JOURNAL OF ENGINEERING SCIENCES

Volume 8, Issue 2 (2021)

Chernysh Y., Plyatsuk L., Roubik H., Yakhnenko O., Skvortsova P., Bataltsev Y. (2021). Application of technological solutions for bioremediation of soils contaminated with heavy metals. Journal of Engineering Sciences, Vol. 8(2), pp. H8-H16, doi: 10.21272/jes.2021.8(2).h2

Application of Technological Solutions for Bioremediation of Soils Contaminated with Heavy Metals

Chernysh Y.[1,2*], Plyatsuk L.[1,2], Roubik H.[2,3], Yakhnenko O.[1], Skvortsova P.[1], Bataltsev Y.[1]

[1] Sumy State University, 2, Rymskogo-Korsakova St., 40007 Sumy, Ukraine;
[2] International Innovation and Applied Center "Aquatic Artery", 2, Rymskogo-Korsakova St., Sumy, 40007, Ukraine;
[3] Czech University of Life Sciences Prague, Faculty of Tropical AgriSciences, 129, Kamycka St., 165 00 Prague, Czech Republic

Article info:
Submitted: September 2, 2021
Accepted for publication: November 24, 2021
Available online: November 29, 2021

***Corresponding email:**
e.chernish@ssu.edu.ua

Abstract. This article focuses on studying biotechnologies for remediation of soils contaminated with heavy metals to determine further the most effective methods for cleaning soils from the action of toxicants with their subsequent implementation in practice. The soil restoration methods were analyzed, their advantages and disadvantages were identified, making it possible to establish that biological methods are the safest and most environmentally friendly. The expediency of using biological methods lies in the possibility of breeding strains of microorganisms that destroy soil toxicants. However, the efficiency of microbial cultures is not equally high due to a narrow range of favorable conditions for functioning, the risk of manifestation of the phenomenon of degeneration of microorganisms until the required level of soil purification is achieved. This confirms the prospects for the further development of this direction and the search for ways to eliminate certain disadvantages of biological methods. For an integrated biotechnological solution to soil remediation, a scheme of aerobic plants was developed, which is characterized by two stages: aerobic soil cultivation with biocomposite and a phytoremediation stage for additional purification and control of the content of toxicants in the soil.

Keywords: soil contamination, biotechnologies, remediation, heavy metals, biocomposite

1 Introduction

One of the most dangerous to human and animal health pollutants is heavy metals (HM). They do not decompose in the environment and accumulate in the tissues of living organisms [1]. Developing effective ways to preserve and protect the environment requires the definition and constant monitoring of the distribution of toxicants in the ecosystem, particularly in the edaphotope, according to their distribution by soil profile. At the same time, the need for comprehensive application of measures for their immobilization into immobile forms is of paramount importance. An ecologically safe method of soil decontamination is traditionally considered to be phytoremediation, due to which the process of removing toxicants occurs without destroying the soil structure and reducing its fertility [2, 3]. The latest biotechnological methods of detoxification and restoration of soil complexes of natural and anthropogenic landscapes are being developed.

Scientific and methodological issues of soil remediation and other components of the geological environment from toxic pollutants are being developed within a new scientific direction – geopurgology.

Among the measures to reduce the impact of pollutants on the soil should be identified primary: chemical, technological, mechanical, and biotechnological. The formed block diagram for these areas is shown in Figure 1.

Phytoremediation is considered the most promising and ecologically safe method of decontamination of contaminated soils. The purification mechanism is based mainly on the use of hyperaccumulator plants, which can remove toxicants from the environment in high concentrations and show tolerance to their action.

The main advantage of phytotechnologies is that removing pollutants occurs without destroying the soil structure and reducing its fertility.

