

INVESTIGATION OF THE DIRECTIONS OF USING A HYBRID COMPOSITION BIOPRODUCT FOR DETOXIFICATION OF A SOIL ECOSYSTEM CONTAMINATED WITH HEAVY METALS AND OIL PRODUCTS

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ABSTRACT

Contamination of soils with heavy metals (HM) and petroleum products is an extremely important and urgent problem of humankind. Their danger determined by the ability to accumulate in the soil, to join the trophic chains and transmitted in the system soil - plant - man. The aim of the study was to investigate the effect of a hybrid biological product for the reclamation of contaminated lands. To achieve this goal, we conducted a section of the soil profile at the site of soil sampling for analysis of HM content near the phosphogypsum heap. The study of quantitative and qualitative changes in the fractional composition of the soil complex of gray forest soil was carried out with the introduction of increasing doses of organo-mineral complexes. The elemental composition determined by X-ray fluorescence analysis. The optical density of cell suspensions was measured using the method of photometric absorption of culture on a Multiscan analyzer. The obtained results of the study were analyzed using the VOSviewer software. The taxonomic classification also carried out using electronic databases KEGG database, made a comparative characterization of the impact of different organo-mineral compositions on the degree of reduction of mobile forms of HM in the soil and cluster visualization of the combined use of bioprocesses for soil detoxification. According to the results of research for biochemical purification of soils from HM contamination, a biopreparation with an external coating with a biodegradable polymer film, biomass of microorganisms with a lyophilized film and a mineral base of phosphogypsum was proposed.

KEYWORDS

bioremediation, heavy metals, petroleum hydrocarbons, soil, hybrid bioproduct

INTRODUCTION

Petroleum hydrocarbons (PH) and heavy metals (HM) can influence soil ecosystems sufficiently to result in significant losses in soil quality. ADENIYI & AFOLABI (2002) studied cadmium, chromium, copper, lead, and nickel as HM associated with soil pollution by oil products. Biological soil remediation is an environmentally friendly and safe approach to clean up soils contaminated with trace amounts of heavy metals such as Cu, Pb and Zn, as well as petroleum hydrocarbons. According to the study, the content of heavy metals in alfalfa plants was limited and followed the order: Zn > Cu > Pb. HM were concentrated in the roots of plants (AGNELLO et al., 2016a). For

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bioremediation of soils (ALI et al., 2002), contaminated with hydrocarbons and heavy metals, it was proposed to use transconjugate bacteria with resistance to As and Cd. It was proved that transconjugate bacteria are already part of the local soil microflora. The unicellular bacteria were associated with the following species in decreasing order of predominance: *Bacillus subtilis*, *Corynebacterium pseudotuberculosis*, *Brevibacterium linens*, *Alcaligenes faecalis*, *Enterobacter aerogenes*, and *Chromobacterium orangeum*. Filamentous forms have been associated with *Nocardia corallina*, *Streptomyces flavovirens*, *Micromonospora chalybeata*, and *Nocardia paraffinea*. These isolates can use hydrocarbons as their sole source of carbon and energy. Low concentrations of As and, to a lesser extent, Cd increase the potential for consumption of hydrocarbons by individual isolates.

Simultaneously with an increase in metabolic activity at the community level and microbial load, a gradual decrease in ecotoxicity was observed (ALISI et al., 2009). Moreover, some heavy metals have a positive effect on the decontamination of oil polluted soils in the case of biological treatment. Stimulation of microbial activity is observed at low concentrations of HM, nevertheless, it was reported an inhibitory effect of toxic metals, in particular nickel, on the biodegradation and mineralization of organic contaminants such as petroleum hydrocarbons (AL-SALEH & OBUKWE, 2009). The lowest biodegradation efficiency of the aliphatic (26%) as well as the aromatic (32%) fraction was noted for the system, which contained diesel oil and heavy metals (D + HM). The presence of polycyclic aromatic hydrocarbons (PAHs) in the D + HM + PAH system increased the removal efficiency of aromatic to a level similar to the D system (58%), whereas the biodegradation efficiency of the aliphatic fraction was lower (36%). Comparison of D and D + HM as well as D + PAH and D + PAH+HM indicates that the highest residual concentrations of hydrocarbons (which correspond to the lowest biodegradation efficiencies) were observed in systems which included heavy metals (CZARNY et al., 2020). The significant increase of *Acinetobacter* abundance in systems, which included heavy metals, was most likely associated with adaptation mechanisms, which allow this genus to proliferate in the presence of such contaminants. Several studies confirm the presence of this bacterial class in environmental niches contaminated with high concentrations of hydrocarbons and heavy metals (MARKOWICZ et al., 2016). A recent report regarding the natural attenuation of soils co-contaminated with aged heavy metals and hydrocarbons indicated that bacteria belonging to the *Acinetobacter* class were predominant (45%) in one of the investigated samples (KHUDUR et al., 2018).

