

INTERRELATIONS BETWEEN DNA CONCENTRATION AND PHYSICOCHEMICAL PARAMETERS IN THE LOESS SOIL PROFILE

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Abstract. The purpose of presented study was determination of the impact of selected physicochemical parameters, strongly influencing the soil biological life: water potential (pF), oxygen availability for microorganisms (ODR), redox potential (Eh), content of Mg, Ca and total organic carbon (TOC) on soil DNA concentration. Undisturbed loess soil material was taken to metal cylinders (100 cm^3) from four depths (0-20; 20-40; 40-60 and 60-80 cm), what make possibility for description of DNA content and its distribution in the whole soil profile. Our results revealed significant ($p < 0.05$) positive relationships between soil DNA content and measured values of ODR ($r = 0.94^{***}$), Eh ($r = 0.52^{**}$) and TOC ($r = 0.98^{***}$), what were confirmed by high values of correlation coefficients (r). Whereas, significant negative interrelationships between soil DNA and pF ($r = -0.57^*$) or Mg content ($r = -0.79^{***}$) were determined. However, in the current experiment conditions, we did not found significant correlation between Ca presence and DNA content ($p > 0.05$). Significant ($p < 0.05$) decrease of DNA concentration by 62.8% with an increase of soil depth was noted, what was undoubtedly connected with spatial distribution of microorganisms in the soil profile and its likes for surface layers colonization.

Key words: DNA content, soil water potential, oxygen availability, redox potential, divalent cations

INTRODUCTION

Soil environment by many researchers is compared to the “black box”, due to the fact that immense amount of biological information is contained inside the soil. Some of investigations demonstrated that there is sufficient DNA in 1 g of natural soil (not cultivated) to spread the genetic information over 2.142 km (Raes *et al.* 2007, Trevors 2010). This is an enormous amount of DNA but does not inform about the number of genes expressed and the duration of gene expression

in that gram of soil. One should bear in mind that the extraction of DNA from soil samples can never be 100% efficient and in reality may vary from a few μg to almost 200 μg DNA per gram of dry soil weight. This might be the reason why most authors have reported smaller values (from 1 to about 50 μg total DNA g^{-1} dry weight) depending on the method of isolation and soil sample (Lerat *et al.* 2005, Sagova-Mareckova *et al.* 2008). The general content of DNA is differentiated in relation to: the soil types, soil conditions, numbers of microorganisms, type of cultivation, and/or climate.

DNA, as the signature of life, has been extensively studied in a wide range of environments. Recently, DNA analysis has become central to work on natural gene exchange, soil bioremediation, genetically modified organisms, exobiology and paleontology. However, fundamental questions about what happens to DNA in the environment remain unanswered (Pote *et al.* 2007).

The total soil DNA includes both intracellular as extracellular forms of DNA (Pietramellara *et al.* 2009). According to various researchers (Ceccherini *et al.* 2003, Levy-Booth *et al.* 2007) once released into the environment DNA may:

- persist by binding to soil minerals or humic substances,
- be degraded by microbial DNases, and used as a nutrient for plant and microbial growth, and/or
- be incorporated into a bacterial genome as a possible source of genetic diversity.

Numerous experiments have demonstrated the mechanisms and importance of DNA adsorption onto soil components such as sand particles, clays minerals, or humic compounds (Romanowski *et al.* 1991, Khanna and Stotzky 1992, Poly *et al.* 2000). These results suggest that free DNA is rapidly degraded in the environment, but the adsorption of DNA onto soil components retards DNA degradation and constitutes a major mechanism of DNA molecule persistence in soil. So it is possible that DNA remains stable in the environment (Pote *et al.* 2007).

DNA is net negatively-charged, thus it can absorb easily to net positively-charged surfaces, but in the case of net negatively-charged surfaces DNA molecules require cations to mediate adsorption. Lorenz and Wackernagel (1994) indicated that divalent (Mg^{2+} , Ca^{2+}) cations are substantially more efficient DNA adsorption mediators than monovalent cations (Na^+ , K^+ , NH_4^+).