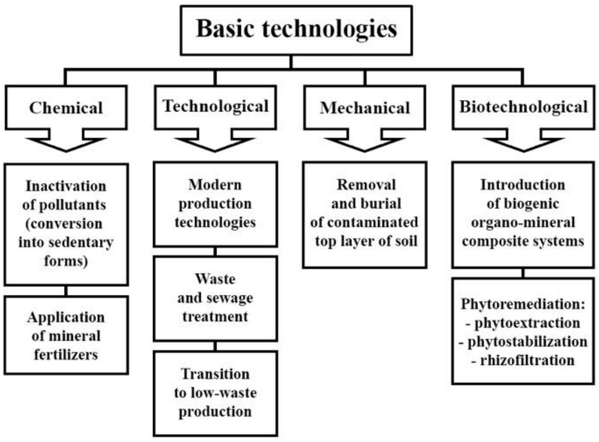

Figure 1 – Remediation technologies for contaminated soil

To remediate the soil from heavy metals used [4-6]: phytoextraction (absorption and accumulation of pollutants in the plant), phytostabilization (reduction of pollutant mobility and/or accumulation in the root system of the accumulating plant or in the rhizome) or rhizofiltration (metals are absorbed and bound in the root systems of phytore).

The work aims to study the influence of biotechnological tillage on the localization of HM in the soil complex.

To achieve this aim, the following tasks were solved:

- to study the processes of heavy metals in the soil complex concerning other components of the ecosystem;
- to determine the directions of soil bioremediation in the process of cleaning them from HM;
- development of a technological scheme for implementing complex biotechnology for remediation of soils contaminated with heavy metals.

2 Methodological approach to the processes of heavy metals fixation in soils using biotechnological techniques

Soil microorganisms can influence the bioavailability and absorption of heavy metals and can also promote growth and reduce the toxic effect of pollutants on plants. Approximately 80 % of aboveground plants have symbiotic associations of microorganisms. The ability to exist in an environment contaminated with heavy metals is characteristic of many species inhabiting the rhizosphere. The reason for the stability of the soil microbiota can be both innate and acquired mechanisms of adaptation.

Microorganisms (internal bioremediation) can be introduced into the contaminated soil or isolated from a specific environment and then into the contaminated one (bioaugmentation).

Bioaugmentation – the introduction (in the form of biological products) of specialized microorganisms, alien to the habitat, which has been previously isolated from natural sources or specially genetically modified and selected. There is the destruction of pollutants by different types of microorganisms due to the activation of aboriginal microflora or the introduction of certain cultures of microorganisms into the soil, the use of complex biological products and other methods to create an optimal environment for the development of certain groups microorganisms decompose pollutants. The soil becomes suitable for growing plants. Introduced microorganisms decompose the bulk of contaminants, reduce their negative impact on biota and thus stimulate self-cleaning processes [7].

Table 1 shows the results of the analysis of the properties of different types of organic fertilizers.

Table 1 – Advantages and disadvantages of different types of organic fertilizers

Name of organic fertilizer	Advantages	Disadvantages
Manure	Cheap and affordable organic fertilizer contains macro-and micronutrients in large quantities. The aftereffect of the applied fertilizer lasts 3-5 years. Contributes to the gradual humus formation and soil structure	The composition is not constant, it depends on the type of animal, its feed, litter and method of maintenance, storage of manure and its humidity. Requires special mechanisms for introduction. Requires determination of the concentration of substances to not provoke toxic effects. It may contain geohelminth eggs and cause additional biocontamination. Improper storage and application can cause contamination of soil, groundwater, and surface water with excessive amounts of nitrogen and phosphorus compounds. It may contain large amounts of dissolved salts, including chlorides and sulfates, which can cause secondary salinization of the soil.