AGARRY et al. (2014) proved the effect of nickel metal, poultry dung as biostimulant, and stubborn grass (*Sporobolus pyramidalis*) during phytoremediation on gasoline biodegradation and growth rate of intrinsic soil microbial species. This study showed reduction of total petroleum hydrocarbon (TPH) concentration at the level 52.7%, 57.6%, 60.5%, and 62.4% for the samples with initial nickel content at 0, 2.5, 7.5, and 12.5 mg/kg, respectively at the end of the remediation time (42 days). Based on this observation the importance of indigenous microflora in nickel uptake from heavy metal contaminated soil. Moreover, the best result of TPH degradation at 95.8% was indicated for combined biostimulation and phytoremediation treatment.

The highest degree of TPH removal (68%) from co-contaminated soil with HM as Cu, Pb and Zn was proved for bioaugmentation assisted phytoremediation treatment, followed by bioaugmentation with *Pseudomonas aeruginosa* (59%), phytoremediation with alfalfa (*Medicago sativa L.*) (47%), and natural attenuation (37%). In this case the contribution between plant and bacteria seemed to be additive rather than synergistic (AGNELLO et al., 2016a,b). This effect was additionally enhanced by the joint application of citric acid and Tween® 80.

ATAGANA (2011) proved the capability of *Chromolaena odorata* (L) to grow in the presence of different concentrations (between 100 mg · kg⁻¹ and 2,000 mg · kg⁻¹) of heavy metals (Zn, Cd and Ni) in soil contaminated 50,000 mg · kg⁻¹ oil crude. The growth of the plant was sustained for 180 days during which period the plant was able to cause the removal of both the contaminant oil by 82% and the present heavy metals by up to 65%. Benefits of biological techniques during soil decontamination treatment. There are naturally in soil indigenous bacteria such as *Bacillus subtilis*, *Corynebacterium pseudotuberculosis*, *Enterobacter aerogenes*, *Nocardia corallina*, and *Streptomyces flavovirens* with the combined potential for As and Cd resistance and hydrocarbon utilization (ALI et al., 2012).

HAMDI et al. (2007) observed that the bioremediation efficacy was more likely to rely on the selectivity and specialisation of added microorganisms rather than on nutrient load. ALISI et al. (2009) used ten bacterial strains in the consortium ENEA-LAM for bioaugmentation as follows *Pseudomonas jessenii*, *Pseudomonas resinovorans*, *Arthrobacter* sp., *Rhodococcus erythropolis*, *Arthrobacter* sp., *Arthrobacter* sp., *Exiguobacterium* sp., *Delftia tsuruhatensis*, *Bacillus cereus*, *Pseudomonas fluorescens*. These strains were selected from the native microbial community for their multiple resistances to heavy metals Diesel oil biodegradation in this case was effective.

Four types of biostimulants such as rice husk (RH), chicken manure (CM), rice husk and chicken manure in ratio 3:1 (RC_3:1), chicken manure and rice husk in ratio 3:1 (CR_3:1) showed different potential in heavy metals reduction during soil decontamination. In particular, high efficiency in Co removing had RC_3:1 (75.8%) and RH (69%); Zn reduction – CR_3:1 (89.6%) and CM (89%); Cd reduction – CM (65.5%) and RH (63.6%). Nevertheless, RH and CR_3:1 treatments indicated best results in petroleum hydrocarbons removal in comparison with other samples (ADAMS et al., 2017)

The biosorption of heavy metals by biofilms can be optimized using a supporting medium for microorganisms. A study by SOHAIB et al. (2020) evaluated the suitability and effectiveness of compost, biogas slurry, corn cob and zeolite as carriers for maintaining the survival of rhizobacteria and improving the metabolic activity of multi-strain bacterial consortia. The survival rate of gusA-labeled *Pseudomonas putida*, *Serratia ficaria* and *Pseudomonas fluorescens* strains was assessed for 90 days. The results showed that vehicle-based inoculation further enhanced the efficiency of the multi-strain consortium (SOHAIB et al., 2020).