When setting foot on soil, most people are unaware they stand on a outstandingly diverse community of plants, animals and microbes, as there are billions and trillions of individuals. A study by Whitman *et al.* (1998) and Trevors (2010) revealed that just one gram of the surface soil layer on a dry weight basis contained 10^{10} of viruses, 10^{8-10} of bacteria (10^6 aerobic heterotrophs and 10^8 *Actinobacteria*), 10^6 of fungi, 10^6 of algae, 10^5 of protozoa and 10^2 of nematodes. However, a single gram of arable soil may contain between 10^9 - 10^{10} of bacterial

cells; on average $5.95 \cdot 10^9$ cells g^{-1} of soil (Fierer *et al.* 2007). Among these huge biodiversity only 1% bacteria species are known (Kirk *et al.* 2004, Fierer *et al.* 2007), remained 99% are still an enigma for researchers. We should also take into account that among known soil bacteria species only 0.3% are cultivated in laboratory conditions (Amann *et al.* 1995).

Due to this fact, understanding of relations between microorganisms biodiversity, content of genetic information enclosed in extracted form of soil DNA and environment functioning has become one of the main challenge facing the soil biology and microbiology. This challenge is more than complicated as well as life in the soil has been subjected to direct and indirect effects of soil heterogenic physicochemical processes. An important soil factors which are considered to be the most critical proximal regulator of microbial activities are as follows: soil moisture, oxygen availability for microorganisms and plant roots, and/or redox potential.

Thus, the aim of presented study was determination of the impact of selected physicochemical parameters, indirectly influencing the soil biological life: *pF*, *ODR*, *Eh*, content of Mg, Ca and *TOC* on soil DNA concentration, as well as description of DNA distribution in the loess soil profile.

MATERIALS AND METHODS

Loess soil taken in September 2009 from the forest in Konopnica (nearby Lublin) was used for experiment. Soil material was taken from four depths: 0-20, 20-40, 40-60, and 60-80 cm, in order to make possible an observation of investigated parameters variability in the whole soil profile. Each of described below laboratory analyses were conducted threefold. Selection of the loess profile for our investigations was not accidental, as firstly this soil type is representative for The Lublin District Region and secondly what is important loess is homogenous soil in the whole profile and thus it is perfect for model experiments. The relevant characteristics of the loess soil are reported in Table 1.

Table 1. Basic characteristics of the loess soil

Depth (cm)	Granulometric composition (%)				pH (in H ₂ O)	TOC (%)
	1-0.1 (mm)	0.1-0.02 (mm)	0.02-0.002 (mm)	< 0.002 (mm)		
0-20	4	43	35	18	5.4	1.47
20-40	3	46	35	16	5.7	0.31
40-60	8	42	39	11	6.2	0.21
60-80	6	45	34	15	6.8	0.15

Soil samples were transferred to plastic cylinders ($h = 5$ cm, $V = 100$ cm³) and placed on a plate inside the stainless-steel pressure chamber, containing a porous plate saturated with water at the bottom, in order to obtain a hydraulic contact between a sample and the porous plate. The laboratory set LAB o12 (Soil Moisture Equipment Company, USA) was used and the pressure was applied for the following water potentials (pF): 0, 1.0, 1.5 and 2.0, corresponding to a range of available water, and its quality for microorganisms and plant roots.

ODR was measured by an ODR -meter manufactured by the Institute of Agrophysics, Polish Academy of Soil Sciences (Lublin), based on Lemon and Ericsson method (1952), modified by Malicki and Bieganowski (1999). The ODR technique used a set of platinum microelectrodes as cathodes and a reference anode cell. Four platinum wire electrodes (0.5×4 mm) were placed at a depth of 2 cm and polarised to -0.65 V versus the saturated calomel electrode for 4 min. The data were recorded in three replicates for each sample.

Eh measurements were performed with pIONeer 65 device (Radiometer Analytical S.A.). The measurements were taken after stabilisation of the readings.

