UDC 631.465

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ACTIVITIES OF ENZYMES PARTICIPATING IN REDOX POTENTIAL IN THE TWO DEPTHS OF TAGAN PEATLAND

The redox potential of soils seems to be a critical environmental factor because it governs the chemical and biochemical form of many compounds and their availability for plants and soil microorganisms. Dissolved and total organic carbon, total nitrogen and activity of reeducates, xanthine oxidase, phenol oxidase and peroxides were determined in the peat samples. The aim of this study is to compare the activity of enzymes participating in redox potential at two depths of profile Tagan peatland: acrotelm and catotelm. The study has shown the impact of two depths of profile peatland on activities enzymes participating in redox potential like nitrate reductase, xanthine oxidase, phenol oxidase and peroxidase. In soil profiles activity of xanthine oxidase, phenol oxidase and peroxidase increased with the depth in the points 2, 3 and 4 and also xanthine oxidase in point 1. Together with extending into the depth of soil, there was the increase in nitrate reductase activity in points 1, 3 and the decrease in points 2, 4.

Key words: *peat soils, activity of nitrate reductase, xanthine oxidase, phenol oxidase and peroxidase.*

Oxidation-reduction reactions play a key role in ecologically important biogeochemical processes in soil and influence on soil chemical, biochemical, physical and biological properties. The redox potential is a critical environmental factor because it governs the chemical and biochemical form of many compounds and their availability for plants and soil microorganisms, and it also influences on the products of microbial metabolism in soil. Furthermore, oxidation and reduction of organic matter are intimately linked with energy transformations which may form the basis of an energy-yielding metabolism [1–3].

Peat has traditionally been characterized in the Russian literature as possessing a hydrologically active, low-density upper layer, and a higher-density, hydrologically inactive lower layer. This classification was formalized by Ingram [4] who identified the upper layer as the acrotelm and the lower layer as the catotelm. The acrotelm contains roots and decomposing plant material, typically possesses a relatively a high hydraulic conductivity (~1 cm s^{-1}), and crucially is defined as the zone through which the water table fluctuates. The catotelm is the water saturated anaerobic zone that comprises layers of defense peat with small pore spaces and hydraulic conductivity 3–5 orders of magnitude lower [5–7].

Peatland ecosystems are characterized by anaerobic conditions. Due to lack of oxygen, the decomposition process is very slow and hence contributes to the accumulation of huge amounts of organic matter. In contrast to a mineral soil system where the organic matter content makes up only a small fraction, the organic matter is an integral and substantial part of peat. In soil, the degradation and polymerization of organic compounds are responsible for the formation of humic molecules, which are characterized by a complex polymeric structure that contains mainly carboxylic and phenolic functional groups [8]. Mineralization of organic carbon or organic nitrogen conversions into CO_2 and NH_4^+ can be carried out by a variety of microbial species. Microbially mediated transformation of organic matter, and specifically nitrogen-containing functional groups, occurs during microbial respiration through which the acquisition of organic forms of nitrogen appears to be mediated by redox chemistry [3, 9, 10]. The activity of microbially derived extracellular hydrolytic and oxidative enzyme activities in peat, that responsible for the conversion of organic matter from high to low molecular weight compounds found in dissolved organic carbon (DOC), is principally regulated by temperature, redox potential, nutrient availability, and changes of pH [11, 12].

Soil organic matter transformation is strongly effected by the activities of soil microorganisms, which use many enzymes in their metabolic pathways. Nitrate reductase, xanthine oxidase, phenol oxidase and peroxidase are present in natural soil, and may originate from microorganisms, other organism, degradation of plants and root exudates of plants.

The aim of this study is to compare then activity of enzymes participating in redox potential at two depths of profile Tagan peatland.

Soils samples were taken from four places marked as No 1, 2, 3 and 4 each from two intervals 0–25 and 50–75 cm of the peatland Tagan which is located near Tomsk, Western Siberia, Russia. Place No 1 in both layers represents grasses peat with the degree of the decomposition ranged from 25–35 % (pH 5.57–5.79). Point 2 was characterized by wooden and wooden grasses peat with 30–35 % degree of the decomposition (pH 5.70–6.21). There was buckbean peat in the points 3 and 4 (pH 5.20–5.44). However, 1.5 m depth of sapropel was located in point 4.

Dissolved organic carbon and total organic carbon (TOC), nitrate reductase, xanthine oxidase, phenol oxidase and peroxidase activity were determined in the soil samples.

Samples were air-dried and crushed to pass a 1 mm mesh sieve. These 10 sub-samples were mixed for preparing a "mean sample", which was used for the potentiometric determination of pH (in 1N KCl), and for the measurements of TOC and DOC. For the estimation of DOC, air-dried soil samples in deionized water were heated at 100 °C by two hours under reflux condenser. Extracts were separated by the mean filter paper and analyzed on TOC 5050A a facilities (Shimadzu, Japan).

