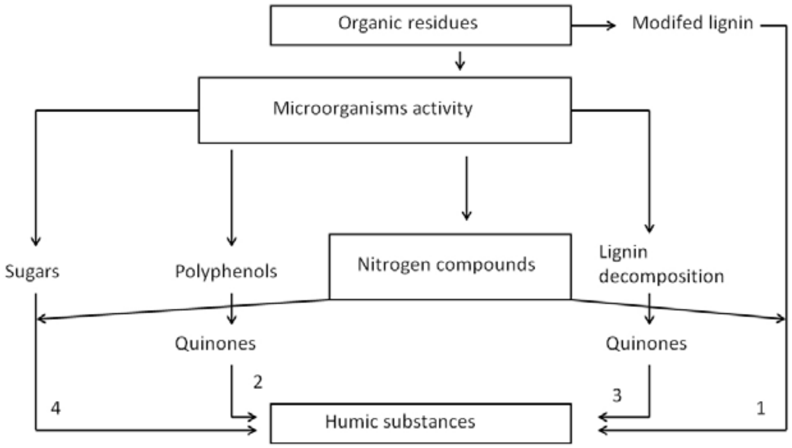

Fig. 4. Different mechanisms of soil organic matter formation (STEVENSON, 1994).

The first of them – the lignin theory of Waksman (Fig. 4 way 1) - says that humic substances are formed from the lignin modified by the activity of microorganisms. Modifications involve the de-methylation increasing the number of phenolic groups and shortening the side chains of the carbon to carboxyl groups. Next, the modified lignin are enriched in the nitrogen as a result of the abiotic condensation with polypeptides according to form Schiff's bases. Humic acids formed in this way undergo fragmentation to fulvic acids. According to the second and third mechanism (Figure 4, way 2, 3) polyphenols that appear due to the demethylation and the enzymatic oxidation of the lignin or cellulose are oxidized to quinones. Enzymes - phenol oxidases play an active role here. Quinones undergo the condensation with polypeptides that are produced by soil microorganisms, thereby they enrich the appearing products (humic acids) in the nitrogen. Many systems that catalyze the processes of the oxidation of polyphenols to quinones other than the activity of microorganisms were found in the soil environment. These are clay minerals, hydroxides and amphoteric metal ions (Fe, Mn, Al) (STEPANOV, 2008). The fourth way of the formation of humic substances in the terrestrial environment is the condensation of simple sugars and amino acids thanks to Maillard's reaction (STEINBERG, 2008). Glucosamines appear in the huge amount of the substrates that next undergo a series of reactions including the dehydration leading to the formation of dark-coloured polymers with features similar to humic acids (STEVENSON, 1994; SIVAKOVA *et al.*, 2011). Humic acids may be also formed by the auto-oxidation of unsaturated fatty acids - a lipid model (INBAR *et al.*, 1989). These processes are typical for the surface layer of sea areas, where fatty acids of the algae origin accumulate. This mechanism is catalyzed by light and ions of heavy metals.

Humic acids can be formed only in the microbiological way. It has been proven that sulfur bacteria and archaea are capable to produce the brown biopolymers containing elements of an aromatic carbon with properties similar to the humic acids in its structure from the aliphatic substrates (MARYGANOVA *et al.*, 2013).

Observing the process of the humification of the organic matter in composts, many researchers show that the content of the fraction of humic substances increase relatively, but generally there are more humic acids and less fulvic acids (CHEFTEZ *et al*., 1996; HSU *et al*., 1999). However, at the same time other studies show that the total fraction of humic substances does not change in the whole period of composting – the humification. Other authors show that the direction of changes of the carbon content of the fraction of the humic acids and fulvic acids is ambiguous (CHEFTEZ *et al*., 1996) or even that the fraction of humic acids is decrease during maturing of the compost (VEEKEN *et al*., 2000). These significant discrepancies of the obtained results show the diversity of organic materials undergoing composting.

7. Structure of the NOM

Studying the organic matter of the soil, of the composts, of the sludge or water it is very important to describe the structure that clearly reflects the physicochemical properties of the tested substance. The problem of the chemical structure of naturally occurring chemical substances has been bothering researchers for nearly two centuries and it has still remained in many aspects clearly not solved. Particular difficulties appear during attempting to determine the structure of humic substances.

