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IMPACT OF FLOODING ON SOIL ENZYME ACTIVITY IN ENVIRONMENTALLY SENSITIVE AREAS

Petra HULIČOVÁ[1], Danica FAZEKAŠOVÁ[1*] & Juraj FAZEKAŠ[1]

[1]University of Prešov, Faculty of Management, Department of Environmental Management, 081 16 Prešov, Ul. 17. Novembra č. 1, Slovakia, petra.hulicova@unipo.sk, danica.fazekasova@unipo.sk, juraj.fazekas@smail.unipo.sk

**Corresponding author. Email: danica.fazekasova@unipo.sk*

Abstract: Frequent and excessive flooding not only affects the yield of crop but also the processes that take place in the soil. In the present study, we monitored the activity of soil enzymes (activity of urease, acid phosphatase and alkaline phosphatase, catalase) and hydrophysical soil properties of different ecosystems (arable land, permanent grassland and forest land) in the years 2012–2014 in environmentally sensitive areas that are affected by irregular flooding. Dry polder Besa is in the southeastern part of the Eastern lowlands of Slovakia and construction of dry polder Besa necessitated the flood situation in the rivers of the Eastern lowlands. Measured values of monitored enzymes (activity of urease, acid phosphatase and alkaline phosphatase, catalase) pointed out the differences in their activity between the types of ecosystems and the depth of the soil profile. Statistical testing showed some correlation dependence between almost all selected biological and hydrophysical parameters, as well as the enzymes themselves. Based on the obtained results, we can conclude that the occurrence of continuous water on the soil surface for a certain period of time can have a negative impact on the hydrophysical and biological properties of the soil.

Keywords: ecosystem, flooded area, hydrophysical soil properties, soil enzymes

1. INTRODUCTION

The consequences of flooding are reflected in changes in soil structure, consequently affecting the reception, retention and transfer of water in the soil and disruption of water and air regimes in the soil, which result in a reduction in the infiltration and drainage capacity (Tsheboeng et al., 2014). Loss of structure causes the deterioration of physical, chemical and biological soil properties. There is limited gas exchange between soil and the atmosphere, which leads to the creation so-called anaerobic conditions, thereby hampering the conditions necessary for the intake of essential nutrients. A concentration of CO_2 exacerbates the lack of oxygen and reduction processes occur in flooded soils. This has a toxic effect on the plant roots, resulting in their death and, subsequently, a decrease in yield. The nitrogen cycle changes and denitrification predominates. This slows the decomposition of organic matter and increases soil pH, depending on the duration of flooding. This, in turn, changes the salt content and macronutrients and micronutrients in the soil (Kotorová et al., 2010).

Water is a key regulatory factor of life and activity of soil microbiocenosis. It affects the physical and chemical processes in the soil and determines the habitat of resident microorganisms. According to Šantrůčková (2001), the amount of water in the soil, in the free space between the solid particles, is one of the most important factors that influence plant growth and soil biological activity. Water is part of all living organisms but it is also the environment in which all vital processes take place. Microorganisms extract their food, in the form of soluble organic and mineral substances, from soil solution. The amount of water in the soil affects the activity of the organisms and the transformation processes of soil. It ensures the movement of nutrients in the soil profile, and passive movement of microorganisms. Soil water mainly affects seasonal population dynamics and activity of soil microorganisms (Javoreková et al., 2008; Barral & Paradelo, 2009).

The basic characteristics of the water regime of the soil are referred to as moisture and the moisture potential. Moisture determines the volume of water in the soil and is a function of time and space. The moisture potential characterises the energetic level of water in the soil (Kotorová et al., 2010; Łydżba et al., 2014).

Soil biology is a significant component of soil quality and microorganisms play vital roles in soil fertility and primary production through organic matter decomposition and nutrient cycling (Castaldi et al., 2004). Enzymes are important activators of the life processes of soil and are known to be a suitable indicator of healthy soils. Soil enzymes play an important role in maintaining soil ecology, physical and chemical characteristics, fertility and in the overall process of decomposition of organic matter in the soil system (Nielsen and Winding, 2002). Soil enzyme activity is a reliable indicator of the biological condition of the soil and can be used to quickly obtain relevant results on changes in soil conditions (Dindar et al., 2015; Makoi & Ndakineni, 2008; Dick, 1997).