TOC was determined with use of automatic analyser $TOC-V$ SSM 5000A (Shimadzu).

Mg and Ca concentrations were determined by AAS technique (FAAS method) with use Z-8200 Hitachi Spectrophotometer (Japan). Each samples were replicated three times. Divalent cations content are expressed as a mg kg⁻¹.

Total DNA was extracted from 250 mg of soil at full water capacity (pF 0) and field capacity (pF 2) conditions, using GeneMatrix Soil DNA Purification Kit (EURx 1.4., Poland). The used amount of soil (250 mg) was strongly connected with protocol procedure enclosed to Soil DNA Purification Kit. Soil samples added to the bead solution were vortexed for 10 min followed by centrifugation at 10.000 x g for 30 s; the supernatant was re-centrifuged after protein precipitation and then purified by passing through a spin column by centrifuging at 10.000 x g for 30 s. The DNA concentration in each purified extract was quantified by UV spectroscopy (UV-1800 Shimadzu) at 260 nm and expressed as µg DNA per g⁻¹ dry soil. The extracted DNA was stored at -60°C .

Analyses of variance were conducted with Statistica 8.0 program (STATSOFT USA) to test differences among tested soil parameters. Least significant differences (Duncan's LSD) were calculated to find significant differences between investigated factors and soil DNA concentration. The differences were declared significant at the $p < 0.05$.

RESULTS AND DISCUSSION

Distribution of DNA in the loess soil profile is presented in Figure 1. By using the GeneMatrix soil DNA isolation kit, we were able to receive 1.3-3.5 $\mu\text{g g}^{-1}$, dependently from the depth. The highest amount of soil DNA in surface layer was estimated. It was connected with well known spatial distribution of microorganisms and its preferences for surface layers occupation. With increase of soil depth to the layer of 40-60 cm linear drop of DNA content by 62.8% in comparison to surface layer was observed. DNA concentration remained on the constant level (1.3 $\mu\text{g g}^{-1}$) also in the deepest part of the loess profile (60-80 cm).

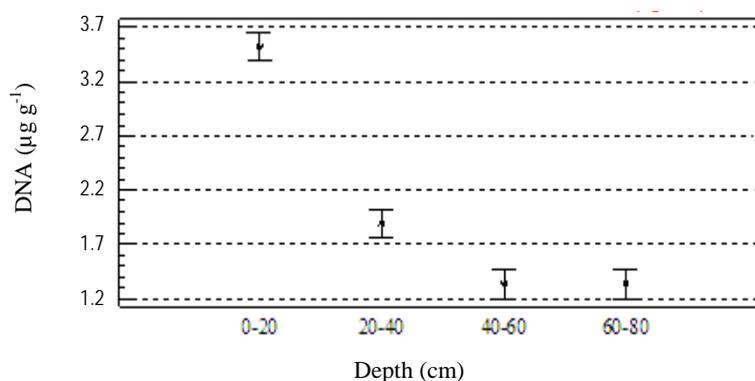


Fig. 1. Differentiations of DNA concentration at four depths of the loess soil profile ($n = 48$, $p = 0.001$, ANOVA, 95% LSD intervals)

However, we should always underline that extraction of DNA from soil samples is never 100% efficient and can vary from a $1\mu\text{g}$ to almost $200\mu\text{g}$ DNA per g dry weight soil, depending on the method and sample. Pote *et al.* (2007) received total DNA concentration ranged from 6.7 to $50.4\mu\text{g g}^{-1}$, using Ultraclean soil DNA Kit (MoBio Labs, Solana Beach, USA), whilst Udawatta *et al.* (2008) isolated $16.5\mu\text{g DNA g}^{-1}$, using Soil Isolation DNA Kit (MoBio Laboratories, Carlsbad, CA). Generally obtained amounts of soil DNA varied between 1 - $50\mu\text{g g}^{-1}$ (Agnelli *et al.* 2004, Raes *et al.* 2007), thus our results are compatible with other studies.

