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EVALUATION OF TRANSFORMATION OF THE QUALITY COMPOSITION OF ORGANIC MATTER AND PARTICLES SIZE DISTRIBUTION OF SOLUTION OF HUMIC ACID OF SODDY-PODZOLIC SOIL AFTER LONG-TERM APPLICATION OF VARIOUS FERTILIZATION SYSTEMS

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Abstract

Soil organic matter play crucial role in soil functions, physicochemical properties and structure stability, which determined main soil properties - fertility. The objective is to study transformation of the quality composition of organic matter of soddy-podzolic soil with the application of various fertilization systems in relation to deleted of nano-, micro-size particles distribution of alkali solutions of isolated humic acids (HAs) from soil. Via UV-visible spectroscopy and gel-permeation size-exclusion chromatograph, determined structural molecular complexity of isolated HAs and by dynamic light scattering and visual observing of dried drops of HA solutions was evaluate particles size of HA in solution at micro- and nano-level of secondary or colloid structural organization. Revealed relation between fraction compositions of organic matter that causing by fertilizer systems and molecular complexity with particles size distribution of isolated HA in alkali solution. We showed that under long-term application of mineral and organo-mineral fertilization systems significant increase ratio of small nanoparticles population and decrease of big microparticles in alkali HA solution of soddy-podzolic soil with highest degree of fulvatiozation.

Key words: *soddy-podzolic soil, soil organic matter, fertilizers, humic acid, particles size distribution.*

INTRODUCTION

Under climate changes and escalating emissions of greenhouse gases from soils, more and more attention focused on soil organic matter (SOM) and its stabilization in soil under tillage, fertilizers or amendments. SOM play crucial role in soil functions, physicochemical properties and structure stability, which determined main soil properties - fertility (Angelico et al., 2023; Wu et al., 2024; Yang et al., 2020). The amount of SOM is therefore a very important parameter, but the qualitative parameters can decide extent on its quantity, and are more important in the characterization of changes in SOM transformation and deep processes of humification at molecular and nano-levels (de Aguiar, 2022; Fragouli, 2023).

The process of humification includes both the decomposition of plant materials with a high molecular weight (biodegradation), and the re-association of decomposition compounds products by means of polymerization \ condensation reactions, as well as molecular self-assembly and intermolecular aggregation that determine degree of structural randomness of particles size and molecular weights polydispersity of humic biomolecular associations. It mean that the polydispersity is due to the variety of possible variants of intermolecular re-associations and re-aggregations, which form humification products determined dynamics and stability of SOM (Beckett, 1987; Fragouli, 2023; Tombácz, 1999).

Humic substances (HS) main and most reactive component of SOM exist in the form of self-organized hydrophilic-hydrophobic micelles of aggregate particles of different sizes, which under certain conditions can undergo transformations, changing the balance of the quality of organic matter in soils (Angelico et al., 2023; de Aguiar, 2022; Wu et al., 2024). Molecular structural composition of HS effect on the functioning of soil organic carbon and sequestration of carbon in soil humic matter that was not always in an inert or stable sate (Drososa et al., 2020; Li et al., 2019; Song et al., 2014). In order to evaluating and monitoring of SOM quality and process of organic transformation need to have a more clear understanding of HS complex nature and their properties. In these case determine relations between parameters of SOM quality and directions of humification with structural characteristics and physico-chemical properties of isolated soil HS fractions is very important in modern humic chemistry and in evaluation of quality and stability of organic matter under soil perturbations (Escalona et al., 2023; Jiang et al., 2021; Jiménez-González et al., 2020; Olk et al., 2019a; 2019b; Vinci et al., 2021; Yang et al., 2020). Therefore, development and applications of new methodical approach play key role in humic chemistry improving and assessing of SOM into molecular and nano-level (Angelico et al., 2023; Jiménez-González et al., 2020; Jovanović et al., 2013; Olk et al., 2019a; 2019b;).