During the past 50 years, a lot of models of the structure have appeared. Among them couple of them should be distinguished: a model of the structure of Dragunov's humic acids from 1948, Kasatoczkin's from 1951, the model of the structure of fulvic acids presented by Schnitzer and Khan in 1972 and by Buffle, the model of humic acids according to Stevenson from 1982 (STEVENSON, 1994) and a model of Schulten, Plage and Schnitzer presented in 1991 (SCHULTEN *et al*., 1991; YARKOVA and GYUL'MALIEV, 2012). These structures are shown in Fig. 5. They differ greatly because of the elementary composition, the molecule shape, the size of structural units and the nature of the connections.

It is assumed that humic substances are a mixture of a large number of chemical individuals of a polymeric feature. The latest studies also indicate the alkyl-aromatic structure of humic acid molecules (GONET, 1993; CHAIKOVSKAYA *et al*., 2008). But the aromatic rings of the hydroxyphenol type and the aliphatic structures of the type of carbohydrate residues, proteins and amino acids are connected with oxygen bridges (-O-), nitrogen (-N-), sulfur (-S-) and $-CH_2-$,-NH- groups and other (GONET, 1993; BENZOSIKOV and LODYGIN, 2009; EFANOV and CHERNENKO, 2010). The functional groups: carboxyl, phenolic and alcohol, quinones and ketones, methoxy, amino and amide are a very important element of the structure that determines a range of properties of humic substances. They are chemically connected mainly with the aromatic part through, as it could be seen thanks to the example of Schulten and co-workers (Fig. 5), they are also partly connected with the alkyl group.

The shape of the humic acid molecule in the solution strongly depends on the composition of the solvent (concentration, pH and ionic strength). It is assumed that they are spherocolloids with the porous (spongy) structure, the molecular weight from 2000 to about 300 000 g/mol. The low molecular fractions constitute fulvic acids and the high molecular fractions constitute humic acids (KOWALKOWSKI, 2009; KULIKOVA and PERMINOVA, 2010a; KULIKOVA and PERMINOVA, 2010b).

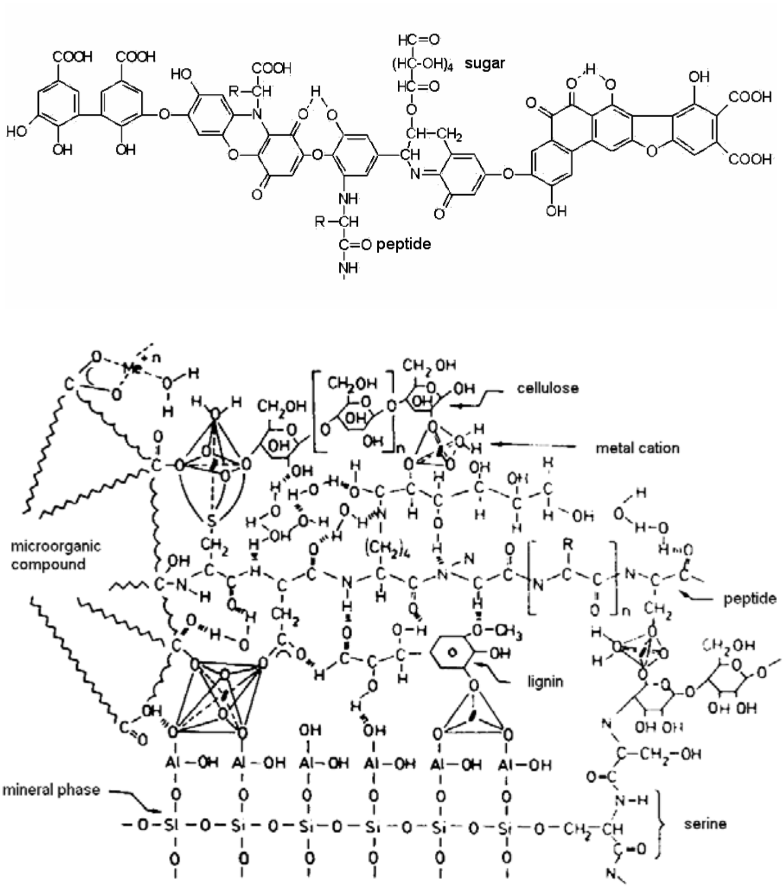

Fig. 5. Models of the humic substances structure (SCHULTEN *et al.*, 1991; GONET, 1993; STEVENSON, 1994).

To conclude, the natural organic matter has the elementary composition similar to other natural polymers (Table 1).

Table 1. Elemental analysis of soil organic matter (DZIADOWIEC, 1993; PSHINKO, 2009; KLUCACKOVA, 2010).

