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Humic Substances in the Early Biosphere[1]

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Abstract—Humic substances and their organic–mineral compounds are the first stage in transformation of biotic residues into stable geopolymers, which comprise the main reservoir of organic C in the biosphere. Early appearance of HS in the Earth's history is of principal importance for the understanding of geo-biological processes on land in the past. However, there is no fossil record of HS before land colonization by lignified vegetation (400 Ma). When the first soil HS were formed? Could HS be synthesized in the Precambrian before plants and mosses terrestrialization? The formation of HS occurs in mesophilic aerobic conditions and requires presence of oxidative catalysts and production of aromatic (phenolic) precursors by biota. In this paper, humification processes in algo–myco–bacterial and lichen communities are discussed from actualistic point of view. These communities are considered as a relict ecosystem, which could dominate on land during the Neoproterozoic–Early Paleozoic (1–0.5 Ga).

Keywords: paleosol, humus, organomineral complexes, early Biosphere

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INTRODUCTION

Evolution and functioning of terrestrial ecosystems are closely linked to soil formation. Soils are organic–mineral product of interaction of the biota with its mineral support under subaerial conditions and differ from other dispersed formations (grunts, weathering crusts) in the presence of organic matter. The latter is composed of dead biotic residues, humus, and organic–mineral compounds. The major part of humus (up to 90%) is represented by humic substances (HS) and their organic–mineral compounds. Heterogeneity and macromolecular structure, along with chemical and physical stabilization, make HS resistant to microbial attack. Humic substances and their organic–mineral compounds represent the first stage in transformation of biotic residues into stable geopolymers, which comprise the main reservoir of organic C in the biosphere. Thus, early appearance of HS in the Earth's history is of principal importance for the understanding of geo-biological processes on land in the past. In this paper, early humification processes on land are discussed on the basis of actualistic approach.

SEARCH FOR FOSSIL HS OR ACTUALISTIC RECONSTRUCTION?

The fossil record of HS is scarce due to processes of erosion and organic matter mineralization. HS are gradually decomposing with time, representing a dynamic reservoir on the geologic scale. The mean residence time of HS in surface soils is about $n \times 10^2$–10^3 years. It was found that, in buried paleosols, about 70% of humus is lost during the first hundreds of years (Demkin et al., 2007), only 6–7% of organic C remains after 10–80 thousand years (Glazovskaya 1996). In paleosols dated millions of years, the C_{org} content usually does not exceed 0.n%, humic substances comprise only several percents of humus and are enriched by C (70% versus 50% in present-day HS) (Ivanov and Khokhlova, 2008). The extremely low content in soils, chemical alteration, and lithification complicate identification and analysis of humus fossils. Most recalcitrant soil HS are those tightly bound to dispersed minerals or preserved in peats. For example, the oldest fossilized soil organic matter was found in Late Silurian–Early Devonian hystosols (420–400 Ma) and represented thin layers of peat composed of remnants of vascular plants (Retallack 2003). The oldest characterized humic and fulvic acids were found in complexes with palygorskite in the Carboniferous Serpukhovian paleosol (330 Ma) formed under higher vegetation in arid climate (Alekseeva et al., 2010).

Generally, the Devonian appearance of higher plants (400 Ma) is the lower documented limit for the soil formation. Since that time, soils developed as a space for the root system. However, it is evident that soils as the upper organic–mineral layer of the lithosphere appeared much earlier. Soil humus formation is

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closely related to land colonization by autotrophic biota and its organotrophic satellites. It is generally believed that the first primitive soils were formed under cyano–bacterial communities about 2 Ga. They represented organic–mineral films or coatings on the surface of wet solid rocks or mineral grains (Dobrovolsky 2006). Early stages of land colonization were also represented by algo–bacterial communities, microfungi, and lichens, which dominated on land in the Proterozoic and Early Paleozoic 1.5–0.5 Ga (Sokolov and Fedonkin, 1988; Karatygin 1993; Zavarzin, 2011). Mosses appeared in the Late Proterozoic (700 Ma) (Heckmann et al., 2001). Thus, during 1.5 b.y. before lignified plants terrestrialization, representatives of the algo–myco–bacterial world formed the most stable system in the Earth's history, with the source of primary production of organic C in subaerial conditions. These pioneer organisms were responsible for the transformation of their mineral substrate and primary accumulation of organic matter in soils. But could microbial communities of the Precambrian produce soil humic substances analogous to that of the modern biosphere? In the absence of direct paleontological evidence, the actualistic approach can be used. It is necessary to (1) analyze existing humification theories with respect of their applicability to the early biosphere and (2) find relict communities in modern ecosystems, which can serve as a model for investigation of ancient humification processes.