Immobilization of microorganisms on fixed beds is a common procedure in various purification systems, often using granular activated carbon as a support matrix for immobilization (CARPIO et al., 2014).

The purpose of this study is to estimate the effect of a hybrid biological product for the reclamation of contaminated land, which relates to the biochemical direction of stimulating the natural protective properties of the soil complex from the action of oil and heavy metals.

MATERIALS AND METHODS

Research methods

Conditions for conducting a microfield experiment. The study of quantitative and qualitative changes in the fractional composition of the soil complex of gray forest soil was carried out with the introduction of increasing doses of organo-mineral complexes. This preserves the whole set of natural soil and environmental factors. The experiment was performed in plexiglass blocks with a perforated bottom with an area of 0.20 m² (0.4 m × 0.5 m). The blocks were filled with gray forest soil from the territory with a high level of man-caused load, containing lead at the level of 17.6–21.2 mg / kg and cadmium - 0.55–1.00 mg / kg (gross form).

Perennial grasses were grown in the blocks, using increasing doses of biocomposite - a product of anaerobic conversion of sewage sludge (SS) and phosphogypsum. Every season, the natural vegetation that grew in blocks was mowed and removed from the soil surface. The soil in the blocks was dug to a depth of 0–20 cm, partially removed from the blocks, mixed and backfilled into the blocks at random. At the same time, perennial grasses were used, which are used during crop rotations (clover, a mixture with cereals). The room temperature was maintained at 295–298 K.

The experiment was carried out according to the scheme of application of a hybrid biological product at the rate of: 1) 25 t / ha; 2) 50 t / ha; 3) 75 t / ha. It was mixed with a 0–20 cm layer of soil before sowing the crop. The experiment was repeated three times. Soil samples were taken after harvesting from a layer of 0–20 cm. Soil samples from each replication were mixed and dried in the open air to remove existing plant and mesofauna residues during sieving through a sieve with a hole diameter of 3 mm. Air-dry samples were used to determine the HM fractions.

The study of changes in the fractional composition of the soil complex was conducted to compare the effect of hybrid biological product as a product of anaerobic processing of sludge and phosphogypsum with the effect of other organo-mineral compositions on the degree of HM mobility in soil. The following organo-mineral compositions were used for experimental research:

- organo-mineral compost based on a mixture of phosphogypsum (10 wt.%), superphosphate (1 wt.%) and cattle humus;
- a combination of a mixture of sodium humate with superphosphate (1: 1).

According to the results of experimental experiments, the percentage of mobile forms of HM released by the extractant (ammonium acetate buffer solution - AAB) from their gross content before and after tillage with organo-mineral compositions, the degree of reduction of HM mobility in the soil with the introduction of various formula:

$$D_{\text{HM}} = \frac{b_0 - b_1}{b_0} \cdot 100\%$$

where D_{HM} is the degree of reduction of mobility of heavy metals in the soil, %; b_0 is the initial percentage of mobile forms of HM, %; b_1 is the percentage of mobile forms of HM after processing, %.

Microscopic analysis was performed using images of the surface of the object with high spatial resolution and depth of field in reflected electrons (BSE) using scanning electron microscopy SEM-EDX (using energy-dispersing analyzer) in combination with microanalysis to create maps of the mineral composition of soil samples and biocomposite based on SS and phosphogypsum.

The effect of different doses of biocomposite on soil biome is assessed by changes in the kinetic parameters of the development of rhizosphere microorganisms, in particular by the accumulation of microbial biomass.

The method of determining microbial carbon in the soil is based on the respiratory response of microorganisms in accordance with the method by which microbial biomass is calculated as follows (KAISER et al., 1992):

$$C_{\text{microb}}=30.0 \cdot 10^{-3} \text{ cm}^3\text{CO}_2\text{-C/g}^{-1}\cdot\text{h}^{-1}.$$

Determination of organic carbon is carried out by the method of Turin, which is based on the decomposition of organic matter by potassium dichromate in an acidic environment in accordance with existing methods (DSTU 7855: 2015 Soil quality. Determination of the group composition of humus by the method of Tyurin in the modification of Konova and Belchikova. Kyiv, 2016. 13 p.).

The initial incubation period is characterized by increased CO_2 emissions due to the redistribution of nutrients in the soil microzones during mixing, separated by a determination period (up to 5 hours). The average values are calculated over a 25-hour interval with a relatively constant rate of CO_2 release.

Measurement of optical density (OD) of cell suspensions was performed by photometric absorption of culture on a Multiscan analyzer. The optical density, measured at 600 nm, reflects the concentration of cells in the nutrient medium. In this case, the bacteria are at the logarithmic stage of growth.