Research in the field of soil enzymatic activity mainly focuses on the relationship between the enzyme activity and various parameters of soil environment, such as moisture, temperature, soil aeration, pH, texture, organic carbon content and quality of humus components, macronutrients and content of clay minerals. Among other factors, it is soil biocenosis, vegetation cover, agricultural engineering and cultivation systems and, not least, the influence of xenobiotics and contaminants (Effron et al., 2004; Kizilkaya et al., 2004).

The territory of the dry polder Besa contains different ecosystems (forests, natural grassland, aquatic ecosystems and agro-ecosystems) and has a high degree of biodiversity. Ecological stability in this area is disturbed for long and irregular intervals by human activities and the artificial flooding of the polder.

The aim of this study was to investigate the effects of flooding on the enzymatic activity of soils (activity of urease, catalase, acid phosphatase and alkaline phosphatase) and to determine statistically significant differences in soil biological activity and hydrophysical properties depending on the type of ecosystem (arable land, permanent grassland and forest land) and soil depth in environmentally sensitive areas of the dry polder Besa, which is disrupted by artificial flooding.

2. MATERIAL AND METHODS

2.1 Study area

The research was performed in the dry polder Besa, which is a dry reservoir in the southeastern part of the Eastern lowlands, Slovakia, the construction of which required the flooding of the rivers. Besa polder is irregularly inundated land; the last saturation was in 2010. The climate in the Eastern lowlands is moderate continental, characterised by a warm-dry to moderately dry climate and cold winters. The dominant soil type is Fluvisols with clay subtype in the study area. In the past, the area of Besa polder was significantly influenced by the ecosystems in the area and this effect persists even today. The Ramsar locality is located in the polder territory, covering an area of 2.654 hectares and which is included in the Natura 2000 network, with the habitats and species having the highest degree of protection (Kováč et al., 2009).

2.2 Soil assays

Soil sampling for quantification of selected biological and hydrophysical properties of soil took place in the spring–summer season (May–June) from 2012 to 2014 from five research profiles at three depths of soil profile (Table 1). Disturbed samples were taken to determine the biological parameters of soil; undisturbed samples were taken to determinate hydrophysical soil parameters. A Kopecky physical cylinder with a capacity of 100 cm^3 was used to collect soil from three soil depths: 0–20 cm, 20–40 cm and 40–60 cm, in quadruplicate, during the months of May to June (2012–2014).

Table 1. Characteristics of sampling fields.

Profile	Ecosystem	GPS	Soil texture
1.	arable land	N48° 31' 43.3" E21° 56' 08.2"	sandy-loam
2.	permanent grassland	N48° 32' 39.1" E21° 55' 34.4"	clay-loam
3.	permanent grassland	N48° 31' 51.5" E21° 56' 57.0"	clayey
4.	permanent grassland	N48° 31' 46.2" E21° 57' 59.0"	clay
5.	forest land	N48° 31' 25.0" E21° 55' 44.1"	clayey

Urease activity (URE) was measured using the method described by Galstjan (Chazijev, 1976). This method is based on spectrophotometric measured of the ammonia content (at 410 nm), which is created, along with carbon oxide, in a hydrolysis of urea (substratum) catalyzed by urease. In order to determine the content of ammonia by this method, toluene and phosphate buffer (pH 6.7), 2M KCl, Seignet’s salt and Nessler’s agent was used. Acid (ACP) and alkaline phosphatase (ALP) was measured using the method described by Chazijev, modified by Grejtovský (1991). The soil was incubated with phenyl phosphate and the phenol amount was determined by 4-aminoantipyrin spectrophotometric (at 510 nm). Catalase activity (CAT) was measured using method described by Chazijev (1976). This method is based on measuring the volume of oxygen released for 10 minutes from 10 g of the soil sample after addition of 20 ml of 3% H_2O_2. Available water capacity (θP %), wilting point (θV %), non-capillary porosity (θNK %) and soil moisture (%) were determined using the method described by Hrivňáková et al., (2011).