	Element content [%]			
	C	**H**	**O**	**N**
Humic acids	52.0-62.0	3.0-5.5	30.0-33.0	3.5-5.0
Fulvic acids	44.0-49.0	3.5-5.0	44.0-49.0	2.0-4.0
Peptides	50.0-55.0	6.5-7.3	19.0-24.0	15.0-19.0
Cellulose	44.4	6.2	49.4	-
Sugars	45.4	6.1	48.5	-
Lignin	62.0-69.0	5.0-6.5	26.0-33.0	-

The elementary composition of the natural organic substances is a feature that can be used to their identification and the conclusion of the structure, origin and energy or sorption (DZIADOWIEC, 1993; PSHINKO, 2009; KLUCACKOVA, 2010).

8. Significance of the NOM in environment

From all ecosystems in the terrestrial environment the role and the importance of the organic matter for the soil are the most known. The soil organic matter consisted of about 90% with humic substances affects the main physical, chemical and biological features taking part in all processes occurring in the soil. The key functions of the soil organic matter commonly include (DZIADOWIEC, 1993; EPSTEIN, 1997):

- the participation in the formation of soils and shaping their properties,
- the participation in the processes of ion exchange,
- the participation in the biological cycle of elements,
- providing the energy and carbon for soil microorganisms,
- providing biogenic elements for higher plants,
- influencing on the plant growth and development.

The organic matter affects the sorption and buffer abilities of soils. It regulates the concentration of soil solutions and their reaction. Specific organic substances (humic substances) are an important part of the sorption complex. Their absorptive ability denoted as the cation exchange capacity (CEC) is higher than the sorption capacity of its mineral part (KLAVINS and PURMALIS, 2010).

Sorption properties of the organic matter are determined by the presence of functional groups such as carboxyl and hydroxyl groups, constant and the degree of their dissociation, mainly in the structure of fractions of humic substances.

The chemical composition and the energy value of humic substances of the soil organic matter indicate that they may be an interesting source of the energy, biogens (carbon and nitrogen) for plants and microorganisms. However, due to their great diversity and uniqueness of the chemical structure, it is a source that is difficult to reach and it is only for a specialized group of microorganisms.

The significance of the soil organic matter fraction for higher plants is conditioned on the effects both indirect and direct (KOWALKOWSKI, 2001). The organic matter affecting the physicochemical properties of the soil environment forms favorable conditions for the plant growth. It is assumed that the plant growth and plant development are conditioned by the availability of nutrients, water, air to the roots as well as light and temperature. But most of these conditions are regulated by the presence of the humus soil and its quality. Many studies have clearly shown that humic substances also affect directly the germination, the seedling growth, the growth of roots and stem (GONET, 2003). The soil organic matter regulates the bioavailability of nutrients to microorganisms and higher plants through their impact on weathering processes, the properties of ion exchange, chelating and buffer properties of the soils. On the other hand, organic matter fractions, in particular low molecular humic acids can penetrate the plant through the root system (or leaves as a result of agrotechnical practices involving spraying humic preparations) and participate in a series of metabolic processes of the plant (DZIADOWIEC, 1973; KOWALKOWSKI 2001). Humic substances after penetrating the plant form redox systems affecting the metabolism of plant cells. In addition, they also influence on the membrane potential of cells by facilitating taking food elements including the nitrogen in the ammonium form and iron (DZIADOWIEC, 1993).

The increased respiration, the increase of the activity of the enzymes and the intensity of the photosynthesis and the increase of the resistance to unfavorable conditions of life were observed among the tested plants. The mechanisms according to which humic substances impact directly on the plants have not been explained yet. The activity of humic substances as for example the growth hormones may be connected with the inhibition of the oxidase of 3-indoleacetic acid and counteracting its decomposition. Maintaining a high activity of this compound has a positive effect on the plant growth (GONET, 2003).

The physicochemical properties (quality) of the organic matter have a significance for many effects connected with the soil fertility and plant growth. It was noticed for example that the soil fertility depends on the quality of the organic matter.

9. Soil organic matter analytical methods

Measuring of SOM directly is difficult. It is substituted by measurement of SOC. A classical procedure to calculate SOM is by multiplying the percentage of organic carbon by a factor. Conversion factors change between 1.4 and 3.3 (LAL, 1979; RASMUSSEN *et al*., 1991) and this large range is connected with the origin of the soils. A conversion factor of 1.72 is most often used. Therefore, to ensure consistency and allow reliable comparison of data, it is advantageous to report results as SOC rather than as SOM.