The special software for identification of necessary ecological-trophic groups of microorganisms and realization of schemes of trophic interactions in associations of various ecological-trophic groups of microorganisms is used in the work. Taxonomic classification was performed using electronic databases KEGG database (Kyoto Encyclopedia of Genes and Genomes), EzBioCloud Database and BacDive (The Bacterial Diversity Metadatabase).

Statistical processing of the obtained data was performed in the packages of computer programs MS Office – Microsoft Excel. The difference in averages was considered significant at a significance level of $p < 0.05$.

RESULTS

Soil profile study

Soils are characterized by an acidic pH value of an average of 5.8 up to 6.5, the absence or camouflage of transitional horizons along the soil profile, weakly expressed eluvial and well-defined illuvial horizon of brown-brown color. Behind the sanitary

protection zone of the dump there are agricultural lands, where the upper horizons of soils are humus-accumulative and part of the eluvium is plowed. Although gray forest soils differ from light gray ones in the best agronomic indicators, they are combined by an acid reaction in the upper horizons, unsaturation of bases, low nutrient content. This group of soils is believed to have some unfavorable physical properties due to poor structure.

The figure shows a photograph of a section of the soil profile at the site of soil sampling for analysis of the content of HM near the phosphogypsum dump. The soil profile was formed in places with minimal parameters of GTKV-IX = 1.18–1.20, which is due to climatic and relief factors due to the additional accumulation of moisture in the wastewater of GTKV-IX decreases to 1.76.

In the profile of gray forest soils near the phosphogypsum heap the following horizons are distinguished (Figure 1): H_0 - turf of dark brown color (up to 2 cm thick); H - humus-accumulative with dark gray turf with siliceous powder (7–11 cm); E_1 - eluvial grayish-whitish color, powdery-lumpy structural composition with carbonate inclusions, contains many plant roots (18–20 cm); E_2 - transitional eluvial-illuvial plate-nut with carbonate inclusions, compacted; gradual transition (6–10 cm); And - illuvial brown lumpy-prismatic (32–35 cm) and P - soil-forming rock from a depth of 60–80 cm.

An important environmental factor is the presence in the soil of easily soluble in water, and therefore mobile fulvic acids, which cause the processes of intensive leaching from the soil profile of many trace elements - Fe, Mn, Zn, Cd, Pb, Sr, V, including HM. This process is also characteristic of gray forest soils.

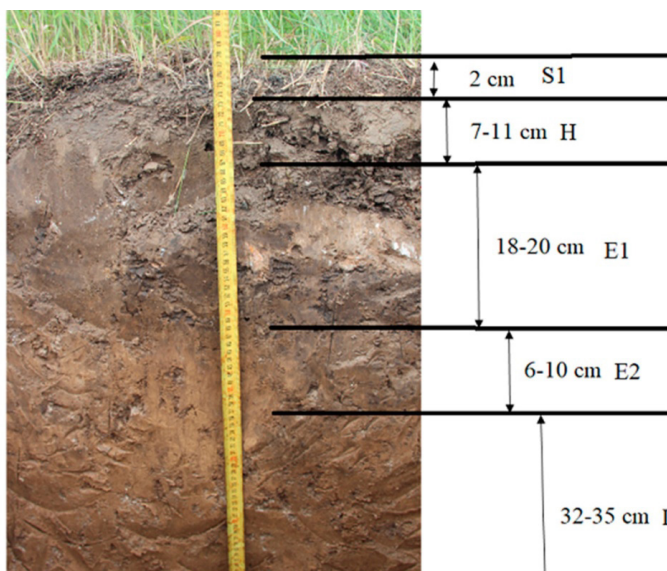


Figure 1. Profile of gray forest soil near the phosphogypsum dump.