2.3 Statistical analysis

Statistica 12 software was used for all data analyses. Spearman's rank coefficient was used to determine of the level of correlation significance between soil properties. The test for differences in means of hydrophysical and soil enzyme activity between arable land, permanent grasslands and forested land was computed using One-way ANOVA. Data were LOG transformed prior to analysis.

3. RESULTS AND DISCUSSION

3.1. Hydrophysical and biological properties

The values of hydrophysical and biological properties are shown in Table 2 and Table 3. For classification and evaluation of the soil water regime hydrolimits of available water capacity, field water capacity, decreased availability point, hygroscopicity are often used as the characteristic values of moisture (Šutor et al., 2002). The available water capacity reached values corresponding for heavy to very heavy soils (Kotorová et al., 2010). The type of ecosystem, year of monitoring and depth of the soil profile significantly influenced the available water capacity (Table 4). The available water capacity was significantly affected by the high point of wilting. The lowest value was recorded on arable land. The results (Table 4) obtained in the study area show that the type of ecosystem, year of monitoring and depth of the soil profile at the wilting point has a significant impact. Kotorová (2007), Kotorová et al., (2010) published similar results for heavy soil of clay-glayed type.

There is mostly air in non-capillary pores. The redundancies of water occur in these pores throughout the depth of the soil profile. The obtained values show a high variability of this parameter and also show a weak airiness of the soil profile. The results of the analysis of variance (Table 4) showed that the type of ecosystem and depth of soil profile significantly influenced the non-capillary porosity. The wide range of non-capillary porosity (1.26–16.22% - Table 2) shows significant spatial variability in Besa polder. Soil moisture is characterised by hydrolimits that point to decreased water availability. The type of ecosystem, year of monitoring and depth of the soil profile significantly affected soil moisture (Table 4).

Table 2. Values of hydrophysical soil properties of sampling fields.

Parameter	Profile	Min	Max	Mean	Std. Dev.
θ_P – Available water capacity (%)	1.	16.4	28.38	20.95	3.61
	2.	17.52	29.93	22.69	4.05
	3.	12.94	22.93	15.05	3.07
	4.	13.1	23.92	18.72	2.97
	5.	14.68	20.03	17.59	1.95
θ_V – Wilting point (%)	1.	8.03	11.72	10.05	1.12
	2.	21.74	25.66	23.45	1.61
	3.	27.09	32.55	28.70	1.65
	4.	28.16	33.88	32.10	1.62
	5.	23.61	29.16	26.63	1.91
θ_{NK} – Non capillary porosity (%)	1.	5.43	10.14	6.87	1.35
	2.	1.26	9.56	5.75	2.75
	3.	3.81	12.42	7.33	2.51
	4.	1.5	8.71	5.15	2.19
	5.	2.03	16.22	6.98	4.27
Moisture (%)	1.	9.55	13.79	11.64	1.57
	2.	21.85	30.35	25.29	2.91
	3.	22.79	29.73	25.80	2.32
	4.	23.93	40.25	33.36	5.13
	5.	21.68	26.53	23.51	1.74

The urease enzyme is responsible for the hydrolysis of urea to carbon dioxide and ammonia and acts on carbon nitrogen bonds other than the peptide linkage (Bremner & Mulvaney, 1978). Activity of urease varied depending on the ecosystem type. The average value of urease activity was the lowest in the permanent grassland (0.02 mg $NH_4 \pm N$ g^{-1} 24 h^{-1}); the highest values were in arable land (0.58 mg $NH_4 \pm N$ g^{-1} 24 h^{-1} (Table 3). Kizilkaya & Ekberli (2008) reported that soil urease activity significantly increases with an increase in the concentration of urea. Studies by Skujins (1978); Wittmann et al. (2004) and Dengiz et al., (2007) pointed out that urease activity decreased with the increase of soil depth; this was also confirmed by our results (Figure 1). The highest values in all monitored ecosystems

(0.58 mg $NH_4 \pm N$ g^{-1} $24\ h^{-1}$) were recorded at a depth of 0–20 cm. The type of ecosystem, year of monitoring and depth of the soil profile significantly affected activity of urease (Table 5). According to Klosea & Tabatabai (2000), the urease soil is more often closely linked with organic matter or clay minerals, which are characterised by the largest surface area and carry an electric charge. Lower values of soil urease activity are likely to be influenced by the texture of the soil (McGarity & Myers, 1967) and tillage (Saviozzi et al., 2001).