The obtained results include also retention characteristics of the loess soil determined for the range from full water capacity conditions (pF 0), till to more importantly from the agricultural point of view – the field water capacity (pF 2.2), i.e. the value, corresponding to water retention by soil due to gravitation forces, for 2 days after an intense downpour. Prepared pF -curves, illustrating the relations between soil water content and pF value are presented in Figure 2.

Loess soil displayed different ability for retaining of water, thus full water capacity (pF 0) ranged from 30-20% v/v, meanwhile pF 2.0 scoped between 15-8% v/v of water content (Fig. 2). The observed differences were connected with soil depth, as surface layers (0-20, 20-40 cm) were characterized by highest abilities for water keeping, whereas in the deeper parts of the profile (40-60, 60-80 cm) much lower values of water were retained. Naturally, with growing values of pF the reduction of water retention abilities was noted.

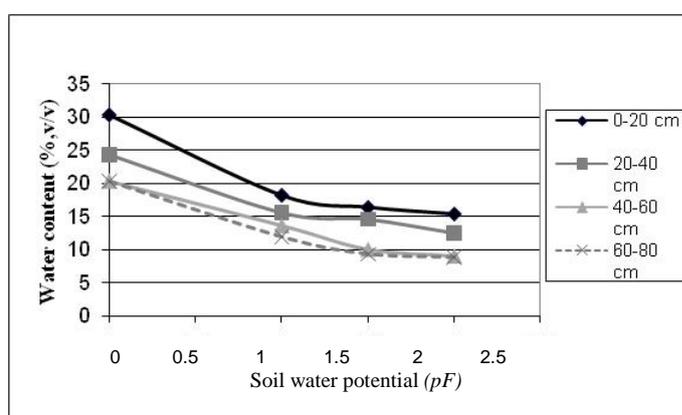


Fig. 2. The relationship between soil water content and water potential value (pF curves) at 4 depths of loess soil profile

Mentioned differences in water retaining are undoubtedly affected by both proportions of textural components, compaction and amount of organic carbon (Tab. 1). Walczak *et al.* (2002) and Brzezińska *et al.* (2005) reported that because macropore continuity is of major importance to the aeration status of the soil, the effect of soil compaction on other aeration properties depend on soil hydro-physical status. It is also considered that water retention may be affected by changes in soil organic matter and consequently the increase in organic matter level meant higher water content (Walczak *et al.* 2002, Zhuang *et al.* 2008). Even though in the case of investigated loess soil the range of TOC constitutes only 0.15 to 1.47%, it affects nearly all soil physical, chemical as well as biological properties. Thus, our results are compatible with this statement, as we observed the highest retention ability in the surface layer – rich in $C_{org.}$ (1.47%), while the lowest capability for keeping the water in subsoil (60-80 cm) characterized by poor level of $C_{org.}$ (0.15%) was noted. Moreover, observed in the current experiment almost 89% drop of TOC content (Tab. 1) in the deepest part of the loess profile in relation to the surface layer was agreed with Turski and Witkowska-Walczak (2004) study.

ODR measurements give information about the presence of O_2 , available in the soil for plant roots and microorganisms, whereas *Eh* is concerned with all redox transformations, resulting from the actual composition of the soil gas phase and soil solution. The changeable values of *ODR* and *Eh* in the investigated soil profile are presented in Figure 3. In general *ODR* level varied in the range from 76 to $121 \mu g O_2 m^{-2} s^{-1}$, whilst *Eh* varied between 702-712 mV. These results confirmed the fact that loess soil is good oxygenated in the whole profile.