Soil samples for chemical analysis were dried to an air-dry state according to GOST 5180-84. Air-dry samples were stored in glass containers. Determination of the elemental composition was carried out using X-ray fluorescence analysis (Figure 2)

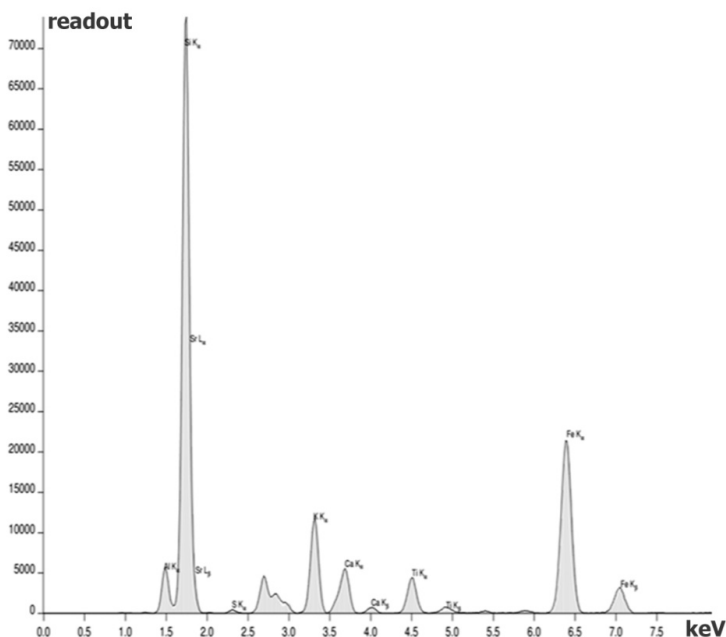


Figure 2. Spectrogram of a mixed soil sample of a plot of agricultural land (private village - the area closest to the phosphogypsum dump massif).

Substantiation of biochemical transformation of complex hydrocarbon compounds and heavy metals: experimental modeling

The biological product is offered with an external coating of biodegradable polymer film, biomass of microorganisms in lyophilized form and a mineral substrate based on phosphogypsum.

While a nutrient recycling strategy is expected to be a tool for combating particularly diffuse sources, it is also important to look for opportunities in existing point sources, and especially where results can be achieved quickly and cost-effectively. Phosphogypsum contains useful elements (Ca, S, P, Mg, K, Na, microelements), and the biopolymer film contains organic carbon compounds for the growth of the necessary ecological and trophic groups of microorganisms-destroyers of oil. The general scheme of the hybrid biological product is presented in Figure 3.

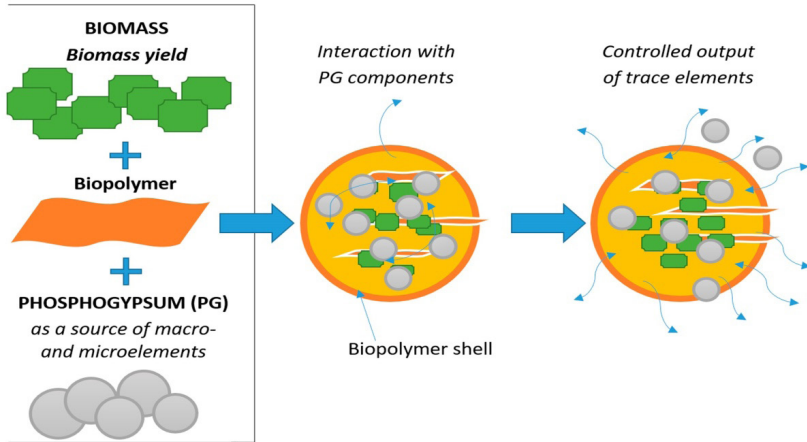


Figure 3. Scheme of the composition of the hybrid biological product for soil decontamination.

A method of obtaining a granular hybrid biological product containing immobilized microorganisms-destroyers of complex petroleum hydrocarbons (genera *Rhodococcus*, *Acinetobacter*, *Pseudomonas*), which includes immobilization of microorganisms in the form of a suspension in 6-8% solution of sodium alginate to which is added by mass fraction 4-7 levels of 10-11 mass fractions and biopolymer based on plant waste with a mass fraction of 7-10; granulation is carried out in a rotating plate granulator with mixing of immobilized microorganisms with a mineral additive of phosphogypsum and biopolymer, at a plate rotation speed of 75-80 rpm. and granulation times - 15-30 minutes, and get granules, which are additionally covered with a biopolymer film. The granules are obtained with a diameter of 5-6.95 mm (Figure 4).

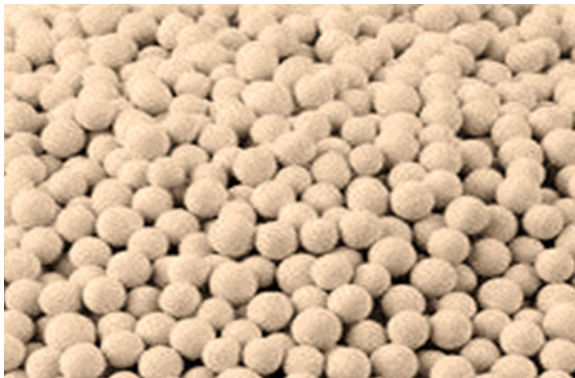


Figure 4. General view of the hybrid biological product.