In the last decades evaluation of SOM quality significant transformation due to modern analytical methods and understanding of composition, structural organization and properties of soil HS fractions (humic\fulvic and humins). Like a molecular compositions, parameters of the secondary structural or colloid organization of HS, is an important indicators of SOM transformation, especially when caused by the impact of different land use (Escalona et al., 2023; Jiang et al., 2021; Jovanović et al., 2013; Wu et al., 2024).

Therefore, objectives of these study was explore the relationship between transformation of soddy-podzolic organic matter fraction composition under long-term fertilizer systems and nano-, micro-size particles distribution characteristics of solutions of isolated humic acid (HA) fraction evaluated via dynamic light scattering (DLS) and dry drop methods.

MATERIALS AND METHODS

The research was conducted in the laboratory of organic fertilizers and humus of the National Scientific Center «Institute for soil science and agrochemistry research named after O.N. Sokolovsky» in soil samples taken in a long-term field experiment on the territory of the Volyn State Agricultural Research Station of the Institute of Agriculture of Western Polissya NAAS of Ukraine, located in the village of Rokyny, Lutsk district, Volyn region, in the natural and climatic zone of Polissya. Soil samples was taken from soddy-podzolic soil of the field for the study of long-term use different fertilizers systems (60 years). Provided soil samples in triplicate repetition collected from 20 cm soil profile with variants: control (without fertilizers), mineral fertilizer ($N_{540}P_{510}K_{450}$), organo-mineral fertilizer (manure + $N_{540}P_{510}K_{450}$). Indexes of quality of SOM were determined using group and fractional analysis of soddy-podzolic soil samples by the method of Tyurin in the modification of Ponomarova and Plotnikova (DSTU 7828:2015).

Spectroscopic (UV-visible), gel-permeation methods of size-exclusion chromatograph (SEC) as well as DLS electrochemical technics (particle size diameter and polydispersity index, zeta potential) with visual observing of fractal aggregates in dried drop were applied to evaluate the differences in the behavior, molecular and colloid structural micro-organization of the Has in alkali media isolated from long-term fertilized soddy-podzolic soil.

Isolating of HA fractions from soil and preparing of study HA solutions. The isolation of the HA fraction that correspond to second HA-2 fraction at fractionation of humus by Tyurin method from the 0-20 cm layer of soddy-podzolic soil was carried out according to standard method of alkali-acid extraction. 100 g of prepared soil samples was stirred in 500 ml of 0.1 N NaOH for 24 h. After 24 h the slurry was centrifuged at 6000 rpm for 15 min to separate the supernatant containing the humic substances of humic and fulvic acid fractions from the mineral residues and humin fraction.

Concentrate HCl was added to the supernatant to acidify it to pH 1 for precipitation of HA fraction. After standing for 16 h, the suspension with humic acids was centrifuged at 6000 rpm for 15 min to obtain the HA precipitate. To remove mineral residues such as fine clay silicates, the precipitate was further dissolving in 0.1 N NaOH and periodic shaking for 2 h and performed HA re-precipitation. After that re-precipitating of HA was carry out by adding 6 M HCl to acidify the mixture to pH = 1, and allowing the suspension to settle for 16 h. This precipitated HA fraction was wash by distilled water and separated by centrifuging at 6000 rpm for 15 min, and dried for finely ground.

Solutions of isolated HAs fraction from soil samples were prepare by dissolving of 10 mg of isolated finely ground HA by 0.1. N NaOH in 100 ml flask with next stirring until complete dissolution. Prepared study solutions consisted of three alkali HA solu°tions from the soil with different fertilizers: HA solution from the soil without fertilizers; HA solution from the soil with mineral fertilizer; HA solution from the soil with organo-mineral fertilizer.

Size-exclusion chromatograph. To characterizing, the molecular-mass distribution of isolated HA solutions performed by SEC via permeation gel chromatography on column (2×40 cm) using Sephadex G-200 elution of humic fractions by distilled water with UV detection at 280 nm using UIT SFU-0170 spectrophotometer.

UV-visible spectra. To obtain UV-visible specters of absorption of HA solution used Hitachi spectrophotometer in the spectral range 200-600 nm. Measuring specters carry out in quartz cuvettes. Water solution of 0.1. n NaOH was used as a comparison standard when recording absorption spectra and removing alkali absorption in HA solutions. After placing the cuvettes with HA solution in a spectrophotometer under temperature control of 25°C waited for 5 minutes for a relative equilibrium of dynamic process in humic solution.