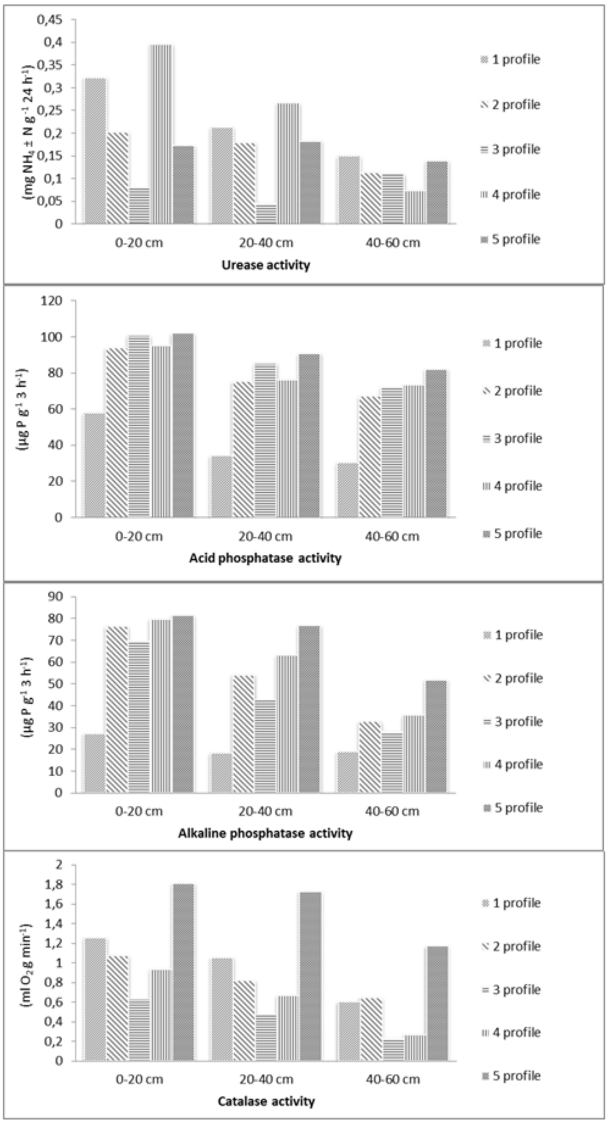

Figure 1. The average values of soil enzyme activity depending on the depth in the investigated area, expressed by descriptive statistics.

Phosphatases belong to a broad group of enzymes that are able to stimulate the hydrolysis of esters and anhydrides of phosphoric acid that play an important role in the cycle of phosphorus. They catalyse the degradation of organic phosphorous, which is unavailable to plants and convert it to inorganic phosphate, which is available to plants (Šarapatka, 2002). The acid phosphatase is typical for acid soils and the alkaline phosphatase is typical in neutral or alkaline soils. Soil reaction, temperature, organic matter content and, especially, moisture affect phosphatase activity. For this reason, phosphatase activity in soil varies seasonally (Nannipieri et al., 2002). Several authors have reported in their studies that phosphatase activity increases with an increase in soil moisture, as compared to the optimal moisture (Speir et al., 2003). Phosphatase activity decreases with the increase in soil depth, mainly due to the smaller amount of microorganisms in the lower layers of soil (Chazijev, 1976). The value of acid phosphatase activity was the lowest on arable land (26.3 µg P g^{-1} $3\ h^{-1}$) and the highest on forested land (117.41 µg P g^{-1} $3\ h^{-1}$). In the case of alkaline phosphatase activity, the lowest value was recorded on arable land (10.53 µg P g^{-1} $3\ h^{-1}$) and highest value on forested land (83.18 µg P g^{-1} $3\ h^{-1}$) (Table 3). The type of ecosystem, year of monitoring and depth of the soil profile significantly affected activity of alkaline and acid phosphatase (Table 5).

Table 3. Values of soil enzymes activity of sampling fields.