Name of organic fertilizer	Advantages	Disadvantages
Digest	It has a balanced composition of macro and micronutrients. It's an "environmentally friendly" fertilizer, because after the mesophilic mode, it does not contain components of pathogenic microflora, worm eggs, weed seeds. Promotes the development of typical soil microflora. It is a fermented material, quickly humified or mineralized depending on the soil conditions. Contributes to the structure of the soil. Increases soil moisture content. Stimulates the development of bacterial microflora and inhibits the development of actinomycetes and mycoflora.	The composition is not constant and depends on the raw material and the fermentation period. Productivity as a biofertilizer depends on humidity and depth of application. It has an alkaline pH, better have a stimulating effect on acidic soils. The liquid fraction of the digestate has a high concentration of certain nutrients (phosphorus, ammonia) and requires prior preparation before application (dilution with water). Does not have a selective inhibitory or stimulating effect concerning certain types of microorganisms. The digestate obtained during the fermentation of the sludge of treatment plants preliminarily requires laboratory chemical studies to prevent the ingress of contaminants into the soil in a mobile form.
Vermicompost	Contains 4–8 times more humus content Than regular compost. Easy to transport. Improves soil structure. Enriches with organic residues serve as a nutrient substrate for certain groups of soil microorganisms.	The need to dispose of biomass of live worms, to ensure that they do not enter natural ecosystems. The need to maintain certain conditions for the activity of worms. Have enough space for vermicomposting.
Biofertilizers – micro-biological inoculants - specialized drugs based on microorganisms and enzymes	Do not harm the soil ecosystem. Contain natural strains of microorganisms that can facilitate the assimilation of certain chemical elements in other members of the soil biota. Direct stimulation of growth through the synthesis of valuable substances for plants. Resistant to water leaching. Do not contain pathogenic microflora. Cause increased availability of nutrients, synthesis of growth hormones, decomposition of stress mediators.	Requires certain conditions during storage of the drug. They do not contain the necessary ready-made substances necessary for developing soil microflora or plants but only stimulate the chemical transformation of substances present in the soil. You can not accurately predict the speed of the result. They operate in certain climatic conditions and require a particular time of application (from spring to autumn, in a relatively warm period).
Biopesticides – indirect stimulation of the development of certain crops due to the suppression and displacement of phytopathogens or bacteria that inhibit the growth of plants or beneficial microflora	High efficiency. Non-toxic. Do not cause gradual resistance in the organism on which they act. Effective for different plant crops and in different soil and climatic conditions. Often compatible for use together with biofertilizers.	Have a short storage method. More expensive than conventional pesticides. Stop active action after a specific time. Requirements for deadlines. Have only a narrow focus on practical action.

The expediency of their use is due to the lack of developed natural microbiological coenosis with pollution.

Advantages of use:

– selectivity;

– the possibility of removing strains of microorganisms that destroy soil toxicants.

Disadvantages of use:

– the efficiency of microbial cultures is not equally high due to the narrow range of favorable operating conditions;

– the risk of manifestation of the phenomenon of degeneration of microorganisms before reaching the required level of soil purification;

– the risk of violation of natural microbiocenoses with the use of microbial cultures.

Bacteria of the genera Actinomyces, Arthrobacter, Thiobacterium, Desulfoiomaculum, Pseudomonas, Bacillus, and other fungi with bacterial genes are used for purification. In complex cases of soil contamination, the most effective is its purification with complex biological products containing a range of crops and nutrients at the same time.

For the biodegradation of HM in soils, a method of their purification [8] has been developed by treatment with a culture of bacteria (strain Bacillus fastidious VKPM B-4368) in a liquid mineral nutrient medium containing carbohydrates. The bioleaching process is carried out until the pH value is equal to 4.0–6.0 with the consumption of the liquid component in the amount of 0.4–10.0 mass parts per 1 mass part of the cultivated soil. The method is effective but is material-intensive (bringing pH 2.8 to pH 4.0–6.0 requires a large amount of neutralizing substance, the cost of 0.4-10.0 mass parts of nutrient medium per 1 mass part of the soil is also resource-intensive) [9-10].

The efficiency of phytotechnology use is determined by the phytoavailability of heavy metals in the soil environment. Effectors are used to enhance remediation, mainly chelated compounds, but sometimes microorganisms are used. Common effectors are ethylenediaminetetraacetic acid, citric acid, diethylenetriaminepentaacetic acid, ethylene glycol tetraacetic acid, and others. Synthetic chelating compounds are considered more effective, but have a severe disadvantage of use – many soluble and mobile forms of metals [11-13].