Dynamic light scattering (DLS) measuring. The particles size distribution (PSD) indexes and Zeta-potential (ZP) of HA solutions investigated by DLS via Zetasizer MAL1151890. Measuring triplicated and average values presented. DLS technique measures the diffusion of particles moving under Brownian motion, and a size distribution using the Stokes-Einstein relationship.

Drying drop methods of HA solutions. To obtain fractal micro-aggregates of HA solutions, the drying drop method (DDR) used which described in work (Song et al., 2014). A drop of HA solution with a volume of 3 μl without any pretreatment was placed for drying on a clean, fat-free, dry glass slide surface. The samples were left to dry under room conditions (27°C and humidity 55) and after 24 h examined visual morphology of obtained space-time organizations of fractal aggregates via a optic microscope at 100x with digital camera.

RESULTS AND DISCUSSIONS

Quality of organic matter of soddy-podzolic soil after long-term fertilizers. The transformation of organic matter of soddy-podzolic soil under long-term application of fertilization characterized by increasing of total organic carbon, fulvic and labile humic fraction but decreasing of mature HA fraction that lead to lowering of humification degree. In Table 1 represented results of indexes of quality of soddy-podzolic SOM.

Table 1. Indexes of quality of soddy-podzolic organic matter after long-term mineral and organo-mineral fertilizers

Indexes	Control soil without fertilizers	Mineral fertilizer (NPK)	Organo-mineral fertilizer (NPK + manure)
C_{total}, %	0.57	0.64	0.73
C_{HA}, % to C_{total}	47.4	35.9	35.6
Humification degree	Very high	high	high
C_{FA}, % to C_{total}	50.8	62.5	61.6
C_{HA}/C_{FA}	0.93	0.57	0.57
Humus type	Humat-fulvat	fulvat	fulvat
$C_{HA\text{-}1}$, % to total HA content	14.8	30.4	57.6
$C_{HA\text{-}2}$, % to total HA content	33.3	8.7	19.2

Revealed that fertilization increased of total soil organic carbon that associated with enhanced biotransformation activity of SOM as a result of entering in soil of fresh fertilizer source (NPK and manure) of transformation. Under long-term agricultural use of sod-podzolic soil under fertilization, the content of humus in the arable layer increased, but the degree of humification of organic matter changed from very high to high degree. Lowering of humification degree under fertilization of soddy-podzolic soil due to decreasing content of HA and increasing of fulvic fraction that lead to formation of fulvat humus state. Results indicate increasing of content of labile HA fraction (HA-1) in soddy-podzolic soil after fertilizer applications. Accumulation of labile humic fraction HA-1 is due to intense neo-formation of mobile HS fractions such as labile humic and fulvic acids cause by activation of biotransformation of fresh SOM. Showed that under organo-mineral fertilizer in soddy-podzolic soil form largest content of labile humic fraction (HA-1) which caused formation most mobile humus system compare to control soil without fertilizer and soil with mineral fertilizer. A decrease in the content of stable and polymerized mature humic fraction (HA-2) in the soil after the application of fertilizers was revealed compared to the control soil, especially on variants with mineral fertilizer. Results showed that increasing of total organic carbon in soddy-podzolic soil after fertilization occur by accumulation of labile HS fractions of HA and fulvic (FA).

SEC of HA solutions. Isolated HA fractions from soddy-podzolic soil correspond to stable mature humic fraction of soil (HA-2) that characterized by SEC and UV-visible spectroscopy revealed high molecular size and concentration of stable aromatic condensed benzoic structures in control soil without fertilizers and with highest total content of HA and stable mature fraction (HA-2). Increasing of molecular size and with high concentration of condensed aromatic structures promote formation more complex and stable HA structure that due higher hydrophobic-hydrophilic molecular heterogeneities (Debska et al., 2007; McAdams et al., 2018).