NATURE AND FORMATION OF HUMIC SUBSTANCES

Humic substances are heterogeneous dark-colored compounds formed as by-products during oxidative biotransformation of plant and microbial residues. The heterogeneity of HS results from the diverse precursor material, but generally, the humification process (formation of HS) selects those compounds which are most stable under given bioclimatic conditions (Orlov 1990). Judging from the solubility in alkali, HS are operationally divided into humic acids (HA), insoluble at pH < 2 (10–100 kDa of molecular weight); fulvic acids (FA), soluble at all pH values (0.5–10 kDa); and the insoluble residue humin, which consists mostly of aliphatic compounds tightly bound to mineral particles. Humus acids (HA and FA) are most important within HS due to their high reactivity. They represent polydisperse mixture of dark-colored compounds, which are composed of aromatic rings, aliphatic chains, N- and S-containing heterocycles, and bear different functional groups, among which carboxylic and phenolic are most abundant (Stevenson 1994).

Synthesis of soil humus acids is heterophase process in aerobic mesophilic conditions (pH 4–7, t = 10–30°C). It is initiated by phenoxy radicals, quinones, and semiquinones, which are formed during catalytic oxidation of phenolic compounds by extracellular fungal oxidoreductases (laccases, peroxidases, tyrosinases). Minerals, bearing transition elements (Fe, Mn) can also serve as catalysts (Huang, 2000), although their oxidation power is lower than that of enzymes. After catalytic oxidation, phenols and their oxidation products undergo spontaneous coupling with nitrogenous compounds, lipids, carbohydrates, etc. with the formation of polymers via C–C, C–O, N–N, and C–N bonds. It is widely accepted that humus acids are formed by two main pathways: (1) as by-products of oxidative biotransformation of plant and microbial macromolecules (lignin, melanins); (2) as condensation products of biotically produced low molecular weight phenolics (Stevenson, 1994). In both cases, the presence of catalysts and solid phase are essential for accelerating the humification process.

In the first pathway, the humification process is solid-state fermentation of organic residues by soil fungi. The high molecular weight and insoluble HA are formed during partial transformation of lignin by cellulolytic fungi (brown rot basidiomycetes and soil micromycetes). Fulvic acids and low molecular weight fractions of HA are formed when lignin is decomposed to soluble products by ligninolytic peroxidases of white-rot and litter-decomposing basidiomycetes (reviewed by Zavarzina et al., 2011). In the absence of lignin in plant tissues (e.g., when lichens are decomposed) the main humification products are FA (Parinkina et al., 1998; Abakumov, 2010). Insoluble HA can be formed as transformation products of fungal melanins; however, the scale of the process is questionable.

In the second pathway, humification is heterophasic condensation of low molecular weight and, therefore, soluble precursors on/near the surface of catalytically active solid phases (see review by Zavarzina, 2011). Synthesis of macromolecules in the bulk soil solution is thermodynamically unfavorable because of extremely low precursor concentrations. After catalytic oxidation on the solid-solution interface, phenols and their oxidation products undergo surface/boundary polymerization and become rapidly adsorbed onto the mineral phase. As a result, dark-colored coatings are formed on the mineral grains, a part of humic material is irreversibly bound to solid matrix. Products of abiotic catalysis have lower molecular weights than products of enzymatic synthesis.

It is likely that significant accumulation of high molecular weight humification products in soils began only after massive appearance of lignin in Devonian. Since that time, oxidative biodegradation of lignocellulose (first humification pathway) became the main route of HS formation in soils. Condensation pathway seems to be important at primary stages of pedogenesis in the absence of phenolic polymers in decomposing material. Adsorption of soluble organic compounds on soil minerals, along with heterophasic condensation, can be considered as elementary processes leading to the formation of organic–mineral soils. Synthesis of

HS by heterophasic condensation requires (1) aerobic mesophilic conditions (t = 10–30°C, pH 4–7); (2) sufficient water supply; (3) catalytically active mineral phase; (4) well-developed primary production, which serves as a source of phenolic precursors.