Biosurfactants are amphiphilic compounds containing both hydrophilic and hydrophobic portions, referred to as head and tail. Compared with chemical surfactants, biosurfactants derived from plants and microorganisms have shown better performance considered suitable in removing heavy metal from contaminated soil. The mechanism of heavy metal removal from contaminated soil using ionic biosurfactants is shown in Figure 2.

[15] studied the effect of the sequence of addition of OH-Al species, protein molecules (albumin) and montmorillonite, and pH on the nature and properties of the final protein-OH-Al-montmorillonite complexes.

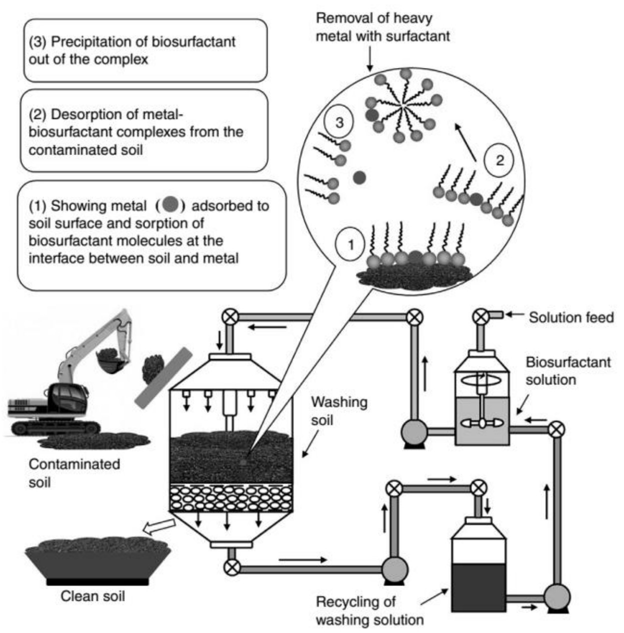

Figure 2 – Processing contaminated soil using biosurfactants for heavy metal removal [14]

Sorption of elements in cationic form onto soil components may be either enhanced or inhibited by the presence of organic acids (as oxalic, citric, malic, tartaric, malonic acid) and aminoacids through different processes, including alteration of surface charge, site competition, formation of different complexes in solution, ternary complex formed on the surfaces of soil components. Ternary surfaces complexes may be classified as type A, in which the metal ion is bonded to both the sorbent and the organic ligand, or type B, in which the ligand is bonded to the sorbent between the surface and the metal ion (Figure 3) [15].

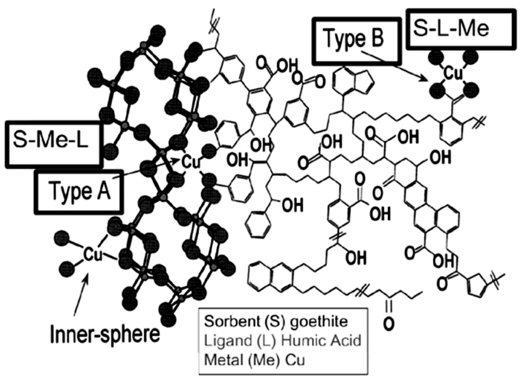

Figure 3 – Illustration of some possible bonding configurations of Cu (Me) on goethite (S)–organic matter (L) complexes: inner sphere complexation of Cu at goethite sites, and type-A-(S-Me-L) and B-(S-L-Me) ternary complexes [15]

The highly dispersed fraction consists of minerals with a layered crystalline structure, in which the layers are connected less firmly than the ions in the structures of clastic minerals.