The obtained SEC profiles of molecular-mass distribution of isolated HA are mainly represent of peak of accumulation of heterogeneous low-weight molecular fraction (Figure 1).

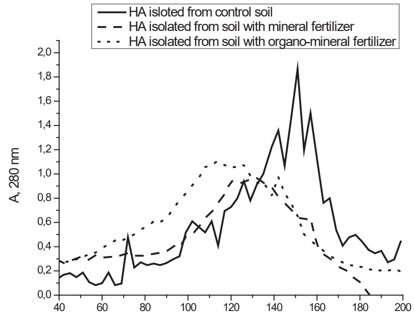


Figure 1. SEC profile of HA isolated from soddy-podzolic soil with fertilizers

The highest absorption at 280 nm of main peak observed in HA isolated from control soil without fertilizer. Application of fertilizers in soddy-podzolic soil decrease intensity of absorption of peak and broad the chromatogram that due to increase of molecular dispersity of HA. Revealed increasing of main chromatogram peak in HA from soil with organo-mineral fertilizer compare to HA from soil with mineral fertilizer.

Increasing absorption intensity at 280 nm of main SEC peak indicate higher concentration of heterogeneous low-weight stable (hydrophobic) aromatic structures that accumulate and under self-organization and intermolecular aggregation increase molecular size and complexity of hydrophobic-hydrophilic heterogeneities of soil HA (Kučerík et al., 2007; Piccolo et al., 1996). Higher molecular dispersity of HA is due to domination of small molecular size structures that spread in samples as result of weak intermolecular self-aggregation (Baigorri et al., 2007a, 2007b; Jovanovic et al., 2022). SEC results indicate decreasing of molecular size of isolated HA from soil with fertilizers as results of higher molecular dispersity and lower of molecular heterogeneity compare to HA from soil without fertilizer.

UV\visible spectra of humic solutions. On Figure 2 show UV-visible specters of absorbing HA solutions from the soddy-podzolic soil with fertilizers. Obtained specters are characterized by wide specters in the UV range that start to decrease monotonically with an increasing wavelength. The uniformity of the spectra indicates the similarity in general composition and structural organization of HA solutions. The main difference in obtained specters is expressed in intensity of absorption (A), the broad and tilt angle of the spectra line in the UV range. Absorption (A) in the UV range corresponds to various aromatic and phenolic-like structures. The most strong and high intensity of absorbing with broad spectra in the UV range corresponds to the HA solution from control soil without fertilizers, the lowest intensity indicates in the HA solution of soil with mineral fertilizer. Increasing UV absorption (at 254 nm) of HA solution occur to higher concentration of aromatic and phenolic humic structures that lead to stronger prosperity to form big sized complex humic aggregates. Obtained decreasing of UV-visible absorption of HA in solution is due to lowering the concentration of stable aromatic structures in isolated HA from soil with fertilizer. The increasing of UV-visible absorption observed in HA from soil with organo-mineral fertilizer.

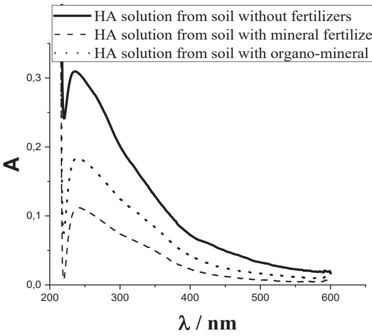
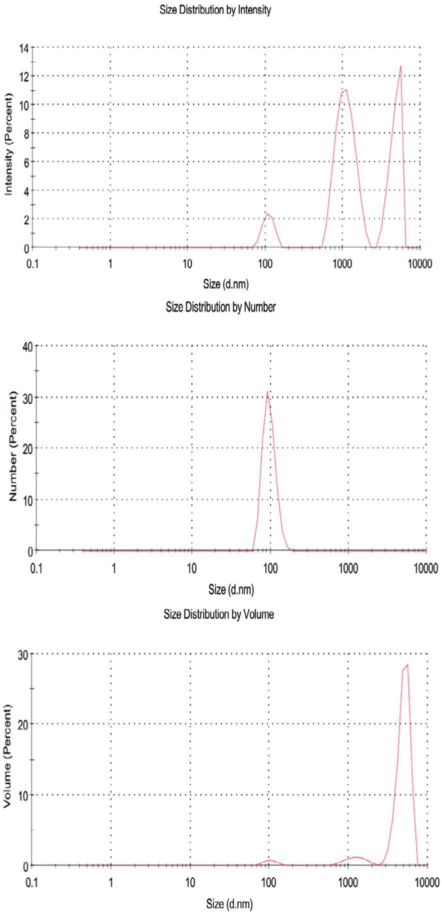