For determine of nitrate reductase, xanthine oxidase, phenol oxidase and peroxidase activity field-moist soil samples were passed a 2 mm mesh sieve. Ten soil samples from each site were pooled together to give the so-called average mixed sample and roots and stones removed.

Nitrate reductase activity was determined by Kandeler method [13]. The standard stock solution was prepared by dissolving 4.9257 g $NaNO_2$ in deionized water and the volume diluted to 1000 mL with deionized water in a volumetric flask. From this solution, working standard was obtained, where the concentration of $N\text{-}NO_2^-$ was equal to 10 µg $N\text{-}NO_2^-$ mL^{-1}. The calibration standards were constructed by adding of 0 (blank reagent), 2.0, 4.0, 8.0 and 10.0 mL working standard in five 100 mL volumetric flasks. 50 mL of potassium chloride solution was added and the volume was made up with deionized water. For colorimetric analysis, 5 mL of each standard, 3 mL of ammonium chloride buffer at pH=8.5 and 2 mL of colour reagent were added. All calibration standards were mixed and allowed to stand for 15 min at 20 °C. Nitrite ions revealed purple complex with sulphanilamide and N-(1-naphtyl)-ethylenediamine hydrochloride determined colorimetrically at wavelength λ_{max}=520 nm using a UV-VIS spectrophotometer Beckman DU®-68 USA. Activity of nitrate reductase in soils was calculated from the early-prepared analytical curve according to the Beer-Walter light absorption law by means of the least squares formulas (1), (Fig. 1, Table 1):

$$A = \varepsilon\, c\, l, \qquad (1)$$

where: A is absorbance, ε is molar absorption coefficient [L mol^{-1} cm^{-1}], c is concentration [mol L^{-1}], l is thickness of layer [1 cm].

KNO_3 is used as a substrate for measurement of activity of nitrate reductase in field-moist soil samples. 5 g soil samples were incubated for 24h at 25 °C with 1 mL of 25 mM KNO_3 solution and 4 mL of 0.9 mM 2.4-dinitrophenol solution and 5 mL of deionized water. The controls were incubated for 24h at –20 °C. After incubation the controls were thawed at 20 °C. Nitrite reductase is inhibited by the addition of 2.4-dinitrophenol. Nitrate released as a result of incubation was extracted with 10 mL of 4 M potassium chloride. Next, samples were centrifuged at 4000 r.p.m. for 10 min, and soil solutions were filtered. For colorimetric analysis 5 mL of each soil extract, 3 mL of ammonium chloride buffer (0.19 M at pH=8.5) and 2 mL of colour reagent, were added. All samples were mixed and allowed to stand for 15 min at 20 °C. The concentration of nitrite was determined colorimetrically at λ_{max}=520 nm from the early-prepared analytical curve according to the Beer-Walter light absorption law by means of the least squares formulas (1), (Fig. 1, Table 1).

Table 1

Molar absorption coefficients (ε), and correlation coefficients (r) for determined compounds

Compounds	Molar absorption coefficient (e) L mol^{-1} cm^{-1}	Correlation coefficient (r)
Nitrite (for measurements of nitrate reductase activity)	21 115±1	0.999
Uric acid (for measurements of xanthine oxidase activity)	2 847±3	0.996
Benzoquinone (for phenol oxidase activity)	1883±4	0.995
Horseradish peroxidase Type II (for measurements of peroxidase activity)	191 799±3	0.997

Xanthine oxidase activity was determined by Krawczyński method [14]. As standard stock solution 0.66 mM of uric acid was used. 11.2 mg of uric acid was dissolved and diluted the volume to 100 mL with deionized water in a volumetric flask. The calibration standards were constructed by addition of 0 (blank reagent); 1.0; 2.0; 3.0; 4.0; 5.0; 6.0; 7.0; 8.0; 9.0 and 10.0 mL working standard solution. To each calibration standard solutions were added respectively 10.0; 9.0; 8.0; 7.0; 6.0; 5.0; 4.0; 3.0; 2.0; 1.0; 0.0 mL deionized water. With calibration standard, solutions were taken 1 mL dilution and added 2 mL of 0.1 M phosphate buffer at pH=7.5. Obtained calibrations standards containing 0.0; 11.2; 22.4; 33.6; 44.8; 56.0; 67.2; 78.4; 89.6; 100.8 and 112 µg mL^{-1}. Absorbances of calibration standards were measured at λ_{max}=290 nm using a UV-VIS spectrophotometer Beckman DU®-68 USA. Activity of xanthine oxidase was calculated from the early-prepared analytical curve according to the Beer-Walter light absorption law by means of the least squares formulas (1), (Fig. 2, Table 1). The calculation of analytical curve for xanthine oxidase activity was similar to nitrite (for nitrate reductase activity).

