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Effect of humic-rich peat extract on plant growth and microbial activity in contaminated soil

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***Abstract* — The aim of this work was to compare the effect of 1 % and 5 % humic substances (HS) on the growth of bean, wheat, rape and cress, as well as microbial activity (respiration, enzyme activity) in sandy loam soil spiked with a complex contamination, derived from municipal waste. The results of 23 days pot vegetation experiment demonstrated the stimulating effect of HS on the plant growth and soil microbial activity.**

***Keywords* — enzyme activity, humic substances, microbial respiration, plant growth, soil remediation.**

I. Introduction

Recently used soil bioremediation technologies *in situ* are known to be efficient and non-expensive. For example, nutrients, catalytic agents and other amendments added to contaminated soil can considerably stimulate the process of biodegradation by autochtonous or/and allochtonous microbial communities [1]–[2]. On the other hand, the role of higher plants in stimulating the soil remediation process is well documented [3]–[5]. Thus, combination of different methodical approaches could sufficiently improve the bioremediation technology outcome.

Humic substances (HS) are one of the most intensively studied soil amendments during previous few decades [6]–[9]. The breakdown products of plant and animal remains, extracted in an alkaline solution, are commonly referred to as HS. They can be extracted from a wide variety of sources, including subbituminous coals, lignites (brown coals), peat, soil, composts, and raw organic waste [10]. HS can be applied as synergists for improving efficiency of fertilizers and activity of microbial inoculants [10].

Possible mechanisms of mediating action of HS between living cell and contaminant were recently suggested by N.A.Perminova with colleagues [11]. The first scenario refers to deactivation of ecotoxicants by HS due to formation of non-toxic and non-bioavailable complexes. It takes place outside the cell and is defined as "exterior effects". The second scenario refers to deactivation of ecotoxicants due to adsorption of HS onto the cell wall or membrane and it is defined as "boundary effects": sorption takes place on the cell surface and implies changes in permeability and structure of the cell membrane. The third scenario refers to amelioration of contaminant toxicity due to activation of systemic resistance to chemical stress. This implies participation of HS in immune response activation and can be defined as "interior" effects [11].

HS are used in clean-up technologies as a natural surfactant for washing of highly polluted soils due to their additional capacity to promote microbial activity, in contrast to synthetic surfactants, for further natural attenuation in washed soils [8], [12] –[13]. HS were reported to increase microbial activity and remedial performance of *Phragmites communis* in wetlands with complex contamination containing hydrocarbons and heavy metals [14]. HS could act as an enhancing agent for phytodegradation of petroleum hydrocarbons in soil contaminated by diesel fuel and heavy metals [15]. Addition of humates to soil contaminated by hydrocarbons caused significantly faster increase of the total amount of phospholipid fatty acids suggesting improvement of the soil microenvironment [16].

Besides, HS are very effective in chelating many plant nutrients and more importantly, in retaining water. This enables humic acids to retain a wide range of nutrients, all in close proximity to plant roots to provide more balanced nutrients for growth [9]. The stimulatory effects of HS on plant growth are dependent on the source, concentration and molecular weight of humic fractions and mainly on different chemical compounds [17]. HS exhibit a hormone-like activity, in particular an auxin-like activity [17].

HS are derived during different humification processes, generating variable and complex molecules mainly composed of carbon, hydrogen, oxygen, nitrogen, sulphur and functional groups (COOH, OH, C = O). But the intensity of a response is dependent on various parameters such as origin, nature of the initial organic matter, transformation processes, concentration of HS, experimental conditions and plant species [18].

The aim of this work was to compare the effect of HS on the growth of bean (*Vicia faba*), wheat (*Triticum spp*.), rape (*Brassica napus*) and cress (*Lepidium sativum*), as well as microbial activity in sandy loam soil spiked with an complex contamination, derived from municipal waste.

II. Materials and Methods

Humic substances were isolated from industrially mined raised bog peat using extraction methods as suggested by Internation Humic Substances Society [19]. Concentration of carbon, hydrogen, nitrogen and sulphur in the isolated humic substances were determined by combustion-gas chromatography technique, using the Elemental Analyzer Model EA-1108 (Carlo Erba Instruments).