Figure 2. UV\visible specters of HA solutions from soil

Fertilizers promote to reduction of HA complexity and molecular heterogeneities of soddy-podzolic soil compare to HA from control soil. The reduction of HA complexity and molecular heterogeneities of soddy-podzolic soil was appear in decreasing of general molecular size and concentration of stable aromatic structures that promote self-organization of high molecular size associations with next intermolecular aggregation into big sized stable humic micellar aggregates structures in solution. The most simple humic molecular organization with low molecular heterogeneities and decreased molecular size was attributed to HA solution from soil with mineral fertilizer as result of fulvatization of humic fractions. However, organo-mineral fertilizer due to manure component as a source of fresh heterogenic aromatic structures leading to complication of HA structures by increasing the molecular size and concentration of stable aromatic structures of HA in solution.

Unfortunate the gel permeation SEC with spectroscopic methods to detailed evaluation of molecular size distribution of HS fractions is limited. The interesting findings in work (Tian et al., 2021) that HS fractions characterized by a very pronounced stable condensed aromatic structure associated with the highest molecular weight and size. Nevertheless, using DLS methods succeeded detailed characterization of PSD at nano- and micro-size distribution range of isolated HA in alkali solutions from soddy-podzolic soil after long-term application of fertilizer systems.

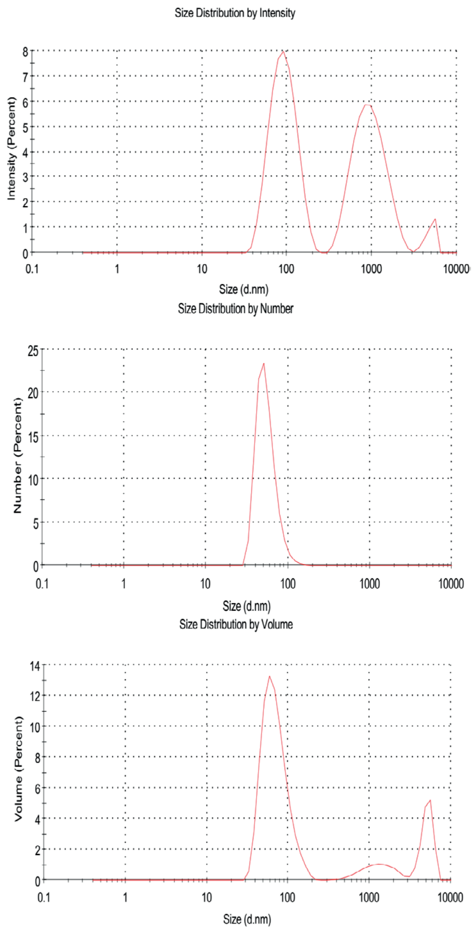
Particles size distribution of HA solutions. PSD reflect aggregation distribution charactersristics and secondary structural organization of HAs in solution. In Figures 3-5, the example of PSD by intensity, number and volume of HA solutions from soil with fertilizer systems shown. According to DLS measuring, results of intensity and volume size distributions of HA solutions had a tri-multimodal character in which three main peak highlight three population of particles size range: peak 1 range at 50-100 nm, peak 2 range at 150-2,000 nm, and peak 3 range at 2,000 nm to more than 1 µm.