Xanthine is used as a substrate for measurement of activity of xanthine oxidase in field-moist soil samples. 0.25 g field-moist soil extracted with 50 mL of phosphate buffer (0.1 M at pH=7.5) using a mechanical shaker for 4 hours. The resultant extracts were filtered and centrifuged at 4000 r.p.m. for 10 min. The assay mixture consisted of 2.5 mL of test solution and 2.0 mL of substrate solution (6.6 mM xanthine). The

mixture was incubated at 37 °C for 30 min. The reaction was then stopped by the addition of 0.5 mL of 0.58 M hydrochloric acid, and the absorbance measured at λ_{max}=290 nm using a UV-VIS spectrophotometer Beckman DU®-68 USA. A control was also prepared in the same way, but the in place of 2.0 mL of phosphate buffer (0.1 M at pH=7.5) xanthine solution was added. Deionized water was used as blank.

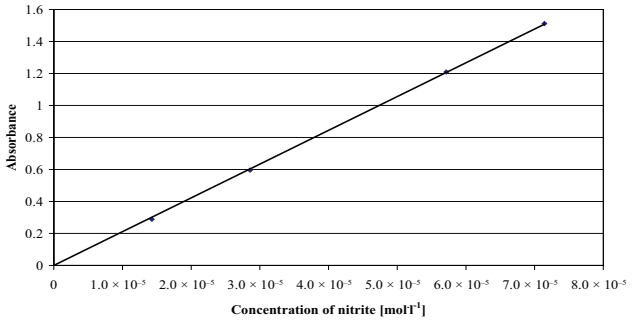

Fig. 1. Analytical curve of the concentrations of nitrite ions

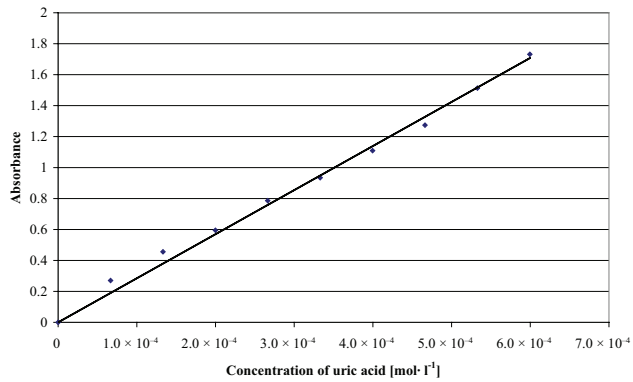

Fig. 2. Analytical curve of the concentrations of uric acid

Phenol oxidase was determined by Perucci method [15]. A standard benzoquinone stock solution was used (200 µg in 1 mL). 20 mg of benzoquinone was dissolved and diluted the volume to 100 mL with 1 M phosphate buffer at pH=6.5 in a volumetric flask. The calibration standards were constructed by adding of 0 (blank reagent); 1.25; 2.5; 6.25 and 12.5 mL working standard in five 25 mL volumetric flasks. 1.5 mL of 0.2 M proline was added to each calibration standards of colour reagent. After 0.5 h, calibration standards were diluted the volume to 25 mL with 1 M phosphate buffer at pH=6.5 in a volumetric flask. Obtained calibration standards contain 0.0; 10.0; 20.0; 50.0 and 100.0 µg mL^{-1} of benzoquinone. Absorbances of the red colour intensity of calibration standards were measured colorimetrically at λ_{max}=525 nm using a UV-VIS spectrophotometer Beckman DU®-68 USA. Activity of phenol oxidase was calculated from the early-prepared analytical curve according to the Beer-Walter light absorption law by means of the least squares formulas (1), (Fig. 3, Table 1). The calculation of analytical curve for phenol oxidase activity was similar to nitrite (for nitrate reductase activity), and xanthine oxidase activity.

Reagent of 0.1 M phosphate (pH=6.5), containing 0.2 M of catechol, was oxygenated for 3 minutes and incubated for 10 minutes at 30 °C. Then, 1.0 g of field-moist soil was added to 3 mL of reagent solution (obtained by mixing 1.5 mL of catechol solution with 1.5 mL of 0.2 M of proline solution) and 2 mL of phosphate buffer (0.1 M at pH=6.5). The mixture was incubated for 20 min at 30 °C and the reaction stopped by cooling in an ice-bath and adding 5 mL of ethanol. The mixture was centrifuged at 4 000 r.p.m. at 4 °C for 10 min and filtered. The absorbance of the supernatant fraction was measured at λ_{max}=525 nm using a UV-VIS spectrophotometer Beckman DU®-68 USA. Assays without soil and without catechol were carried out simultaneously as controls. Phosphate buffer was used as blank.