Pot experiments were performed under laboratory conditions during 23 days. Sandy loam soil used in this study was previously sampled in the site situated nearby the municipal waste storage place. 32 g (50 mL) of soil (dry weight) in each pot was amended with 0.5 mL or 2.5 mL extract of humic substances (HSE), corresponding to 1 vol % or 5 vol %, respectively. Chemical characteristics of soil and HSE are summarized in Table I. Contaminated soil was

prepared by spiking 20 mL of infiltrate to 32 g of soil. Preliminary testing of infiltrate showed its high phytotoxicity. Non-contaminated soil with 0 %, 1 % and 5 % HSE was designated as samples No. 1, 2, 3, while contaminated soil — as samples No. 4, 5 and 6, respectively. 15 seeds of bean (*Vicia faba*) and 30 seeds of wheat *Triticum spp*.), rape (*Brassica napus*) and cress (*Lepidum sativum*) per one pot were sown. Each variant was performed in duplicate. During the vegetation experiment soil moisture was maintained up to 60 % of water holding capacity. Pots were randomly placed under laboratory conditions with 12/12 light cycle.

Dry weight of soil and harvested plants was determined by drying of samples at +105 °C till constant weight. For microbiological testing and fermentative activity 1 g of soil was taken in duplicate. pH_{H2O} (1:2.5) and redox potential were measured by electrode Hanna pH213. The number of colony forming units (CFU) was determined using Tryptone Glucose Yeast Extract Agar (TGA) (Sifin, Germany). CFU were counted after plate incubation at 28 °C during 48h. Soil urease activity was determined by the colorimetric method by the N-NH_4^+ formation in the urea-amended soil sample (after 24h incubation at 37 °C) [20]. FDA hydrolysis activity was determined by hydrolysis of fluorescein diacetate [21]. Microbial respiration was determined according to L.M.Zibilske [22] with some modifications. The CO_2 released from soil (30 g) amended with 1 % glucose after 24h incubation at 25 °C was trapped in 5 mL of 0.05 mol L^{-1} NaOH and determined by titration with 0.05 mol L^{-1} HCl. Content of carbon and sulphur were measured using the C, S analyzer (ELTRA). Total ammonium amount was determined according to ISO 5983-2:2005. An extract of humic substances was kindly provided by JSC "LKT". Soil toxicity study was performed using germination test according to EPA 712-C-96-152 [23].

Statistical analysis

The statistical data analysis was performed using the Single factor ANOVA *Excel* software for the significance level $\alpha = 0.05$.

III. Results and Discussion

A. Elemental Composition of Studied Humic Substances [24–25]

Elemental composition of studied humic substances was detected as follows: C 52 %; H 5 %, N 1.6 %, S 0.5 % and ash 2 %. Content of oxygen, with range 32–42 %, was determined by mass balance. The elemental composition of HS from peat in Latvia is of similar magnitude to those for peat HS from other regions of the world. The peat HS were analyzed using van Krevelen graphs as frequently applied for studies of HS and the C biogeochemical cycle. The index of atomic ratios O/C, H/C and N/C is useful in identification of structural changes and the degree of maturity of HS obtained from different environments. The relation between H/C atomic ratio and O/C atomic ratio of HS of different decomposition degree — beginning with bog plants up to brown coal, lignite and coal — reveals changes in the associated elemental composition (Fig. 1). Fig. 1 can be considered as a graphical representation of the humification process, indicating the degree of maturity and intensity of degradation processes such as dehydrogenation (reduction of H/C ratio), decarboxylation (reduction of O/C ratio), demethylation occurring during the decay of peat-forming plants, and peat humus maturation continuing up to coal. These changes are especially evident if atomic ratios of peat-forming plants (Sector 3 in Fig. 1) are compared to the atomic ratio of organic matter of a high decomposition degree (low moor peat, coal Sectors 4, 5 in Fig. 1). From the point of view of chemistry, peat HS have an intermediate position (Sector 5 in Fig. 1) between the living organic matter and coal organic matter, and their structure is formed in a process in which more labile structures (carbohydrates, amino acids, etc.) are destroyed, but thermodynamically more stable aromatic and polyaromatic structures emerge. Comparatively, the studied peat HS are at the beginning of the transformation process of living organic matter.