Parameter	Pr.	Min	Max	Mean	Std. Dev.
URE – Urease activity (mg $NH_4 \pm N$ $g^{-1} 24\ h^{-1}$)	1.	0.14	0.58	0.23	0.14
	2.	0.08	0.3	0.17	0.06
	3.	0.02	0.19	0.08	0.06
	4.	0.02	0.56	0.25	0.21
	5.	0.12	0.22	0.17	0.03
ACP – Acid phosphatase activity (µg P g^{-1} $3\ h^{-1}$)	1.	26.32	60.34	40.92	13.45
	2.	59.23	99.05	78.87	14.87
	3.	60.68	115.19	86.17	16.33
	4.	60.59	102.02	81.59	15.84
	5.	80.35	117.41	91.80	11.62
ALP – Alkaline phosphatase activity (µg P g^{-1} $3\ h^{-1}$)	1.	10.53	17.42	21.56	8.54
	2.	30.43	80.62	54.46	19.71
	3.	18.25	82.2	46.7	21.30
	4.	31.23	82.27	59.49	20.22
	5.	50.47	83.18	70.03	13.76
CAT – Catalase activity (ml O_2 g min^{-1})	1.	0.44	1.29	0.97	0.31
	2.	0.59	1.13	0.85	0.20
	3.	0.13	0.75	0.45	0.20
	4.	0.20	1.20	0.62	0.33
	5.	0.93	1.89	1.57	0.33

Notes: Pr. - Profile

Catalase is an antioxidant enzyme that is released into the soil in order to decompose hydrogen peroxide, to avoid cell damage. Its effects are detoxifying, which protects organisms (Nelson and Cox, 2000). The values of catalase activity were the highest in forest ecosystems (1.89 ml O_2 g min^{-1}), while the lowest values of catalase activity were on permanent grassland (0.13 ml O_2 g min^{-1} – Table 3). These findings may be related to the results of studies of Gömöryová (2008), according to which the higher values of catalase activity on forested land are related to chemically different sources of organic matter as a source of nutrients and energy for microorganisms. Some studies have shown that enzymatic activity may be limited to the surface layer of soil, whilst the oxidases and peroxidases are highly active at great depths (Zhang et al., 2005; Venkatesan & Senthurpandian, 2006). Monitoring of catalase activity did not confirm the statements of these authors but did show that the highest values occur in the upper layers of the soil profile (Fig. 1). The type of ecosystem and depth of the soil profile significantly affected activity of catalase (Table 5). Ladd (1978) stated that catalase activity is very stable soil parameter whose activity declines with soil depth and is also significantly correlated with organic carbon content.

Table 4. Variance analyses of hydrophysical parameters.

Parameter	Source of variation	Degree of freedom	F-value
θ_V – Wilting point (%)	ecosystem	2	4428.45++
	year	3	17.19++
	depth	2	52.63++
	repeat	3	0.65-
	residual	133	
	total	143	
θ_P – Available water capacity (%)	ecosystem	2	70.70++
	year	3	4.12++
	depth	2	77.84++
	repeat	3	0.17-
	residual	133	
	total	143	
θ_{NK} – Non capillary porosity (%)	ecosystem	2	8.39++
	year	3	0.15-
	depth	2	34.16++
	repeat	3	0.01-
	residual	133	
	total	143	
Moisture (%)	ecosystem	2	1776.14++
	year	3	105.05++
	depth	2	8.45++
	repeat	3	0.40-
	residual	133	
	total	143	

Notes: ++ P < 0.01 + P < 0.05; P = effect of factor significant at the level α = 0.05 or α = 0.01;

3.2. Correlations between soil enzyme activity and hydrophysical soil parameters

Though the statistical correlation testing of the enzymatic activity and the physical parameters of the soil (Table 6) we found a significant negative correlation between the activity of urease and the wilting point (P < 0.05) and a significant positive dependence of the activity of urease with the available water capacity of non-capillary porosity (P < 0.01). According to Šnajdr et al., (2008), urease activity does not always correlate with the availability of water in the soil, which is also consistent with our results.

Table 5. Variance analyses of soil enzyme activity.