Table 2 – Methods of soil biocleaning

The process of bio-remediation	Gist	Sort	Advantages	Disadvantages
Biodegradation of pollutants using microorganisms	Destruction or transformation of contaminants under the influence of enzymatic systems of microorganisms, decomposition of toxic forms of HM compounds to less toxic ones, transformation from active-mobile form of HM compounds into difficult or immobile inaccessible biota, or absorbed on the surface of secondary clay soil minerals.	Internal bioremediation due to naturally occurring species in this type of soil. Bioaugmentation (introducing certain cultured or removed from another environment species of organisms).	The possibility of using artificially created to extract a particular type of toxicant strains. Specific selectivity of action on the toxicant. Relatively easy application in the absence of a developed natural microbiological coenosis. Can be used in situ and ex situ. The cleaning process can be carried out in the near-surface part of the soil using optional aerobes and anaerobes. Less depends on the seasonal phenomena of the atmosphere due to more stable conditions of the soil environment.	The efficiency of microbial cultures is not equally high due to the narrow range of favorable operating conditions. The risk of the phenomenon of degeneration of microorganisms to achieve the required level of soil purification, the need for constant monitoring of species composition. The need together with a specific strain of microorganisms to make additional nutrient medium (mineral or organic components) the risk of violation of natural microbiocenoses with introduced microbial cultures.
Phytoreme-diation	The use of hyperaccumulator plants that are tolerant to the action of HM and can remove certain toxicants from the environment in high concentrations, accumulating them in certain parts of the body.	Phytoextraction (absorption and accumulation of pollutants in the plant). Phytostabilization (reduction of pollutant mobility and/or accumulation in the root system of the accumulating plant or in the rhizosphere). Rhizofiltration (metals are absorbed and bound in the root systems of phytoremediaries)	Can be used in situ, without spending money on the transfer and return of soil after cleaning, are more environmentally friendly for the environment. It is possible, if necessary, to completely remove from the contaminated area of the plant, the body of which contains absorbed from the soil HM. After the death of the body of plants and transformation into a component of humus or the formation of organo-mineral complexes of soil with clay minerals, there is a binding of HM compounds in the stationary component, at the same time there is an improvement in the structural state of the soil, trophic and gas regimes, improvement of the properties of the soil system as a whole.	Do not show selective action only for a certain type of pollutant. Die with an exaggeration of a certain amount of toxicant due to violation of enzymatic regulatory systems. Cleansing occurs only during the growing season. Cleaning occurs primarily in the surface layers of the soil, where the root systems of plants reach. Long-term slow uncontrolled process, which depends on the state of the plant organism.

This feature of these minerals contributes to the sorption of scattered heavy metals, and their concentration in this fraction is higher than in fine-grained and in the soil as a whole [16].

A new direction of scientists' research is the use of alternative immobilizers and the evaluation of their effectiveness compared to traditional means.

Table 2 shows a comparative description of methods of biological soil treatment.

In the work of Adejumo Sifau et al. (2011) investigated the effect of inorganic fertilizer (NPK, 100 kgN/ha), compost from cassava (Manihot utilissima Pohl.), and titonia (Tithonia diversifolia) at concentrations of 20 and 40 tn/ha on the process of immobilization of Pb in the soil in which corn was grown (Zea mays L.). It was found that the concentration of mobile lead in the soil environment with the use of compost from titanium and cassava in the amount of 40 tn/ha, decreased by 72 % and 67 %, and at 20 tn/ha – by 66 % and 49 %. The addition of compost helped to increase the height of the plant by 89–94 %, as well as the area of leaves and their number (especially when applying to the soil compost from titanium at a concentration of 40 tn/ha). Activation of biodegradation of pollutants by maintaining the optimal temperature is carried out by covering the contaminated soil with black polyethylene film in winter to increase the temperature. In summer – use a transparent film to reduce evaporation from the surface [11].

4 Results and Discussion

4.1 Technological intensification of soil bioremediation processes

Various bioreactor designs can be used for ex situ purification of contaminated soils and other materials, including fixed bed reactors (solid-phase bioreactor) and suspension with stirring (sludge bioreactor).