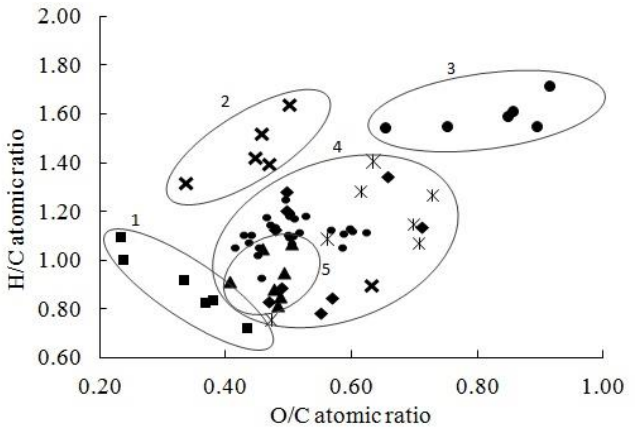

Fig. 1. Van Krevelen graph (H/C *vs.* O/C atomic ratio) of bog plants (⬢); HS isolated from peat samples from bogs in Latvia (•); reference peat HS and peat HS (★); soil HS (◆); HS from different coals and lignite (■), sedimentary HS ( ) and aquatic HS (▲).

TABLE I

Concentration of the total nitrogen, carbon, sulphur, and ash in soil and extract of humic substances (HSE)

Sample	Total N, %	C, %	S, ppm	Dry weight, %	Ash, %	pH	Redox, mV
HSE	0.040	1.44	205.9	2.24	4.43	7.97	–72.2
Soil	0.290	8.36	595.3	26.29	52.65	6.19	28.5

B. Characteristics of Plant Biomass After Vegetation Experiment

Growth of seedlings was characterized in dynamics during the 23 days vegetation experiment. Thus, a stimulating effect of HSE (especially in the set with 1 % HSE) in contaminated soil was observed for bean at 16th day of the experiment (Fig. 2). In turn, this effect for wheat was revealed at the earlier stages of growth, i.e., at the 5th day after sowing.

Fig. 3 illustrates the length and dry weight of wheat and bean seedlings after the 23 days vegetation experiment. Addition of 1 % HSE to contaminated sandy loam soil considerably stimulated the growth of wheat under tested conditions. Addition of infiltrate to soil notably inhibited the growth of bean, as compared to non-contaminated soil. While an amendment of contaminated soil with HSE likely improved growth conditions for bean in concentration-dependent manner.

Statistical analysis of data indicated that the plant response to the presence of HSE and infiltrate is species-specific. In particular, the length of above-ground parts significantly differed between non-contaminated and contaminated soils for all tested plant species, i.e., the p value for wheat, bean, rape and cress was found to be $p < 0.000001$, $p < 0.01$, $p < 0.0000001$ and $p < 0.025$, respectively. In non-contaminated soil, the length of shoot significantly differed for bean ($p < 0.001$) and cress ($p < 0.00001$) in dependence on HSE added. In turn, wheat and rape significantly responded to the presence of HSE in contaminated soil. Regarding the length of root, statistically significant differences were detected for bean and rape between non-contaminated and contaminated soils, as well as for rape in contaminated soil with different concentrations of HSE. Dry weight of seedlings significantly differed between types of treatment only in the variants with wheat.

Stimulating effect of humates at the initial phase of growth was reported by M. Šmídova [6]. Thus, 100 mg L^{-1} of sodium humate accelerated the uptake of water by swelling seeds of winter wheat (*Triticum vulgare* Vill.) during the initial phase of swelling. The fact that the seeds take up a sufficient amount of water sooner makes it possible for the activation of enzyme systems which ensure to take place of normal germination, thereby increasing intensity of respiration. The energy released during respiration is then utilized for more rapid growth of the embryo which is morphologically reflected in the rate of germination [6].

Comparison of fresh and dry biomass weight of wheat and bean, grown in the presence of humates, did not reveal any statistically significant changes. However, there was a tendency for bean biomass to increase in the variants with humates. Biomass of rape and cress in this experiment was not measured due to its small quantity. It has been observed that addition of humic substances to soil stimulates root growth and increase of fresh weight in some plants [7], [26]. In the experiments with tomato seedlings it was revealed that the auxin-like activity in humic matter is associated with complex hydrophobic structures whose simplification by hydrolysis may release auxin-like molecules [27].