Size Distribution by Intensity

Size Distribution by Number

Size Distribution by Volume

Figure 3. PSD by intensity, number and volume of HA solutions of control soil without fertilizers

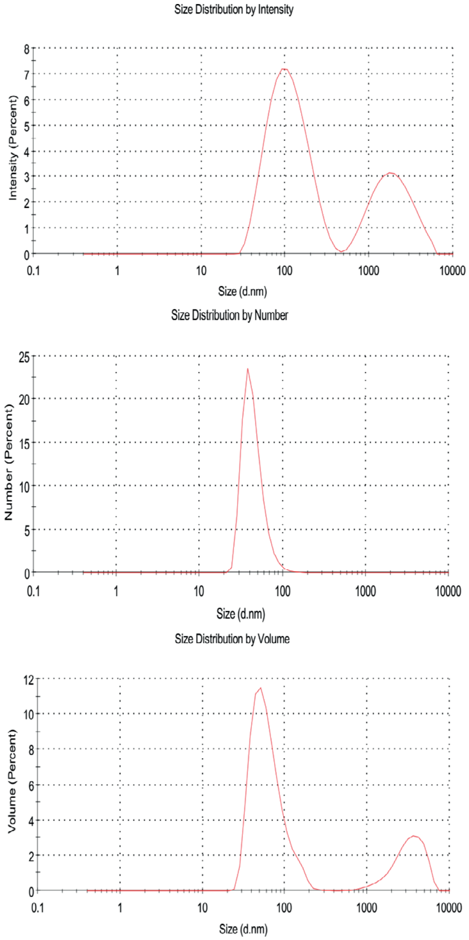

Size Distribution by Intensity

Size Distribution by Number

Size Distribution by Volume

Figure 4. PSD by intensity, number and volume of HA solutions of soil with mineral fertilizer

Tarasevich et al. who analyzed Aldrich HAs in alkali solution divided the same particles size population it into three main particles size fraction ranges called small nanoparticles (30-150 nm), sub-microparticles (200-1000 nm), and big microparticles (1.6-3 µm) with large macroparticles sub-fraction (3-5.6µm) (Dolenko & Trifonova, 2017; Tarasevich et al., 2013). Reveled significant increasing of peak intensity and volume of small-sized nano- and sub-microparticles and decreasing peaks of micro\macroparticles populations in HA solutions from soil with fertilizers compare to HA solution from control soil without fertilizer. The obtained results of re-distribution of PSD by intensity and volume testify significant re-arrangement of colloid or secondary structural organization of HA in solution from soil with fertilizers into the reduction of big sized particles aggregates.


Size Distribution by Intensity

Size Distribution by Number

Size Distribution by Volume

Figure 5. PSD by intensity, number and volume of HA solutions of soil with organo-mineral fertilizer

PSD by number of HA solutions from soil represent by monodisperse distribution of small sized nanoparticles populations. Observed shifting of PSD by number in more small nano-sized range of nanoparticles populations of HA solutions from soil with fertilizer systems (Figures 4 and 5). In HA solution from soil without fertilizer (Figure 3), the volume occupied by nano-particles was much lower but much higher in number in comparison with HA solution from soil with fertilizer systems (Figures 4 and 5). High content of macroparticles size population observed mainly in HA solution from control soil without fertilizer was due to micro- and macroparticles with a small volume of nanoparticles (~100 nm) population. On the contrary, in HA solution from soil with mineral fertilizer detected increasing the intensity and volume PSD of nanoparticles fraction (~100 nm) and decreasing of bigger microparticles population. Observed broadening of PSD peaks of sub-microparticles population in HA solutions from soil with fertilizers. Indicate broadening but decreasing PSD of intensity of overlapped peak of sub- and microparticles populations of HA solution from soil with organo-mineral fertilizer. In HA solution from soil with organo-mineral fertilizer also observed broadening peak of PSD by volume of bigger particles compare to HA solution from soil with mineral fertilizer (Figure 5).

The characteristics of particle sizes of study humic solutions listed in Table 2 that reflect colloid size organization of isolated HAs in solution.