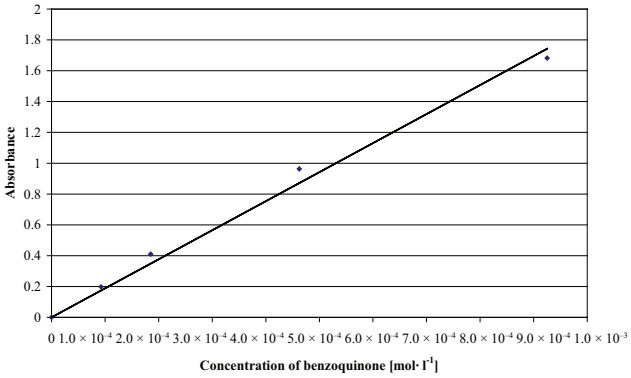

Fig. 3. Analytical curve of the concentrations of benzoquinone

Peroxidase activity in soils was determined by Bartha and Bordeleau method [16]. Horseradish peroxidase Type II (Sigma) was used as enzyme standards. 0.1 g of standard of horseradish peroxidase was dissolved in 0.05 M phosphate buffer at pH=6 in a measuring flask of 1000 mL in volume, in order to prepare standard stock solution. The analytical curve was constructed by adding of 1.0; 2.0; 3.0; 4.0 and 5.0 mL working standard in five 25 mL volumetric flasks. 3.0 mL each standards, 0.5 mL of 0.06 % H_2O_2 in 0.05 M phosphate buffer at pH=6 and 0.1 mL of 0.5 % o-dianisidine in methanol were combined in a 1 cm spectrophotometric cuvette for colorimetric analysis (UV-VIS spectrophotometer Beckman DU®-68 USA). All calibration standards were mixed and allowed to stand for 10 min at 20 °C. The absorbance of the reaction was measured colorimetrically at λ=460 nm is UV-VIS spectrophotometer Beckman DU®-68 USA. Activity of peroxidase was calculated from the early-prepared analytical curve according to the Beer-Walter light absorption law by means of the least squares formulas

(1), (Fig. 4, Table 1). The calculation of analytical curve for peroxidase activity was similar to nitrite (for nitrate reductase activity), xanthine oxidase and phenol oxidase activity.

The field-moist soil was used in all of the experiments. The tested soil was screened through a sieve with 2.0 mm mesh. According to the procedure, 20 g of soil was suspended in 100 mL of 0.05 M phosphate buffer at pH=6 in the flask closed with a cork and agitation on a rotary shaker and incubated for 1 hour at 25 °C. Next, samples were centrifuged at 4000 r.p.m. for 20 min, and soil solutions were filtered. The peroxidase assay was as follows: 0.5 mL of 0.06 % H_2O_2 in 0.05 M phosphate buffer at pH=6, 0.1 mL of 0.5 % o-dianisidine in methanol and 3 mL of soil extract were combined in a 1 cm spectrophotometric cuvette. All the samples were placed at temperature of 20 °C for a period of 10 min. The ingredients were mixed and an increase in the absorbance continuously recorded at λ_{max}=460 nm using a UV-VIS spectrophotometer Beckman DU®-68 USA.

Heat inactivation was achieved by placing the flasks containing the soil extract in a 100 °C water bath for 5 minutes served as control. Further procedure was done as in the case of tested sample but did not contain H_2O_2.

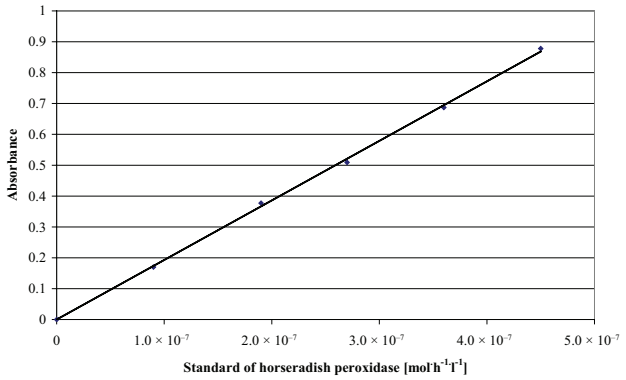

Fig. 4. Analytical curve of the activity of horseradish peroxidase

Catalytic character characterizes chemical, biochemical, physical and biological processes in soil organic matter. Thus, these pathways and their mechanisms occurring in soil organic matter are significantly dependent on the properties of the environment. The pH of the soil seems to be is the most important physicochemical parameter affecting plant growth and behavior of the contaminants in soils. The present study showed small, however, considerable differences in the pH values for both horizons of peat soils.

It is known that organic matter is capable of inducing reduction and oxidation reactions, hence affecting the redox system in the environment. The release of DOC into the soil solution represents a key process for the loss of C from peatlands but is also important since DOC is a substrate and product of microbial metabolism [17, 18]. These include the solubilisation, complexation or sorption and adsoption of metals and hydrophobic organic compounds. Dissolved organic C generally decreases with the depth due to the retention by soil surfaces and is considered to be mostly derived from old organic matter with slow incorporation rate from recently-deposited sources [19, 20].