Physical-chemical properties of soil play an important role in the process of plant growth. Besides, some plants can influence these characteristics during vegetation [28]. Redox potentials are highly variable and therefore are used as an indicator or relative status of the soil. Redox reactions change the speciation and solubility of many elements, create new compounds and alter the biochemistry of soils. In this study, the changes of pH and redox potential in soil after the vegetation experiment were more pronounced in the variants with contaminated soil, in particular for wheat and bean (Fig. 4). Addition of HSE to soil leads to some increase of the pH level due to alkaline properties of HSE (Table I).

Fig. 2. Vegetation experiment in pots after 16 days (A) and 23 days (B).

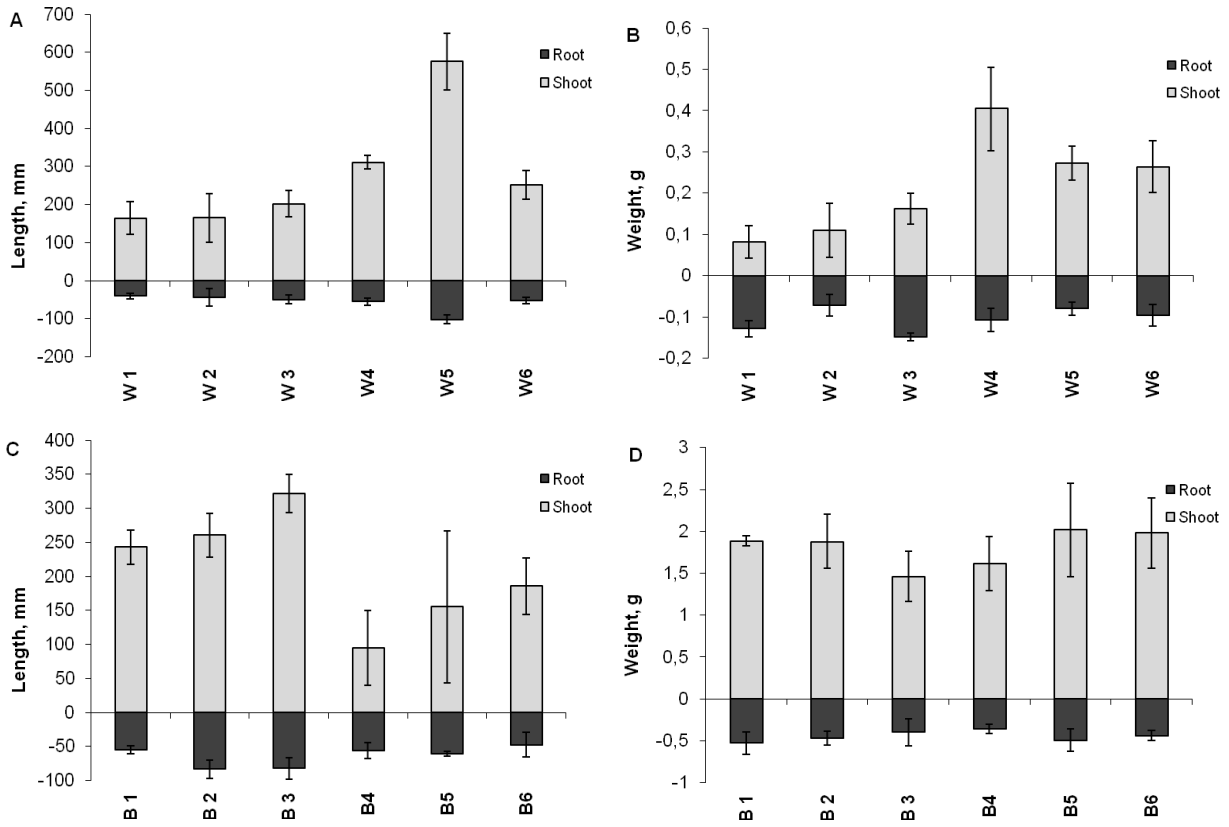

Fig. 3. Length (A, C) and dry weight (B, D) of shoots/roots of tested plants after 23 days vegetation experiment. A, B — wheat; C, D — bean. Numbers indicate as follows: 1, 2, 3 — non-contaminated soil; 4, 5, 6 — contaminated soil; 1, 4 — without HSE; 2, 5 — with 1 % HSE; 3, 6 — with 5 % HSE.