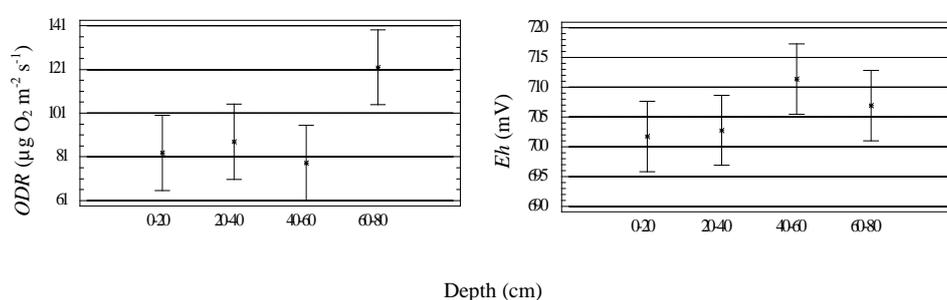


Fig. 3. Differentiations of *ODR* and *Eh* values at four depths of the loess soil profile ($n = 48$, $p = 0.05$, ANOVA, 95% LSD intervals)

Till to the depth of 60 cm we did not find any significant changes ($p > 0.05$) in oxygen availability for microorganisms and plant roots as *ODR* scoped between $76-92 \mu g O_2 m^{-2} s^{-1}$. However, improvement in soil oxygenation in the deepest part of the profile (60-80 cm) was stated, when significant increase ($p < 0.05$) to the level of $121 \mu g O_2 m^{-2} s^{-1}$ in relation to surface layer was registered.

This is consistent with previously published data for *Mollic Gleysol* (Walczak *et al.* 2001), *Eutric Cambisol* (Stepniewska *et al.* 2003) or *Rendzina Leptosol* (Wolińska and Bennicelli 2010). Quite likely, the explanation of this phenomenon involves the differences in granulometric composition of analyzed soil samples (Tab. 1), as the fact, that large granulation favourable for forming of aeration pores was noted in the subsoil layers.

Redox potential level testified about free dissolved oxygen presence in the whole profile of loess soil. Quite lower values (702 mV) at surface (0-20 cm) and subsurface (20-40 cm) layers were measured, whilst in subsoil (40-80 cm) *Eh* ranged between 707-712 mV. Any of registered changes in *Eh* level not exceeded the value of 10 mV, what confirmed homogenous character of the loess soil profile.

Distribution of divalent cations (Mg and Cu) favourable to DNA adsorption on soil particles are shown in Figure 4. On the contrary to *TOC* distribution which displayed reduction by 89% with increase of the depth (Tab. 1), both Mg as Ca

demonstrated growing trend in concentration, reaching the maximal level in the deepest part of the profile.

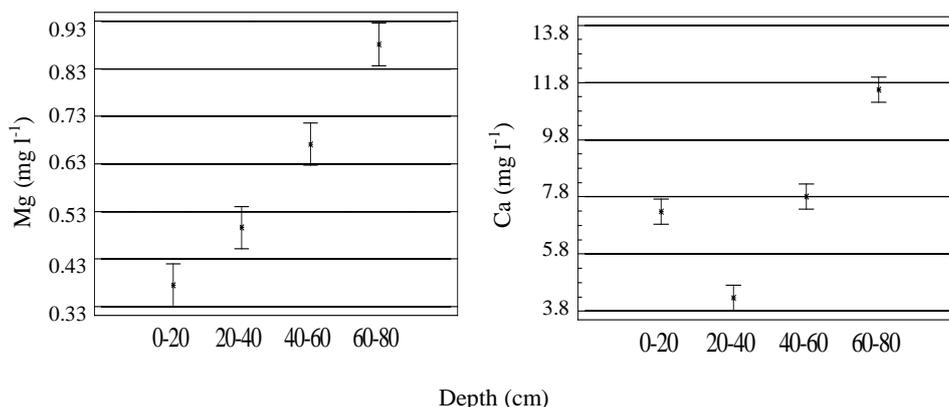


Fig. 4. Differentiations of Mg and Ca concentrations at four depths of the loess soil profile ($n = 48$, $p = 0.001$, ANOVA, 95% LSD intervals)

In the case of Mg linear increase of its content with depth was found and reached 0.87 mg kg^{-1} at the depth of 60-80 cm, what was 2.5 times higher than Mg presence in surface part of the loess profile (0.36 mg kg^{-1}).