Peat is a blond to black organic material (<25 % by weight mineral matter) formed under waterlogged conditions from the partial decomposition of mosses and other bryophytes, sedges, grasses, shrubs and trees. The structure of peat ranges from fibric to sapric, and the relative proportions of C, H, and O vary, depending upon the botanical composition and the degree of decomposition. Typical abundances (moisture and ash free) are in the range 50–60 % C, and 5–6 % H. These elements tend generally to an increase with the increasing degree of decomposition, while the O content (30–40 %) decreases [21].

Our research showed that the dissolved organic carbon concentration ranged from 5.67 to 10.92 g kg^{-1} and total organic matter from 314.0 to 436.8 g kg^{-1} in all of sampling (Table 2). In all cases, concentration of DOC was the highest at 0–25 cm and decreased with depth of profile from 30 to 42 %. However in the points 1, 2, 3 and 4 appeared different phenomena to the changes concentration of TOC. In the soil profiles the concentration of TOC increased with depth from 4 to 28 % (Table 2).

Table 2

Total organic carbon, dissolved organic matter, total nitrogen, and the ratios C/N in peatland Tagan

Sampling sites depth (cm)		*TOC*	*DOC*	N_{total}	*C/N*
		g kg^{-1}			
1	0–25	417.9	10.34	30.24	13.82
	50–75	433.4	7.18	25.76	16.82
2	0–25	395.7	10.32	30.80	12.85
	50–75	425.1	6.65	26.32	16.15
3	0–25	314.0	8.08	24.64	12.74
	50–75	436.8	5.67	26.88	16.25
4	0–25	395.8	10.92	23.52	16.83
	50–75	435.1	6.30	25.20	17.27

Nitrogen is one of important elements in peat soils, which is largely affected by environment factors such as pH, moisture condition, temperature and land use. The nitrogen input in peat ecosystems is mainly in the form of atmospheric deposition from precipitation and dry fall, and nitrogen fixation. It also becomes available to living plants through mineralization and translocation. Nitrogen is lost from the acrotelm through denitrification, grazing, burning, surficial runoff, and erosion. The preferential loss of carbon in the catotelm, results in relative enrichment of nitrogen, and consequently, in a decrease of C/N ratio in the peat [22].

C/N ratio is an indication of the degree of humification of the organic materials. Generally standard C/N ratio in peat soil is from 20.0 to 46.3 % [23]. In all analyzed soil samples the amount of total nitrogen ranged from 23.52 to 30.80 g kg^{-1} (Table 2). Furthermore, these investigations have shown increase of C/N ratio with depth in all of sampling. In the soil profile these values varied at a depth of 0–25 cm from 12.74 to 16.83 and at 50–75 cm from 16.15 to 17.27 (Table 2). However, no significant relationship was found for concentration of total nitrogen, TOC, DOC and activity of nitrate reductase, xanthine oxidase, phenol oxidase and peroxidase.

The results reported by Boyer and Groffman [24] indicate that typically, microorganisms decrease with depth in the soil profile. Microbial processes in soils are often limited by the availability of carbon substrates. Mineralization, mobilization, and podzolization processes all serve to limit the transport of carbon to deeper layers. Members of the soil fauna that are capable of migration within the profile selectively seek out favorable moisture and temperature regime. The results of our research presented in this study weren't confirmed to the investigation obtained by these authors in all of sampling (Table 3).

Table 3

Nitrate reductase, xanthine oxidase, phenol oxidase and peroxidase activity in peatland Tagan

Sampling sites depth (cm)		*Nitrate reductase activity μgN 24h^{-1} g^{-1}*	*Xanthine oxidase activity μmol uric acid h^{-1} g*	*Phenol oxidase activity μmol oxidized catechol h^{-1} g^{-1}*	*Peroxidase activity nmol h^{-1} g*
1.	0–25	0.11	27.22	6.18	6.64
	50–75	0.16	37.43	13.8	5.30
2.	0–25	0.68	22.93	10.85	5.92
	50–75	0.27	33.61	21.65	7.52
3.	0–25	0.35	33.18	15.77	2.30
	50–75	0.46	54.75	20.70	5.00
4.	0–25	0.45	40.43	13.98	1.50
	50–75	0.33	52.05	46.01	4.30

Nitrate reductase is an enzyme involved in the process of denirification. The nitrogen oxides act as terminal electron acceptors in the absence of oxygen. In the anaerobic conditions nitrate ions are reduced to nitrite ions and nitrate reductase is the catalyst of this reaction. Next the formed NO_2^- anions are reduced with the participation of nitrite reductase to N_2O which easy reacts with oxygen. The reduction reaction of N_2O to molecular nitrogen is catalyzed with the nitrous oxide reductase [25, 26]. This process accelerates the pathways of easy decayed organic matter and alkalinity or neutrality of soil.