Parameter	Source of variation	Degree of freedom	F-value
URE – Urease activity (mg $NH_4 \pm N$ g^{-1}24 h^{-1})	ecosystem	2	11.02++
	year	2	18.07++
	depth	2	29.14++
	repeat	3	0.01-
	residual	98	
	total	107	
ALP – Alkaline phosphatase activity (µg P g^{-1} 3 h^{-1})	ecosystem	2	285.14++
	year	2	3.63+
	depth	2	85.62++
	repeat	3	0.03-
	residual	98	
	total	107	
ACP – Acid phosphatase activity (µg P g^{-1} 3 h^{-1})	ecosystem	2	827.53++
	year	2	25.75++
	depth	2	179.63++
	repeat	3	0.18-
	residual	98	
	total	107	
CAT – Catalase activity (ml O_2 g min^{-1})	ecosystem	2	91.61++
	year	2	3.01-
	depth	2	9.56++
	repeat	3	0.01-
	residual	98	
	total	107	

Notes: ++ P < 0.01 + P < 0.05; P = effect of factor significant at the level α = 0.05 or α = 0.01;

Miralles et al., (2007) and Gömöryová et al. (2013) reported a high correlation coefficient between catalase activity and soil moisture that was not confirmed by our results. These authors observed a gradual increase in catalase activity in the long-term waterlogged environment. On the contrary, Nannipieri et al., (2002) found a significant correlation between soil moisture and phosphatase activity, which is also in line with our results. The author presents evidence for an increase in phosphatase activity with increased moisture. The activity of phosphatases, both alkaline and acid, correlates well with the availability of soil water. This indicates that the optimal conditions for microbial flora depends on the balance between moisture and aeration conditions and that excessive dryness is more threatening to microorganisms than excess water in

Table 6. Correlation between soil enzyme activity and hydrophysical soil parameters.

	ACP	ALP	CAT	θ_V	θ_P	θ_{NK}	Moisture
URE	ns	ns	ns	-0.423	0.546**	0.525**	ns
ACP		0.924**	0.432*	0.435*	ns	ns	0.654**
ALP			0.469*	0.438*	ns	ns	0.513*
CAT				ns	ns	ns	ns

Notes: URE = Urease activity; ACP = Acid phosphatase activity; ALP = Alkaline phosphatase activity; CAT = Catalase activity; θ_{NK} = non capillary porosity (%); θ_V = wilting point (%); θ_P = available water capacity (%); moisture (%); ** $p < 0.01$; * $p < 0.05$; ns = non-significant.

pre-filled pores. Vanhala (2002) and Borken et al., (2003) reported that water availability is one of the most important parameters regulating the biological activity in the soil. It directly affects the growth and activity of soil microorganisms and indirectly affects diffusion of soil nutrients in the water film, as well as the supply of oxygen. Water is not the only factor controlling the living conditions of the soil microorganisms. In very wet soils, high water content limits the diffusion of O_2 through the spaces between pores. The range of optimum soil moisture decreases with increasing temperature. According to the above authors, the soil moisture becomes more significant at higher temperatures.

4. CONCLUSION

In this paper we present the results of a study carried out in environmentally sensitive areas of the dry polder Besa, which is disrupted by artificial flooding. Water availability is one of the most important parameters regulating the biological activity in the soil. It directly affects the growth and activity of soil microorganisms and indirectly affects diffusion of soil nutrients in the water film, as well as the supply of oxygen. Measured values of investigated enzymes (activity of urease, acid phosphatase and alkaline phosphatase, catalase) pointed out the differences in their activity between the types of ecosystems and the depth of the soil profile. The highest values of catalase activity and acid and alkaline phosphatase were recorded in forest ecosystems, while urease activity was the lowest in forest ecosystems. In all ecosystems the highest enzyme activity was found in the upper layer of the soil profile.

Statistical testing showed correlation dependence between all selected biological and physical parameters, as well as between enzymes. The amount of water in the soil, in the free space between the solid particles, is one of the most important factors that affect the biological activity of the soil. Lower quality soils are more prone to changes in soil properties, which include the heavy soils with high contents of clay particles present in the area of interest. Based on the results we can conclude that the continuous presence of water on the soil surface for a certain period of time can have a negative impact on the physical and biological properties of the soil.

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