Oxygen supply is absolutely necessary for the humification process. Therefore, humification is limited by the aerobic layer of the weathering profiles and, on the geologic time scale, by the time of oxygenation of the atmosphere. The atmosphere became aerobic not later than 2.4 Ga; however, oxygen concentration became sufficient for the development of subaerial life about 1.5 Ga (Zavarzin, 2011). Most probably, the first humification catalysts were transitional metal ions within soil minerals. Phenoloxidases appeared later; bacteria could be their first producers (Alexander and Zhulin 2000). Thus, the first three conditions necessary for the humification process could operate already in the Early Proterozoic. To understand when the first humus acids were formed, it is necessary to define which of the early land colonizers could produce phenolic compounds in noticeable amount.

LAND COLONIZATION AND SYNTHESIS OF HUMUS ACIDS

Alternatively to the "life came from the sea" approach, it was proposed that colonization of inland proceeded from shallow freshwater bodies (amphibious landscapes) receiving their water supply from the atmosphere (Zavarzin, 2008, 2009, 2011). The route for land colonization looks simple, from ultrafresh water bodies to porous wet ground. Inhabitants of ombrotrophic waters, ombrophiles can be considered as the first mesophilic inhabitants of land, because gydrochemical conditions for them were formed since the appearance of geographic envelope and were more or less the same during the entire history of the biosphere (Zavarzin, 2011). The simplest model of ombrotrophic biocoenosis is a puddle on clayey ground or on a wet surface of solid rocks (Zavarzin and Alekseeva 2009). Typical inhabitants of puddles are cyanobacteria and green algae, along with their heterotrophic satellites, i.e., organotrophic bacteria and microfungi.

The supposition that the first colonizers of inland originate from freshwater reservoirs has no paleontological evidence, because lithification is impossible in ultrafresh conditions. However, even in absence of direct evidence, it is most probable that benthic cyanobacterial and algo-bacterial communities moved onto wetland and formed the basis of the trophic pyramid on the day surface suitable for photosynthesis. Cyanobacteria are the most ancient phototrophic organisms on land, their first microfossils and stromatolites are traced up to the Archean (3.5 Ga). Nearly complete morphological diversity of cyanobacteria arose in the Paleoproterozoic (2.5 Ga), when cyano–bacterial mats became the dominant terrestrial community (Zavarzin, 2008). In addition to cyanobacteria, the early stages of land colonization seem to be represented by algo–bacterial lawns on the shores of water bodies or biofilms on wet ground. Therefore, the only source of organic which could have been on the land surface in the Early Proterozoic seems to be represented by cyano–bacterial and algo–bacterial communities. The cell wall of cyanobacteria and algae is composed of polysaccharides, production of phenoloxidases by these organisms is unknown. Thus, there are at least two constraints for the formation of "true" humus acids in the biosphere of prokaryotes, cyanobacteria, and algae: (1) absence of phenolic precursors in sufficient amount; (2) poor distribution of extracellular phenoloxidases which catalyze the formation of HA of high molecular weight. Cyano–bacterial and algo–bacterial humus could be represented by polysaccharide films on the mineral grains, by hardly hydrolizable compounds of algae and, probably, by melanoidins—the dark-colored polymeric products of sugar–amino acid condensation under UV light (Maillard reaction).

Production of phenoloxidases (laccases, tyrosinases, peroxidases) and phenolic compounds are known in aerobic decomposers, fungi. The appearance of fungi in the Earth's history is rather uncertain. Judging from molecular data, the basic lines of fungi occurred on land 1.2 Ga (Heckmann et al., 2001). Microfungi could substitute organotrophic bacteria in cyano–bacterial and algo–bacterial communities and this substitution was a crucial step in the evolution of terrestrial life. Fungal mycelium seems to be the first adaptation for life in the air. Mycelial organisms started colonization of the atmospheric layer adjacent to the ground and were the first organisms which constructed an aerotop (the space between ground and the top of plant cover) in a few millimeters scale. Microfungi could initiate synthesis of HS, because, in addition to phenoloxidases and various phenolics of low molecular weight, some species produce brown polymeric pigments melanins, which resemble humic acids in physicochemical properties. However, the scale of the process is questionable, because, at present, there is no experimental evidence for significant accumulation of HS under communities of free living algae, bacteria, and microfungi.