The concentration of the suspension of microorganisms is provided by at least 10^{12} CFU / g of granules.

The structural features of such a hybrid biological product in granular form show the properties of controlled release of trace elements and reduce the period of adaptation to the consortium of destructive microorganisms when exposed to an oil spill combined with heavy metal pollution. In the future, after the disposal of complex carbon compounds, there is an accumulation of biomass of destructive microorganisms and sorption of HM with their fixation in insoluble form, which is confirmed by experimental data (Table 1).

Table 1. Experimental data.

Sampling area	The dose of hybrid biological product, kg / m ²	The maximum specific growth rate of microorganisms $\mu\text{m}, \text{h}^{-1}$	Microbial biomass, $\mu\text{gS} / \text{g}$
Soil rhizosphere	2.5	0.254 ± 0.002 0.234 ± 0.002 0.246 ± 0.005	192 \pm 3 188 \pm 5 183 \pm 2
Soil rhizosphere	5.0	0.311 ± 0.002 0.303 ± 0.004 0.317 ± 0.003	253 \pm 3 274 \pm 2 269 \pm 4
Soil rhizosphere	7.5	0.312 ± 0.002 0.310 ± 0.004 0.314 ± 0.002	263 \pm 5 255 \pm 3 277 \pm 3

A comparative characterization of the influence of different organo-mineral compositions on the degree of reduction of mobile forms of HM in the soil is also carried out. As can be seen from Figures 5, 6 when making a biogenic composite, a higher degree of decrease in HM mobility (not less than 70%) was observed in comparison with other organo-mineral complexes.

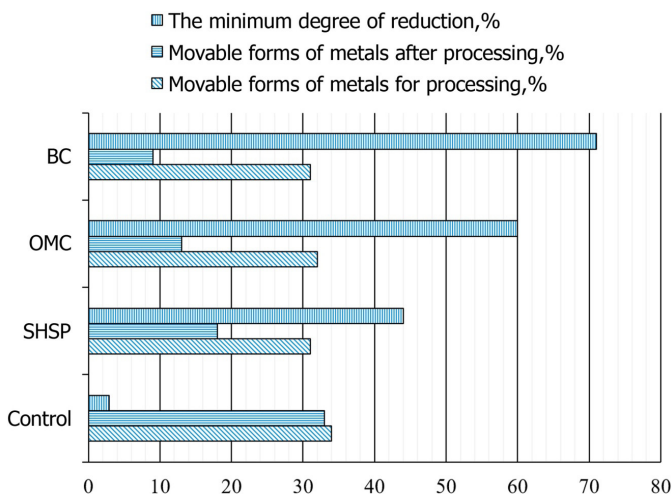


Figure 5. Change in the proportion of mobile forms of heavy metals in the soil with the introduction of various organo-mineral compositions (BC – biologic proposed hybrid composition, OMC – organo-mineral compost based on a mixture of phosphogypsum (10% wt.), Superphosphate (1% wt.) and cattle humus, SHSP – a combination of a mixture of sodium humate and superphosphate).

DISCUSSION

For a more in-depth analysis of the results of the study, a cluster visualization of innovative technological solutions in the field of combined use of bioprocesses for detoxification of soils contaminated with heavy metals and petroleum products was performed.

According to the Scopus database, clusters of innovative research in the field of bioremediation of soils contaminated with heavy metals and petroleum products were formed using VOSviewer software. Three clusters were identified (Figure 6):

- red cluster - biotechnological solutions for the binding of complex hydrocarbons and heavy metals in the matrix structure of various natures with the use of biological agents;
- blue cluster - processes of biodegradation of pollutants, in particular heavy metals, in the restoration of soil ecosystems;
- green cluster - technological solutions of phytoremediation using various combinations of plant species and symbiotic ecological-trophic groups of microorganisms.