SOM studies have included:

1) detailed study of humus chemistry to prediction of the chemical structure of SOM via fractionation procedures,
2) empirical methods to assessment effects of SOM by evaluating field experiments,
3) modeling by soil models (KORSCHENS *et al*. 1998).

The most common methods used to characterize the humic substances in the half of XX centuries were destructive testing methods (wet or dry red-ox processes pyrolysis). Later, a number of non-destructive methods have been developed. These include elementary analysis, the assay of carbon, oxygen, nitrogen - containing components. The properties of humic substances can be described with the classical instrumental analytical methods (potentiometric and conductometric titration, and within the category of spectral methods, UV and VIS, fluorescent, infrared, NMR and ESR spectroscopy). But, different methods may also be used, including for instance X-ray diffraction, surface tension measuring, determination of molecular weight, steam pressure and membrane osmometry, size excluded chromatography (SEC), field flow fractionation (FFF), electrophoresis, ultracentrifuge, viscometry and mass spectrometry. Other methods worth mentioning include light dispersion, X-ray dispersion, electron microscopy and ultrafiltration (HSU *et al*., 1999; PEURAVUORI *et al*., 1998; KELLER *et al*., 2000; KARLEN *et al*., 2003; ZBYTNIEWSKI *et al*., 2005; KOWALKOWSKI, 2010; ZAV'YALOVA, 2012). Sometimes, the simplest methods i.e.. elemental analysis (CHN), give fundamental information about structure of analyzed compounds (Table 1). Between humic and fulvic acids, peptides, cellulose, sugars and lignins huge differences in content of carbon, hydrogen, oxygen and nitrogen are observed (KALEMBASA *et al*., 1973).

Size exclusion chromatography (SEC) and gel permeation chromatography (GPC) with UV-VIS detection is separation technique often used in analysis high molecular analytes (polymers, humic substances). Few selected wavelengths (280 nm, 472 nm, 664 nm) are used to detection. Humic substances possess no maximum absorption in UV-VIS spectrum. From GPC analysis molecular mass of analytes can be assign (LODYGIN *et al.,* 2012).

Nuclear magnetic resonance (NMR) spectroscopy, especially ^{13}C NMR and ^{1}H NMR has been used to predict structure of humic substances. From NMR analysis, distribution of functional groups (aliphatic/aromatic) in molecules is easy to perform. In solid state NMR main disadvantage is amount and purity of sample (CHUNG *et al.,*2012).

Spectroscopic techniques, especially infrared spectroscopy (IR) combined with Fourier transform (FT) having many advantages in analysis of organic molecules (HSs also). The main advantage are: high sensitivity and S/N ratio, many spectra library, small amount sample necessary and simplicity of measure. In the result of FTIR analysis, identification of functional groups in humic substances molecules is easy to carry out (PATRAKOV *et al.,* 2010).

Electrophoretic techniques, capillary zone electrophoresis (CZE), isotachophoresis (ITP) and others, has been used to provide chemical “fingerprints” of humic substances. After electrophoretic analysis, determination of humic substances origin is possible. Additionally different electrophoregram profiles and migration times of humic and fulvic acids have been used to distinguish between young, old, autochthonous and allochthonous natural organic matter. Sometimes, especially isotachophoresis is used to isolation of humic substances fractions (TAKAHASHI *et al.,* 2009, NAGYOVA *et al.,* 2001).

Many information about molecular structure of humic substances can be obtaining from mass spectrometric (MS) techniques (especially electrospray ionization (ESI-MS) mode). Both modes (negative and positive) have provided information on changes in molecule structure during photodegradation. UV spectroscopy do not detect any changes. Coupling of systems (MS/MS, triple or quadrupole) and chemometric methods (library’s) permit identified compounds in samples: benzene, phenol, sugars, organic acids (PICCOLO *et al.,* 2010).

Molecular weight distribution of humic substances can be performed by field flow fractionation (FFF). FFF offers many advantages in comparison to SEC. Main are separating molecules by their size (hydrodynamic) in solvents, without sieve effect in chromatographic column (easy to perform in pratice) (KOWALKOWSKI, 2010).