The availability of organic carbon is one of the most important factors that affect denitrifying activity in soil supplying a source of energy, and substrate for the growth of bacteria. In addition, it participates in an electron exchange [27]. The major products of these reductive processes are gaseous nitrogen species which they evaporate from the soil. The investigation has shown that nitrate reductase activity in the soils ranged at a depth of 0–25 cm from 0.11 to 0.68 μgN $24h^{-1}$ g^{-1} and at a depth of 50–75 cm from 0.16 to 0.46 μgN $24h^{-1}$ g^{-1} in all of sampling (Table 3). It was much higher at a depth of 0–25 cm in point 2 which is characterized by wooden and wooden grasses peat with 35 % degree of the decomposition.

An important role in redox processes in soil plays xanthine oxidase. This enzyme is a metal-flavoprotein containing FAD, molybdenum and iron in the ratio of 2:2:8. Xanthine oxidase is a metal-flavoprotein containing FAD, molybdenum and iron in the ratio of 2:2:8. This enzyme is the last enzyme in the pathway of the degradation of purine derivatives from nucleic acids and is assumed to be a rate-limiting step in purine metabolism. It is also assumed that it takes part in alcohol metabolism; it plays a role in the incorporation of iron in ferritin. Xanthine oxidase oxidizes hypoxanthine and xanthine to uric acid in the purine catabolic pathway. This enzyme is participant in the cycle of nitrogen in soils [28–34]. These studies indicated that xanthine oxidase activity in the soils increased with depth and ranged at a depth of 0–25 cm from 22.93 to 40.43 μmol h^{-1} g^{-1}, and at a depth of 50–75 cm from 33.61 to 54.75 μmol h^{-1} g^{-1} in all of sampling (Table 3). Furthermore, the highest increase activity of this enzyme was confirmed between 0–25 cm and 50–75 cm in point 3, which was 39 %.

Similar trend was shown for the activity of phenol oxidase and peroxidase in the points 2, 3 and 4. The phenol oxidase is enzyme that catalyzes the oxidation of phenolic compounds to quinones, participates in the formation of humic acids, and indicates the capacity of the microflora degrade recalcitrant organic substances. Phenol oxidase is one of the few enzymes able to degrade recalcitrant phenolic materials as lignin. The group includes o-diphenol oxidase (tyrosinase), p-diphenol oxidase (laccases) and polyphenol oxidases [31, 32]. Phenol oxidase enzymes catalyze polyphenol oxidation in the presence of oxygen (O_2) by removing phenolic hydrogen or hydrogenes to form radicals or quinines. These products undergo nucleophilic addition reactions in the presence or absence of free -NH_2 group with the eventual production of humic acid-like polymers. The presence phenol oxidase in soil environments is important in the formation of humic substances a desirable process because the carbon is stored in a stable form [33]. These studies indicated that phenol oxidase activity ranged at a depth of 0–25 cm from 10.85 to 15.77 μmol h^{-1} g^{-1} and at a depth of 50–75 cm from 6.18 to 46.01 μmol h^{-1} g^{-1} (Table 3). Investigations carried out regarding phenol oxidase

activity have shown an increase in its activity in the depth of soils profile in point 2, 3, 4 from 24 to 70 %. The highest increase activity of this enzyme was confirmed from 0–25 cm to 50–75 cm in point 4 which is characterized with 1.5 m depth of sapropel.

Peroxidases are present in natural soil, and may originate from microorganisms, plants or other organisms. This enzyme catalyzes the oxidation of phenols and aromatic amines in the presence of hydrogen peroxide as an electron acceptor in the reactions. The release of carboxyl and methoxyl groups from phenolic substrates is ascribed mainly microbial activity and may lead to CO_2 production in soil [34–38]. Peroxidase has been the most studied because of their role in organic matter degradation and release of nutrients in the soils. As is well known, the role of enzymes in coupling reactions leading to polymerization is limited to the oxidation of the substrates. The oxidative metalloenzym is able to oxidize high redox potential aromatic substrates and has unique catalytic properties. The abundance of relatively stable peroxidases must have a role in the synthesis of macromolecules such as the humic acids (HA). Moreover the polymerization of readily degradable litter, soil organic matter and nitrogen-containing compounds to higher-molecular-weight persistent organic compounds such as HA and fulvic acids (FA) influences on the long-term storage of carbon in soils and the biological availability of soil nitrogen [39–42]. It was observed that activity of peroxidase varied at a depth of 0–25 cm from 1.50 to 6.64 nmol h^{-1} g^{-1} and at a depth of 50–75 cm from 4.30 to 7.52 nmol h^{-1} g^{-1} in all of sampling (Table 3). Furthermore, the highest increase activity of this enzyme in the depth of soil profile was observed in point 4 as in the case of phenol oxidase activity and showing 65 %. Different trend was shown for peroxidase activity in point 1. It was confirmed lowering activity of this enzyme in the soil profile, equal to 20 %.

Conclusions

1. The study has shown impact of two depths of profile peatland on enzymes participating in redox potential like activity of nitrate reductase, xanthine oxidase, phenol oxidase and peroxidase.

2. In the soil profiles activity of xanthine oxidase, phenol oxidase and peroxidase increased with depth in the points 2, 3 and 4 and also xanthine oxidase in point 1.