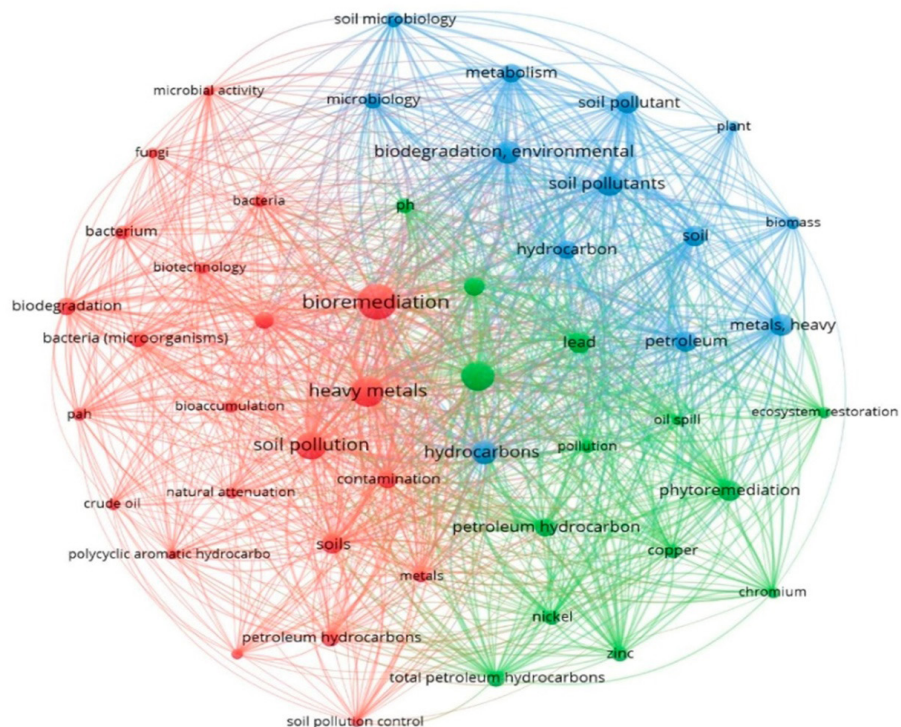


Figure 6. Visual simulation clusters (all clusters are united by common research thematically and regionally).

Fast growth and high above ground biomass yield are among the requisites needed for phytoextraction purposes. In addition, the establishment of a rich root system could create a favourable niche for rhizosphere microorganisms, which is required for rhizodegradation. The results of this study seem to be consistent with those obtained by KIRK et al. (2005), who reported no alfalfa phytotoxicity up to 15.000 mg kg⁻¹ dry weight (DW) of TPH, while they observed that growth of seedlings was stressed and stunted at higher TPH levels (31.000 mg kg⁻¹ DW).

Bioaugmentation with *P. aeruginosa* had a positive effect on plant biomass production. There was an initial trend to improve plant biomass, which became statistically significant for shoots and roots after 90 days. Soil inoculation with *P. aeruginosa* enhanced shoot biomass by 15, 33 and 56% at 30, 60 and 90 days, respectively. Similarly, root biomass was also increased by 13, 19 and 105% at 30, 60 and 90 days, respectively. These results are in accordance with previous studies, in which plant growth promoting ability of *P. aeruginosa* was assessed for other plant species.

Antagonistic effects between metals in multi-metal contaminated soils (FLOGEAC et al., 2007) in addition to the simultaneous presence of organic pollutants and soil ageing could have also contributed (LIN et al., 2008). The extent of metal accumulation in

alfalfa shoots was not influenced by the bioaugmentation treatment. In contrast, soil bioaugmentation had a statistically significant effect on root metal concentrations: Zn content was decreased at 90 days, while Cu content was increased at 60 days. The decrease of metal accumulation when plants were growing in bioaugmented soil could be the consequence of a 'dilution effect' of the increase of plant biomass facilitated by bacteria. In addition, metal immobilization onto bacteria due to biosorption processes could have also contributed to reduce metal availability, as *P. aeruginosa* has been reported to biosorb metals like Cu, Pb and Zn (GABR et al., 2008; PÉREZ SILVA et al., 2009). The distinct impact of bioaugmentation on Cu accumulation by alfalfa may be explained by specific coordination properties (i.e. stability constant of chelating molecule– metal complex) of siderophores (e.g. pyoverdine) produced by *P. aeruginosa* towards particular metals like Cu (CORNU et al., 2014).