Ca content ranged between $4.3\text{--}11.7 \text{ mg kg}^{-1}$ and demonstrated linear growth from the depth of 20-40 cm. Similarly to Mg distribution, also Ca reached almost 3 times higher concentration at the deepest part of the soil profile. These results are however not surprising as it is well known that parent rock material of the loess soil is built by calcium and magnesium carbonate. Higher concentration of Ca in the surface layer (7.3 mg kg^{-1}) in relation to the subsurface (20-40 cm) was unquestionably connected with the process of calcium leaching. Our data are compatible with results of Hajduk *et al.* (2009), who noted similar range of Ca and Mg content in the medium texture soil made from loess.

We also determined an optimal concentrations for divalent cations (Mg and Ca) presence, which facilitate DNA adsorption and its persistence in the investigated loess soil (Fig. 5). In the range of current experiment we found that optimal content of Mg should be between $0.3\text{--}0.53 \text{ mg kg}^{-1}$, whereas in the case of Ca higher concentration between $7\text{--}7.8 \text{ mg kg}^{-1}$ provide an effective DNA bounding.

Nguyen and Chen (2007) hypothesized that specific bridging between the DNA and soil particles in the presence of Ca^{2+} cations may lead to more efficient attachment than in the presence of Mg^{2+} cations, which are only likely to allow for charge neutralization. Also Cai *et al.* (2006) revealed that especially Ca have even a higher

promotive effect. Calcium can form “inner-sphere” complexes with the phosphate groups of the DNA and form bridges between DNA and soil mineral surfaces.

Finally, by using statistical tools correlations between DNA content and *pF*, *ODR*, *Eh*, *TOC*, Mg and Ca concentrations were determined. Statistical relationships described by correlation coefficients (*r*) between DNA concentration and measured soil factors are presented in Table 2.

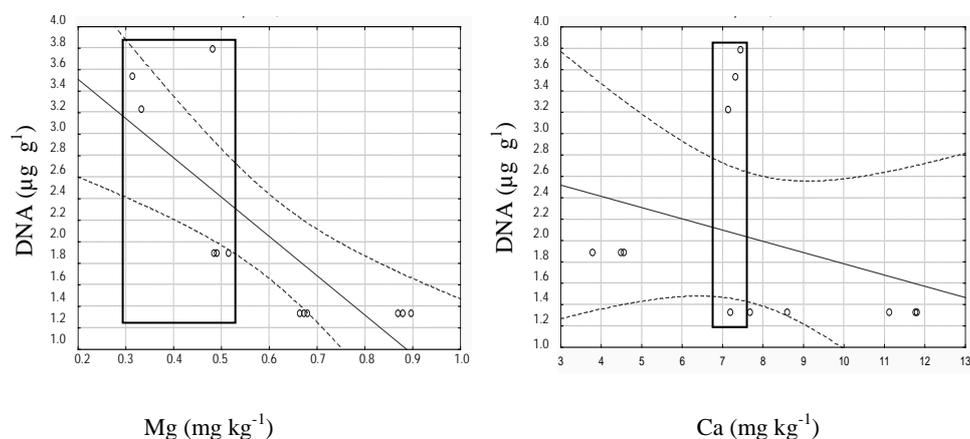


Fig. 5. Optimal range of Mg and Ca concentrations (tagged in rectangle) for DNA persistence at investigated loess soil particles ($n = 48$, $r = -0.79^{***}$ for Mg and $r = -0.30$ n.s. for Ca)

Table 2. Statistical significant relationships between DNA concentration and analyzed parameters ($n = 48$)

Factor	<i>pF</i>	<i>ODR</i>	<i>Eh</i>	Mg	Ca	<i>TOC</i>
c DNA	-0.57*	0.94**	0.52**	-0.79***	-0.30 n.s.	0.98***

*, **, *** – indicate significance at the $p = 0.05$, $p = 0.01$, and $p = 0.001$, respectively, n.s. – not significant differences.