3. Together with extending into the depth of soil, there was an increase in nitrate reductase activity in points 1, 3 and a decrease in points 2, 4.

Acknowledgements

This work was supported by grants No. N N305 3204 36; N N305 121934 founded by Polish Ministry of Education and by RFFR (№№ 09-05-00235, 09-05-00395), Minister of Education and Science (№ 02.740.11.0325).

References

1. Harrison R. M. Understanding our Environment: an Introduction to Environmental Chemistry and Pollution. Birmingham, 1992. 326 p.
2. Gliński J., Sahr K., Stępniewska Z., Brzezińska M. Changes of redox and pH conditions in flooded soil amended with glucose and manganese oxide under laboratory conditions // Z. Planzenenernähr. Bodenk, 1996. № 155. P. 13–17.
3. Tan K. H. Chemical Processes. In Benbi D.K.,I Nieder R. (eds): Handbook of Processes and Modeling in the Soil-Plant System. N. Y., 2003. 762 p.
4. Ingram H. A. P. Soil layers in mires: function and terminology // J. Soil Sci. 1978. № 29. P. 224–227.
5. Moore P. D. Biological processes controlling the development of modern peat-forming ecosystems // Int. J. Coal Geol. 1995. № 28. P. 99–110.
6. Bragg O. M., Tallis J. H. The sensitivity of peat-covered upland landscapes // Catena. 2001. № 42. P. 345–360.
7. Daniels S. M., Agnew C. T., Allot T. E. H., Evans M. G. Water table variability and runoff generation in an eroded peatland // South Pennines, UK. J. Hydrol. 2008. № 361. P. 214–226.
8. Bonnett S. A. F., Ostle N., Freeman Ch. Seasonal variations in decomposition processes in a valley-bottom riparian peatland // Sci. Total Environ. 2006. № 370. P. 561–573.
9. Gondar D., Lopez R., Fiol S. et al. Characterization and acid-base properties of fulvic and humic acids isolated from two horizons of an ombrotrophic peat dog // Geoderma. 2005. № 126. P. 367–374.
10. Fimmen R. L., Cory R. M., Chin Y. P. et al. Probing the oxidation-reduction properties of terrestrially and microbially derived dissolved organic matter // Geochim. Cosmochim. Acta. 2007. № 71. P. 3003–3015.
11. Insam H. Are the soil microbial biomass and basal respiration govemed by the climatic regime? // Soil Biol. Biochem. 1990. № 22. P. 525–532.
12. Freeman C., Liska G., Ostle N. et al. Microbial activity and enzymic decomposition processes following peatland water table drawdown // Plant and Soil. 1996. № 180. P. 121–127.
13. Kandeler E. Nitrate reductase activity. In Schinner F., Öhlinger R., Kandeler E., Margesin R. (eds): Methods in soil biology. Berlin-Heidelberg, 1996. P. 176–179.
14. Krawczyński J. Enzymology Diagnosis in Medical Practice. Warszawa, 1972. P. 182–184. (in Polish).
15. Perucci P., Casucci C., Dumontet S. An improved method to evaluate the o-diphenol oxidase activity of soil // Soil Biol. Biochem. 2000. № 32. P. 1927–1933.