C. Changes of Soil pH and Redox Potential After Vegetation Experiment

Physical-chemical properties of soil play an important role in the process of plant growth. Besides, some plants can influence these characteristics during vegetation [28]. Redox potentials are highly variable and therefore are used as an indicator or relative status of the soil. Redox reactions change the speciation and solubility of many elements, create new compounds and alter the biochemistry of soils. In this study, the changes of pH and redox potential in soil after the vegetation experiment were more pronounced in the variants with contaminated soil, in particular for wheat and bean (Fig. 4). Addition of HSE to soil leads to some increase of the pH level due to alkaline properties of HSE (Table I).

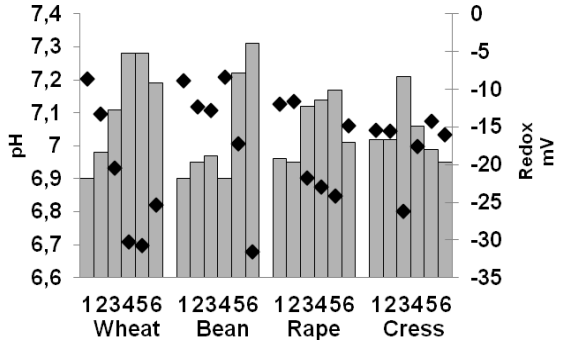

Fig. 4. The pH value and redox potential of soil subjected to different types of treatment with infiltrate and HSE. Numbers indicate as follows: 1, 2, 3 — non-contaminated soil; 4, 5, 6 — contaminated soil; 1, 4 — without HSE; 2, 5 — with1 % HSE; 3, 6 — with 5 % HSE.

D. Soil Microbiological and Biochemical Characteristics After Vegetation Experiment

The total count of aerobic heterotrophic cultivable microorganisms in tested soil was compared. Influence of HSE to the number of CFU in soil was not observed. However, the differences in CFU count were detected in the variants with wheat between sets with non-contaminated and contaminated soils. In particular, the number of CFU in infiltrate-spiked soils after wheat growth was four orders lower, as compared to those in non-contaminated soils. In variants with bean, rape and cress the count of CFU varied in the range of 10^{12}–10^{13} CFU gdw^{-1}. Before the experiment, the concentration of aerobic heterotrophic cultivable microorganisms in soil was 1.7×10^6 CFU gdw^{-1}.

Microbial respiration in soil as a measure of microbial activity is one of the most sufficient indicators of soil pollution. In the present study, addition of 1 % and 5 % HSE to soil resulted in an enhanced respiration of contaminated soil after 23 days vegetation of bean and rape (Fig. 5B). Substrate induced respiration did not reveal any significant differences between plant species tested in these experiments.

Soil biochemical characteristics can provide with additional information on the presence of viable microorganisms as well as on the intensity and on the kind and duration of the effects of pollutants on the metabolic activity of soil microorganisms [29]. The aim of these measurements was to clarify whether the tested plant species in the presence of HSE stimulate microbial activity in contaminated soil or not.