It was found that soil DNA content is positively correlated with *ODR*, *Eh* and *TOC*, what is confirmed by high values of *r* coefficient and mean that increase of each mentioned parameters resulted with growth of DNA content in the loess soil. Negative significant correlations between DNA presence and both *pF* as Mg concentration were determined. Meanwhile, relationship between DNA and Ca content in the range of current experiment did not demonstrated significant character.

Although, study by Sheu *et al.* (2008) and Acosta-Martinez *et al.* (2010) confirmed positive correlation between soil DNA and *TOC* content, even so prior to our study, rather little attention has been paid to the influence of *pF*, *ODR*, and *Eh* on soil DNA presence. Therefore our work focused on these relationships and determination of statistical correlations. Nevertheless, the interpretation of our results has been challenging because of the lack of enough positions in available literature data, with determined *r* coefficients as goodness of fit between investigated soil factors and DNA content. Thus, it is necessary to perform further investigations and selection of another soil types, for explaining and confirming the indicated correlations.

CONCLUSIONS

1. Our results revealed significant ($p < 0.05$) decrease of DNA concentration by ~63% with an increase of soil depth, what was undoubtedly connected with spatial distribution of microorganisms in the soil profile and its likes for surface layers colonization.

2. Significant ($p < 0.05$) positive relationships were determined between soil DNA content and measured aeration status parameters, as *ODR* ($r = 0.94^{***}$) and *Eh* ($r = 0.52^{**}$), however the highest correlations with *TOC* ($r = 0.98^{***}$) was assigned.

3. Significant ($p < 0.05$) negative interrelationships were estimated between soil DNA and as well *pF* ($r = -0.57^*$) as Mg conc. ($r = -0.79^{***}$).

4. The concentration of divalent cations which are optimal for DNA stabilization on loess soil particles ranged between 0.3-0.53 mg kg⁻¹ and 7-7.8 mg kg⁻¹ for Mg and Ca, respectively.

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WSPÓLZALEŻNOŚĆ STĘŻENIA DNA I PARAMETRÓW FIZYKOCHEMICZNYCH W PROFILU GLEBY LESSOWEJ

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Streszczenie. Celem prezentowanej pracy jest określenie wpływu wybranych parametrów fizykochemicznych bezpośrednio warunkujących życie biologiczne gleb: potencjału wodnego (pF); dostępności tlenu dla mikroorganizmów (ODR), potencjału redoks (Eh), zawartości Mg, Ca oraz całkowitego poziomu węgla organicznego (TOC), na zawartość stężenia DNA. Próby gleby lessowej w formie nienaruszonej zostały pobrane do metalowych cylindrów (100 cm^3) z czterech głębokości (0-20; 20-40; 40-60 i 60-80 cm), co pozwoliło na opisanie zarówno zawartości DNA jak i jego rozmieszczenia w całym profilu. Na podstawie wykonanych badań wykazano istotny ($p < 0,05$) dodatni wpływ ODR ($r = 0,94^{***}$), Eh ($r = 0,52^{**}$) oraz TOC ($r = 0,98^{***}$) na zawartość glebowego DNA, co potwierdzają wysokie współczynniki korelacji (r). Natomiast statystycznie istotne ujemne współzależności stwierdzono w przypadku pF ($r = -0,57^*$) oraz zawartości Mg ($r = -0,79^{***}$). Jakkolwiek, w warunkach bieżącego eksperymentu nie znaleziono istotnej korelacji pomiędzy obecnością Ca oraz stężeniem DNA ($p > 0,05$). Wykazano ponadto sukcesywny, istotny spadek ($p < 0,05$) zawartości DNA o 62,8% wraz ze wzrostem głębokości w profilu glebowym, co niewątpliwie związane jest z przestrzennym rozmieszczeniem mikroorganizmów i ich preferencją do zasiedlania powierzchniowych warstw gleby.

Słowa kluczowe: zawartość DNA, potencjał wody glebowej, dostępność tlenu, potencjał redoks, dwuwartościowe kationy