16. Bartha R., Bordeleau L. Cell-free peroxidases in soil // Soil Biol. Biochem. 1969. № 1. P. 139–143.
17. Worral F., Burt T. Predicting the future DOC flux from upland peat catchments // J. Hydrobiol. 2005. № 300. P. 126–139.
18. Qualls R. G., Haines B. L. Biodegradability of dissolved organic matter in forest throughfall, soil solution and stream water // Soil Sci. Soc. Am. J. 1992. № 56. P. 578–586.
19. Marschner B., Bredow A. Temperature effects on release and ecologically relevant properties of dissolved organic carbon and biologically active soil samples // Soil Biol. Biochem. 2002. № 34. P. 459–466.
20. Dou F., Wright A. L., Hons F. M. Depth distribution of soil organic C and N after long-term soybean cropping in Texas // Soil Till. Res. 2007. № 94. P. 530–536.
21. Zaccone C., Miano T. M., Shotyk W. Qualitative comparison between raw peat and related humic acid in an ombrotrophic bog profile // Org. Geochem. 2007. № 38. P. 151–160.
22. Kuhry P., Vitt D. H. Fossil carbon/nitrogen ratios a measure of peat decomposition // Ecology. 1996. № 77/1. P. 271–275.
23. Salimin M. I., Gandaseca S., Ahmed O. H., Majid N. M. A. Comparison of selected chemical properties of peat swamp soil before and after timber harvesting // Am. J. Environ. Sci. 2010. № 6/2. P. 164–167.
24. Boyer J. N., Groffman P. M. Bioavailability of water extractable organic carbon fractions in forest and agricultural soil profiles // Soil Biol. Biochem. 1996. № 28/6. P. 783–790.
25. Fu M. H., Tabatabai M. A. Nitrate reductase activity in soils: effects of trace elements // Soil Biol. Biochem. 1989. № 21. P. 943–946.
26. Ma R. X. Effects of allelochemicals on activity of nitrate reductase // J. Environ. Sci. 2000. № 12. P. 125–128.
27. Lescure C., Mendendes L., Lensi R. et al. Effect of addition of various carbon substrates on dentrification in a vertic Mollsoil // Biol. Fertil. Soils. 1992. № 13. P. 125–129.
28. Fried R., Fried L. W. Xanthine oxidase (Xanthine dexydrogenase). In Bergmeyer H. U. (ed.): Methods of enzymatic analisis. V. 2. 1983. P. 644–649.
29. Hille R., Massey V. Molybdenum – containing hydroxylases: xanthine oxidaes, aldehyde oxidase, and sulfite oxidase. In Thomas S. (ed.): Molybdenum enzymes. N. Y., 1985. P. 443–518.
30. Fujimoto Y., Sakuma S., Tagami T. et al. N-ethylmaleimide inhibits xanthine oxidase activity with no detectable change in xanthine dehydrogenase activity in rabbit liver // Life Sci. 2000. № 68. P. 517–524.
31. Freeman C., Ostle N. J., Fener N., Kang H. A regulatory role for phenol oxidase during decomposition in peatlands // Soil Biol. Biochem. 2004. № 36. P. 1663–1667.
32. Benitez E., Nogales R., Campos M., Ruano F. Biochemical variability of olive-orchard soils under different management systems // Appl. Soil Ecol. 2006. № 32. P. 221–231.
33. Matocha Ch. J., Haszler G. R., Grove J. H. Nitrogen fertilization suppresses soil phenol oxidase enzyme activity in no-tillage systems // Soil Sci. 2004. № 169/10. P. 708–14.
34. Bollag J. M., Chen Ch. M., Sarkar J. M., Loll M. Extraction and purification of a peroxidase from soil // Soil Biol. Biochem. 1987. № 19/1. P. 61–67.
35. Nicell J. A., Wright H. A model of peroxidase activity with inhibition by hydrogen peroxide // Enzym. Microb. Tech. 1997. № 21. P. 302–310.
36. Choinowski T., Blodig W., Winterhalter K. H., Piontek K. The crystal structure of lignin peroxidase at 1.70Å resolution reveals a hydroxyl group on the C^{β} of tryptophan 171: a novel radical site formed during the redox cycle // J. Mol. Biol. 1999. № 286. P. 809–827.
37. Criquet S., Farnet A. M., Tagger S., Le Petit J. Annual variations of phenoloxidase activities in an evergreen oak litter: influence of certain of certainbiotic and abiotic factors // Soil Biol. Biochem. 2000. № 32. P. 1505–1513.
38. Dec J., Haider K., Bollag J. M. Release of substituents from phenolic compounds during oxidative coupling reactions // Chemosphere. 2003. № 52. P. 549–556.
39. Kerstetter R. E., Zepp R. G., Carreira L. H. Peroxidases in grass dew derived from guttation: possible role in polymerization of soil organic matter // Biogeochemistry. 1998. № 42. P. 311–323.
40. Dec J., Haider K., Bollag J. M. Decarboxylation and demethoxylation of naturally occurring phenols during coupling reactions and polymerization // Soil Sci. 2001. № 166/10. P. 660–671.
41. Gostishcheva M. V., Inisheva L. I., Shchegolikhina A. I. Futures of organic matter of peat soils of mire Tagan Tomsk region // Tomsk State Pedagogical University Bulletin. 2010. Issue 3. P. 114–120.
42. Sergeeva M. A., Inisheva L. I. Biochemical processes in oligotrophic landscapes vasjugan bog // Tomsk State Pedagogical University Bulletin. 2008. Issue 4. P. 57–64.

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Received 20.10.2010.

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АКТИВНОСТЬ ЭНЗИМОВ В ОКИСЛИТЕЛЬНО-ВОССТАНОВИТЕЛЬНЫХ ПРОЦЕССАХ НА ДВУХ ГЛУБИНАХ ТАГАНСКОГО БОЛОТА

Редокс потенциал – важный фактор влияния окружающей среды на биологическую среду почв. Подвижный и общий углерод, общий азот и активность отдельных ферментов были определены в торфяных образцах. Цель проведенных исследований – сравнение активности энзимов, участвующих в окислительно-восстановительных процессах по двум глубинам в торфяной залежи Таганского болота, соответствующим деятельному и инертному слою. Исследования показали различие окислительно-восстановительных процессов на разных глубинах профиля болота. Активность ферментов возрастала с глубиной в пунктах 2–4. С углублением происходило увеличение активности нитратредуктазы в пунктах 1, 3 и снижение ее активности в пунктах 2, 4.

Ключевые слова: *торфяные почвы, активность ферментов, нитратредуктаза, пероксидаза, фенолоксидаза.*

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