Indigenous microorganisms of the present soil appeared to be adapted and functional for petroleum hydrocarbons degradation, as demonstrated by the 37% TPH removal in natural attenuation treatment. Although biodegradation is generally considered as the main pathway for pollutant removal in natural attenuation (DECLERCQ et al., 2012) other mechanisms (e.g. volatilization) could have also been involved. Moreover, recent studies support the idea of an active role of alfalfa plants in the rhizospheric degradation of hydrocarbons as the result of the action of plant enzymes released in root exudates (MURATOVA et al., 2014). Finally, an abiotic contribution could be attributed to root exudates, which have been demonstrated to enhance soil desorption of pollutants, improving bioavailability and subsequent biodegradation potential as a result (LEFEVRE et al., 2013). Bioaugmentation with *P. aeruginosa* resulted in even greater remediation efficiency (59%). The present results seem to be consistent with a previous comparative study which demonstrated that bioaugmentation was more effective than natural attenuation on the degradation of light (C12–C23) and heavy (C23–C40) fractions of TPH in soil samples (Bento et al., 2005). It can be hypothesized that the observed increase in TPH removal when soil inoculation was performed are due to *P. aeruginosa* hydrocarbon-degrading ability (JI et al., 2013). The association between alfalfa and *P. aeruginosa* appeared to be particularly effective in terms of TPH removal as a result of the processes mediated by both bacteria and plants. As the bioaugmentation assisted phytoremediation treatment resulted in a 31% increase of TPH removal, relative to natural attenuation, and a 10% and 22% increase in TPH removal was obtained for phytoremediation and bioaugmentation, respectively, in the individual treatments. Although the contribution to TPH removal of bacteria (bioaugmentation treatment) was greater than that of plants (phytoremediation treatment), the advantageous effect of plants is not only limited to the enhancement of pollutant dissipation in the rhizosphere.

Chemically assisted phytoremediation. The higher enzyme activity in the rhizosphere can be explained by different mechanisms. Firstly, the stimulation of microbial activity mediated by rhizodeposition of organic carbon by plants, which creates an environment rich in organic substrates for microorganisms. Secondly, a direct contribution by plants releasing enzymes by roots or by lysis of root cells (NANNIPIERI et al. 2012). Regarding the amendments, soil lipase activity constantly increased over

time in the presence of citric acid alone and in combination with Tween® 80. The positive effect of citric acid on lipase activity may be explained by the mobilization of metal ions in the presence of the organic acid. In the review by SHARMA et al. (2001), the positive effects of metal ions (e.g., Ca, Co, Cu, Fe, Mg) on lipase production by microorganisms were reported, but also inhibition of lipase activity was described by metals (e.g., Ag, Fe, Hg, Zn), possibly as a result of enzyme conformation alteration. Thus, the obtained results of using a hybrid combination biological product are consistent with the results of previous studies and reveal new prospects for the use of secondary resources as mineral feed and sorbent material for the necessary ecological and trophic groups of microorganisms in biodegradation of complex petroleum hydrocarbons and metabolic fixation of heavy metals.

CONCLUSIONS

Effective ways to restore landscapes degraded was determined in the process of the inflow of components of drilling waste, primarily crude oil and heavy metals into the soil. Effective reclamation is possible through the development and implementation of a method of biological remediation of soils as part of a comprehensive technology for their purification.

The practical implementation of the developed approaches will ensure the restoration of impaired functions of natural complexes in oil fields, which are negatively affected at all stages of the technological process of oil production, from drilling exploratory wells to decommissioning of existing wells.

Drilling of wells often takes place on the territory of agricultural lands, which are temporarily withdrawn from their intended use. As a result of oil production, halos are formed with varying degrees of chemical pollution and degradation, which is associated with the loss of fertile properties of the soil.

The biological product is offered with an external coating of biodegradable polymer film, biomass of microorganisms in lyophilized form and a mineral substrate based on phosphogypsum. A comparative characterization of the influence of different organo-mineral compositions on the degree of reduction of mobile forms of HM in the soil is also carried out. The higher degree of decrease in HM mobility (not less than 70%) was observed under BC used in comparison with other organo-mineral complexes.

Further directions of research can be distinguished as follows: to study the patterns of oil geofiltration, which determine the specific effect of toxic effects on the local biota; provide an assessment of the risk of destabilization of ecosystems; to develop and implement technology of biological purification of soils from oil and heavy metals.

The relevance of the planned research is emphasized by the fact that as a result of their implementation it will be possible not only to restore the disturbed functions of natural geosystems, but also to prevent the withdrawal of large areas of land from agricultural use.

ACKNOWLEDGEMENT

Research Project was carried out as planned research projects of the Department of Ecology and Environmental Protection Technologies, Sumy State University, connected

with subjects “Reduction of technogenic loading on the environment of enterprises of chemical, machine-building industry and heat and power engineering” according to the scientific and technical program of the Ministry of Education and Science of Ukraine (state registration No. 0116U006606).

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