Soil organic carbon can by determined by different, classical methods (MOODY *et al.,* 1997; MOSKVIN *et al*., 2005). Acidic wet oxidation (with dichromate) is well known method as the Walkley-Black method (no heating) or Heanes method (externally heated and addition of concentrated sulphuric acid). Results obtained by the Walkley-Black method are not repeatable (recoveries can vary from 56% to 100%). It is result of origin of soil sample. This problem can be solve by dry combustion methods, where all organic carbon is converted to CO_2 in the oxygen or air atmosphere (ZOMEREN *et al.,* 2008). Kalembasa and Jenkinson compare both (dry and wet) methods, and they state that dry method were more accurate (KALEMBASA *et al.,* 1973). Later, Proidakov (PROIDAKOV, 2009) recommended application of infrared detector (NIR) to measurement of content of CO_2 in combustion gases. Table 2 shows most often used analytical techniques used in humic substances analysis.

Table 2. Analytical techniques most often used in humic substances analysis.

Analytical methods	**Literature**
High performance liquid chromatography (HPLC)	HUTTA *et al*., 2003
Gas chromatography (GC)	FABRRI *et al*., 2002
Gel Electrophoresis (PAGE)	BAXTER *et al*., 1992
Gel permeation chromatography (GPC)	CHIN *et al*., 1991 LODYGIN *et al*., 2012
Field Flow Fractionation (FFF)	KOWALKOWSKI, 2010 YOHANNES *et al*., 2005
Capillary zone electrophoresis (CZE)	BAXTER *et al*., 1992 TAKAHASHI *et al*., 2009 ABRAMOV and BEZZUBOV, 2007 LUCIO and SCHMITT-KOPLIN, 2006
Isoelectric focusing (IEF)	CURVETTO *et al*., 1974
Capillary electrochromatography (CEC)	ANDRE and GUILLAUME, 2008
Micellar electrokinetic chromatography (MEKC)	DE MORAES *et al*., 2005
Isotachophoresis (ITP)	KOPACEK *et al*., 1991; NAGYOVA *et al*., 2001
Electron paramagnetic resonance (EPR)	PEUROVUARI *et al*., 1998
Nuclear magnetic resonance (NMR)	KELLER *et al*., 2000 CHUNG *et al*., 2012
Elemental analysis (CHN)	KALEMBASA *et al*., 1973 LISHTVAN *et al*., 2013
Mass spectrometry (MS)	PICCOLO *et al*., 2010
X-ray diffraction (XRD)	THIEME *et al*., 1998
UV-VIS spectroscopy	ZBYTNIEWSKI *et al*., 2005 HSU *et al*., 1999 KUCERIK *et al*., 2009 Leopold *et al*., 2012
IR spectroscopy	HSU *et al*., 1999 PATRAKOV *et al*., 2010 GOSTISHCHEVA *et al*., 2009 MOROS *et al*., 2008
Fluorescence spectroscopy	DIVYA *et al*., 2009 PLAZA *et al*., 2007
Titration	KHIL'KO *et al*., 2011 QUENTEL and FILLELA, 2011
Thermal analysis	ROTARU *et al*., 2008 KUCERIK *et al*., 2006

10. Conclusions

The organic matter is present in all elements of the ecosystem (soils, rivers, oceans). It is very reactive, it is involved in many geochemical cycles and it is essential to the life on the Earth. The soil organic matter is a source of food for the soil fauna and it contributes to the biological diversity of the soil, because it is a source of nutrients such as the nitrogen, phosphorus and sulfur; the soil fertility depends on it at a great rate. The organic carbon reinforces the soil structure, it improves the physical environment, so it allows roots to penetrate the soil.

The organic matter absorbs water (it is able to hold water in an amount equivalent to six times of its weight), so that it is a very important source of life for the flora present in naturally dry and sandy soils. Soils containing the organic matter have a better structure that improves the infiltration of the water and reduces the susceptibility to the compaction and erosion. Their structure has not been explained in a full way, despite many years of research and using many advanced analytical techniques.

To explain the formation and structure of humic substances many models and hypothetic structures have been elaborated. Still occur degradation of organic matter is problem, and next investigation will be conducted with this processes.

Understanding of interactions of natural organic matter (humic substances) with environment (terrestrial, aquatic) is fundamental to comprehend the nature of studied material. Development of technology (new analytical methods) probably solves many instrumental problems. But problem with standards of humic, fulvic substances and humins will be still actual (no repetably).

Acknowledgements: This paper was supported from the budget of Kuyavia and Pomerania and the European Regional Development Fund under the ROP for the years 2007 – 2013: project no. RPKP.05.04.00-04-003/13

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