Table 2. Indexes of Z-average size, PDI, particles size by volume and ZP of HA solutions of soil after long-term mineral and organo-mineral fertilizers

Samples	Z-average size (d. nm)	PDI	Peak 1 by volume, d. nm	Peak 2 by volume, d. nm	Peak 3 by volume, d. nm	ZP, mV
HA solution from control soil	1426	0.669	104,8	1255	4990	-25.1
HA solution from soil with mineral fertilizer	146.8	0.762	71,13	1373	5094	-30.3
HA solution from soil with organo-mineral fertilizer	117.0	0.671	64,81	overlapped	3367	-27.4

As can see (Table 2) fertilization of soddy-podzolic soil decrease the Z-average size (the average size diameters) of humic solutions but not be suitable parameter for humic size characteristics. Z-average particle sizes was determined from intensity, volume and number based DLS records, and represent as average of obtained records of multimodal distribution. Revealed increasing of polydispersity (PDI index) of HA solution from soil with mineral fertilizer that due to higher dispersity of humic system as result of nanoparticles population increasing. Decreasing of PDI observed in HA solution isolated from soil with organo-mineral fertilizer where revealed microparticles population increasing compare to HA solution from soil with mineral fertilizer. In general, the average diameter of the smallest nanoparticles and polydispersity in HA solutions from soil increased after fertilizer application that accompanied also with increasing of negative charge of ZP. In contrast, the humic solution from control soil without fertilizer contained mostly micro- and macroparticles with a diameter around 3-5 μm and lowers index of negative charge of ZP.

In our study, alkali HA solutions revealed relatively high numbers of small nanoparticles population with a diameter around 100 nm and less. Alkali solutions of HA lead to realis large number of smallest sized aggregate particles that was due to “open-folder” humic colloid organization in alkali media (Dolenko & Trifonova, 2017; Klucakova & Veznikova, 2017; Klucakova, 2018). On the basis the obtained PSD results, we used volume based DLS records in order to analyze PSD of study HA solutions from fertilized soil in detail. Volume based PSD of study humic solutions showed a higher volume of small nanoparticles with diameter less than 100 nm in HA solution from soil with fertilizers (Figure 6).

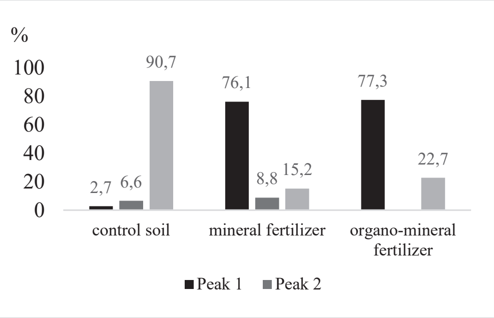


Figure 6. The intensity of peaks of PSD by volume of HA solutions of soil with fertilizers

The volume occupied by large particles populations in humic solution from control soil was much higher in comparison with humic solutions from soil with fertilizers. In control soil without fertilizer observed high volume of microparticles size population aggregates with diameter around 5 µm. Organo-mineral fertilizer promote to some high volume of bigger aggregates particles with a diameter around 3 µm compare to HA solution from soil with mineral fertilizer that mainly consist from small sized nanoparticles population.

Fractal aggregates after dried drop of HA solutions from soil. The method of dried drop of HA solutions was applied for the first time to visual evaluation of ability of fractal aggregates formation. Using a drying drop method of HA solutions from the soil with fertilizers obtained fractal aggregates that represented in Figure 7.