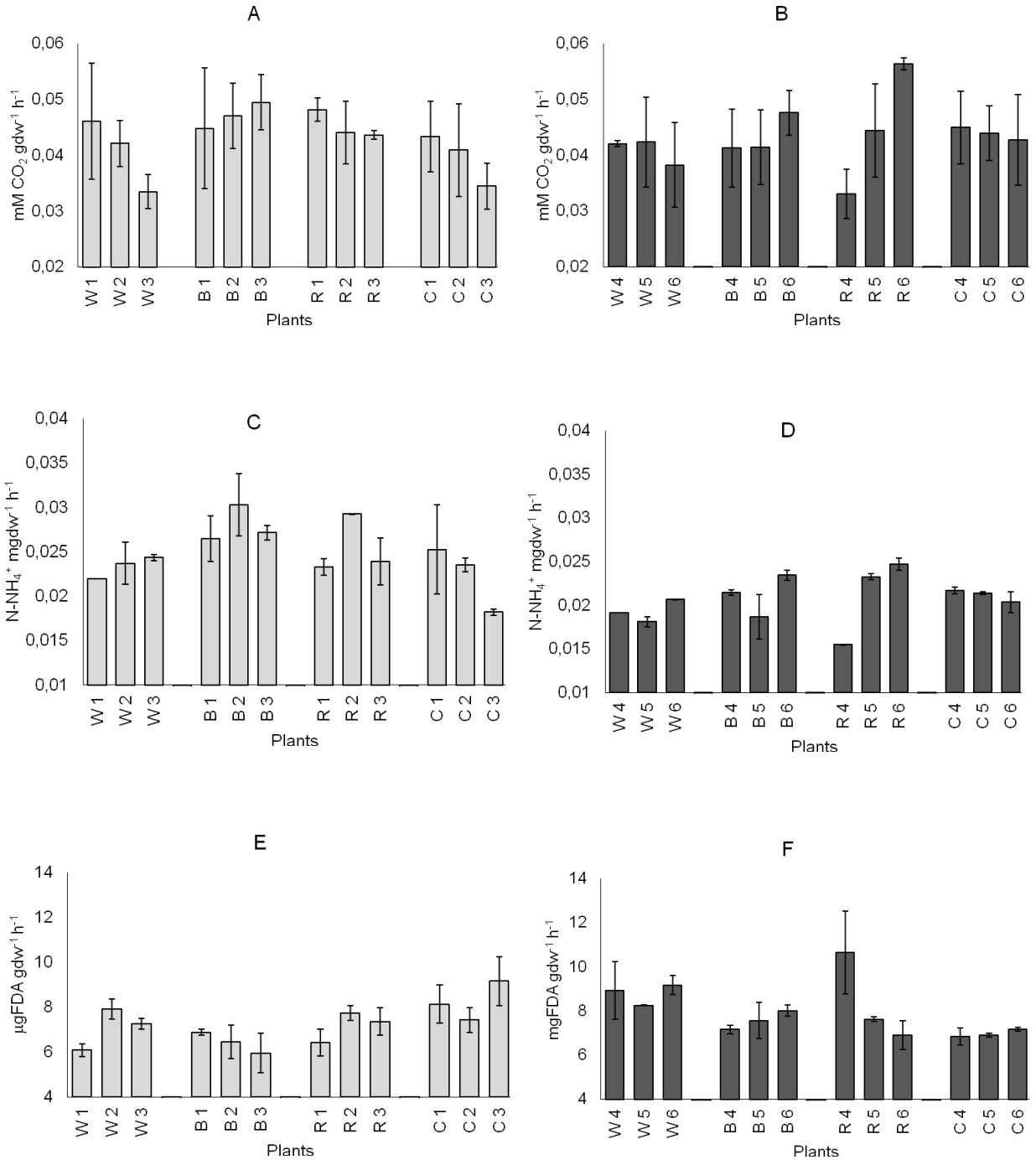

Fig. 5. Microbial substrate induced respiration (A, B), urease (C, D) and fluorescein diacetate hydrolysis (E, F) activity in soil subjected to different types of treatment with infiltrate and HSE. Numbers indicate as follows: 1, 2, 3 — non-contaminated soil; 4, 5, 6 — contaminated soil; 1, 4 — without HSE; 2, 5 — with 1 % HSE; 3, 6 — with 5 % HSE. W, B, R, C — wheat, bean, rape, cress, respectively.

Spectrophotometric determination of the hydrolysis of fluorescein diacetate (FDA) was assessed to be a simple, sensitive and rapid method for determination of microbial activity in soil and litter. It is probably more rewarding to use FDA as a substrate to determine heterotrophic activity than to use it the assessment of biomass [30].

Urease activity in soil is often correlated with the size and/or activity of the microbial community [31]. A large number of microorganisms including bacteria, actinomycetes and fungi can hydrolyze urea. The ratio between intracellular and extracellular urease in soil was reported to be 46:54. The ratio of ureolytic to non-ureolytic bacteria in the population remained unchanged, while addition of available carbon (e. g., glucose) and urea to soil resulted in the increase of both, urease activity and size of bacterial population [31]. It was earlier observed that urease activity in soil can be affected also by cropping systems [32].

The results of the experiments revealed a stimulating effect of HSE to microbial activity in the variants with wheat, bean and rape. An exception was cress salad, where urease and FDA hydrolysis activity was inhibited by HSE in both, contaminated and non-contaminated, soils (Fig. 5, C-F). Statistically significant differences of urease activity among the variants with different HSE concentration in contaminated soil were found for wheat (p = 0.005) and rape (p = 0.027).