The culmination of algo–myco–bacterial community on land is obviously represented by lichens, symbiotic associations of a fungus (structural component, usually ascomycete) with a photobiont –(green algae and/or a cyanobacterium). Lichens are characterized by high tolerance to severe abiotic stresses, such as dessication, rapid rehydration, temperature extremes, and high UV light intensities (Nash, 2008). Lichenization seems to be an adaptation of both bionts for the life on unshaded and xerophytic land surfaces. It is probable that, unlike their components, lichens did not colonize land from water, but were initially adapted for the life in the air. Foliose and fruticose lichens can be considered as first inhabitants of subaerial surfaces, which exposed their photosynthetic component in the atmosphere (Zavarzin, 2011). Exact time of lichenization is unknown, because the fossil record of lichens is scarce. One of possible explanations is that ancient lichens occupied xerophytic habitats subject to weathering. In addition, it is not always possible to identify whether fossils belong to lichen or its separate bionts (Taylor and Osborne, 1996). One of the earliest "terrestrial" fossil lichens is *Farghera robusta,* which was described in Ordovician paleosol in Australia (483 Ma). Fossils resemble the extant foliose lichen *Xanthoparmelia reptans* (Retallack, 2009). The "classic" fossil lichen *Winfrenatia* belongs to Devonian (400 Ma) (Taylor et al., 1995). However, molecular data suggest the possibility of much earlier lichenization. Ascomycetes appeared in the Mesoproterozoic (1458–966 Ma) and could form associations with cyanobacteria, which were widespread on land at that time. Another potential lichen symbionts, green algae, appeared about 1061 ± 109 Ma (Heckmann, 2001). These data suggest that lichenization could occur much earlier than paleontological data suggest.

Lichens are well known as successional predecessors of mosses and higher plants and as organisms which can colonize bare subaerial surfaces. These symbiotic organisms are particularly interesting with respect to early pedogenesis because they can contribute to the formation of both mineral and organic part of the soil. It is well recognized that lichens alter their mineral substrate by hyphal penetration or by production of organic acids and chelating agents. This leads to the formation of fine-earth and secondary minerals (Chen et al., 2000). Humification processes in lichen communities are less understood; however, it is known that mat-forming lichens can serve as significant source of organic remains in soils as well as a source of soluble humification products (Parinkina et al., 1998). In addition, it has recently been shown that living lichens produce significant amounts of water-soluble phenolic compounds, including conjugates of phenolic acids (Zagoskina et al., 2013). Mycobionts of peltigerous lichens produce laccases, tyrosinases, and peroxidases (Laufer et al., 2006a, 2006b; Zavarzina and Zavarzin, 2006; Lisov et al., 2007, 2012; Liers et al., 2012). Both phenolic compounds and enzymes are leachable from thalli by water and, thus, apparently participate in synthesis of HA via heterophase condensation pathway. Our recent laboratory experiments confirm this supposition (Zavarzina, original data). Thus, the formation of HS in noticeable amount can be connected with the development of the lichen cover on land, assuming that ancient lichens produced phenolic compounds and phenoloxidases and were widespread before plants terrestrialization.

CONCLUSIONS

The present-day lichen tundra can be considered as a relict community, which was abundant on land during the Neoproterozoic and initiated accumulation of true humic substances in soils. At present, lichens dominate in extreme habitats on about 6–8% of land surface, mostly in polar and mountainous tundra, Arctic and Antarctic regions, high mountain elevations, and dryland crusts. However, before plants terrestrialization, lichens could be distributed much wider and occupy ordinary habitats. Lichen communities give rise to organic remains, which are commonly associated with fine earth covered by humic film. Such primitive soils, which are characterized by the organic layer and organomineral complexes (cutanes), could provide transition from cyano–bacterial and algo–bacterial films to the true soil.

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