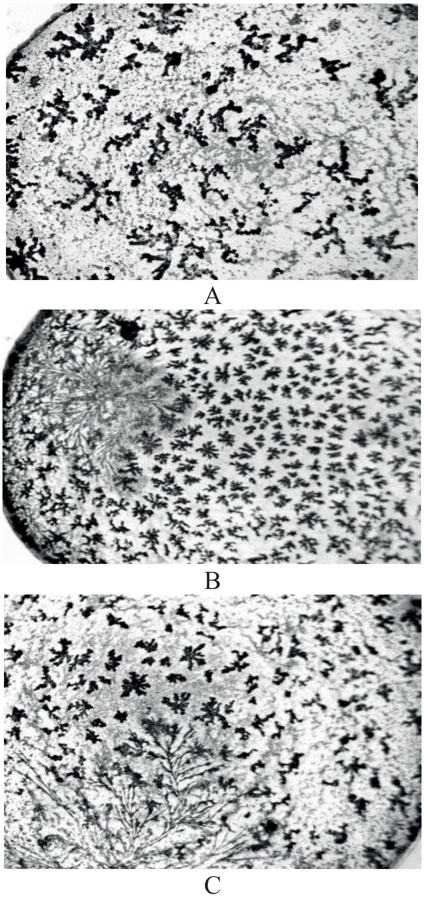


Figure 7. Self-organized fractal aggregates of dried drop of HA solution of control soil without fertilizer (A), soil with mineral fertilizer (B) and with organo-mineral fertilizer (C)

Visual characteristics of fractal aggregates after 24-hour drying of all studied HA solutions belong to the dendritic mass type morphology structure due to Brownian diffusion of particles by diffuse-limited aggregation (DLA) during dehydration self-organization of drops (Österberg et al., 1995; Rizzi et al., 2004). Obtained fractal aggregates of all studied HA solutions characterized by similar morphology type of fractals that indicates the universality of self-aggregation assembling of humic system. Assembling of fractal aggregates under dehydration of colloid solution is due to DLA (cluster-cluster DLA and reaction DLA) when one wandering colloid particle is attached to growth clusters and forms on a microscopic ordered space-time structure (Österberg et al., 1994). The form of this microscopic space-time aggregate structure takes place in the periphery as result of the removal of part of the solution from the center and locally depends on concentration and solution distribution on a glass surf. Visual observation revealed differences in size and number of self-organized fractal aggregates.

As one can see fractal aggregates of HA solution from the soil without fertilizer form most big on size fractal aggregates with low number and the smallest but with a large number of fractal aggregates corresponding to HA solution from soil with fertilizers. Visual detected that under self-organization dehydration of drop of HA solution from soil with mineral fertilizer increase the number of small fractal aggregates comparing even to number of fractal aggregates of dried drop of humic solution from soil with organo-mineral fertilizer.

CONCLUSIONS

Our study of transformation of soddy-podzolic organic matter fraction composition under long-term fertilizer systems was show differences in the structural molecular complexity and colloid particles size distribution between isolated HAs in alkali solutions via UV-visible, SEC, DLS and dried drop methods.

In general, fertilizer systems lead to increasing content of total organic carbon and carbon of labile HA and FA fractions caused by activation of biotransformation of fresh organic matter and intense neoformation of HS.

Nevertheless, observed increasing of total organic carbon and labile HA fractions of soddy-podzolic soil under fertilizers accompanied decreasing content of mature HA fraction due to high mineralization compare to control soil without fertilizers.

Revealed relation between fraction compositions of soddy-podzolic organic matter that causing by fertilizer systems and molecular complexity with particles size distribution of isolated HA in alkali solution. We showed that under long-term application of fertilizers significant increase ratio of small nanoparticles population, decrease of big microparticles in HA solution of soddy-podzolic soil as a results of neoformation of HS, accompanied of increasing content of labile HA and FA fractions.

According to our results, formation of big-sized microparticles of humic aggregates in alkali solution of isolated HAs of soddy-podzolic soil is due to high content of carbon and stable aromatic structures of total HA and mature HA fraction in soil.

Visual confirmed by drying drop methods of HA alkali solutions that long-term fertilizers lead to formation large number of small-sized fractal aggregates space-time structures. After drying drop of HA solution from soil with mineral fertilizer observed manly small fractal aggregates emerged from intermolecular self-aggregation of dominantly small nano-sized colloid particles.

We show that long-term mineral fertilizer of soddy-podzolic soil promote simplification of HAs molecular structure that in alkali solution behave as fulvic-like colloid structural organizations consist from mainly small and mobile nano-sized aggregate particles (less than 100 nm) that influence on mobility of SOM and weak accumulation of neoform labile HSs. However, manure of organo-mineral fertilizer increase the level of structural organization of isolated HA that promote formation of stable big-sized microparticles aggregates in alkali solution that due to stabilization of labile HA and increasing of soil organic matter maturity and quality.

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