IV. Conclusion

Our earlier results demonstrated interrelations among plants, soil microbiota, various amendments and contaminants [2], [33]–[34]. The results of this study indicated a high potential of humic substances in the development of remediation technologies. HSE represents a complex amendment with specific composition, which is dependent on its origin and extraction technology. Chemical testing showed that the studied peat HS are at the beginning of the transformation process of living organic matter.

Addition of infiltrate to soil negatively influenced some parameters tested in this experiment. In particular, the length of above-ground parts significantly differed between non-contaminated and contaminated soils for all tested plant species. Addition of HSE to non-contaminated soil resulted in significant differences for bean and cress plants. In turn, wheat and rape significantly responded to the presence of HSE in contaminated soil. Contaminated soil with HSE after the 23 days vegetation experiment with rape demonstrated a significant increase of microbial substrate induced respiration and urease activity in HSE concentration dependent manner. While FDA hydrolysis in these soil samples showed an opposite dependence.

Plant response to the presence of HS in soil was observed to be a species-specific. Besides, the plants compared in this study are different in terms of their root development and space finally occupied by one plant in soil. These specific properties of each plant species, as well as a treatment rate could noticeably influence the outcome of soil detoxification via stimulation of soil microbial community.

The mechanisms by which HS cause their positive effects on plant growth are not yet fully understood. This study is supposed to be continued in further experiments.

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Olga Muter, Baiba Līmane, Silvija Strikauska, Māris Kļaviņš. Ar humusvielām bagāta kūdras ekstrakta ietekme uz augu augšanu un mikroorganismu aktivitāti piesārņotā augsnē.
Humusvielu kūdras ekstrakts (HKE), kas satur augu un dzīvnieku valsts sadalīšanās produktus, raisa lielu zinātnisko interesi augsnes auglības un revitalizācijas jomā. Darbā izmantojamais HKE tika iegūts kūdras ekstrakcijas rezultātā saskaņā ar standarta metodi. Kūdras humīnskābes satur C 52 %; H 5 %, N 1.6 %, S 0.5 %, O — 32–43 %, pelnus 2 %. Iegūtie rezultāti pēc Van-Klevelena metodes liecināja, ka analizējamais materiāls ir organisko vielu sadalīšanās sākotnējā stadijā. Eksperiments tika veikts laboratorijas apstākļos 23 dienas kultivējot kviešus, pupas, rapsi un kressalātus. Eksperimentiem izmantota smilšmāla augsne, kas iegūta municipālo atkritumu glabātuves apkārtnē. Katrā izmēģinājuma traukā bija 32 g (50 ml) augsnes, 0,5 ml (1 tilp. %) vai 2.5 ml (5 tilp. %) HKE, kā arī 20 ml toksiska infiltrāta, kas iegūts atkritumu glabāšanas vietā. Visu testējamo augu virszemes daļas biomasas pieaugums bija lielāks variantos ar nepiesārņoto augsni. HEK pievienošana stimulēja pupiņu un kressalātu sakņu sistēmas attīstību nepiesārņotā augsnē, bet kviešiem un rapsim – piesārņotā augsnē. HEK stimulējošā ietekme uz augsnes mikroorganismiem tika novērota variantos ar kviešiem, pupiņām un rapsi. Statistiski ticami iegūti ureāzes aktivitātes palielināšanās rezultāti piesārņotā augsnē ar HEK piedevu kviešiem (p = 0.005) un rapsim (p = 0.027). Variantā ar kressalātiem HEK nomāc mikroorganismu ureāzes un fluoresceīna diacetāta aktivitāti. Variantos ar rapsi HEK pievienošana piesārņotai augsnei ievērojami stimulēja augsnes mikrofloras substrāta inducēto elpošanu. Tādejādi HEK ietekmi uz testējamo augu augšanu un augsnes mikroorganismu aktivitāti var novērtēt kā sugas-specifisku. Iegūtie rezultāti liecina, ka HEK efekts ir atkarīgs no augsnes piesārņojuma un humātu iedarbības izraisītā mehānisma komplicēto fizikāli-ķīmisko un bioloģisko procesu kopumā. Optimālai HEK izmantošanai biotehnoloģiskajos procesos augsnes kvalitātes uzlabošanā ir nepieciešami tālāki pētījumi.