Suspension (sludge) reactors are mainly used for the biological treatment of contaminated soil (Figure 4). The amount of soil in suspension reactors with stirring can be up to 30 % of the total volume. Their advantages over solid-phase reactors are easier control, better process control, the ability to provide a higher level of aeration. They are not prone to siltation, which clogs with sediments, suspended particles, and excess biomass. But the design of suspension reactors is more complex, consumes more energy, and requires high costs for exhaust-air purification [18]. Aerobic biogeochemical barriers can be created by the migration of groundwater with high content of Sr^{2+}, Ba^{2+}, Fe^{2+}, Mn^{2+}. Due to the formation of carbonic acid during the oxidation by microorganisms of organic substrates introduced into the contact zone, there is a precipitation of carbonates and bioleaching processes [19]. The development of iron-oxidizing bacteria under aerobic conditions promotes the formation of Fe^{3+}, which interacts with phosphate to form insoluble compounds. The reduction of sulfate is accompanied by HM sulfides, which occur as follows [20]. CH_2O is organic carbon in these reactions, and Me^{2+} denotes the divalent cation HM (Figure 5).

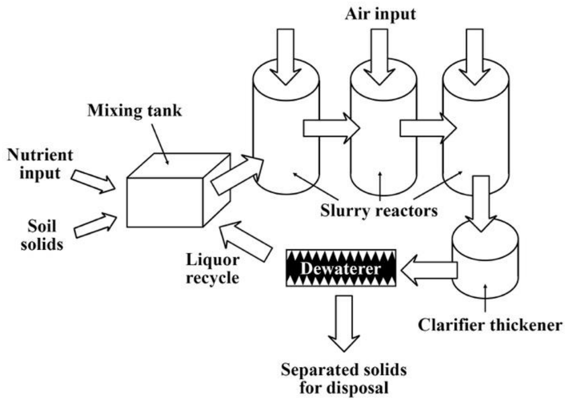

Figure 4 – Tillage using a sludge bioreactor

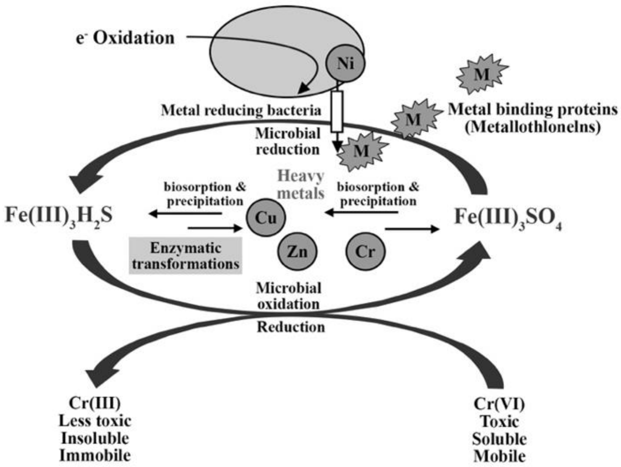

Figure 5 – Precipitation of metals from contaminated soil by microorganisms, based on [20]

The bioscreen can be organized by aerating a section of soil in a direction perpendicular to the movement of groundwater. The pollution can be biologically oxidized or desorbed by the airflow [21].

Methods of electrokinetic activation of biodegradation include the use of electric current, which provides the migration of microorganisms, with its charge, in the contaminated area and increase the speed, efficiency of uniform soil cleaning [22]. Ultrasound is used to activate biodegradation, which includes the destruction of large soil aggregates to increase the availability of contaminants to microorganisms [23].

Electrokinetic remediation is associated with the transmission of a direct electric current of low voltage (20–40 mA/cm^2) with a potential difference of several volts through the ground for a specific time interval from 5–10 min to 120–150 min, which can be carried out in the place of contamination when the electrodes are installed in the soil in situ, or a specially equipped reactor [24].

Electrokinetics processes involve passing a low-intensity electric current between a cathode and an anode embedded in contaminated soil (Figure 6).

Ions and small charged particles, in addition to water, are transported between the electrodes. Anions move towards the positive electrode and cations towards the negative one. An electric gradient initiates movement by electromigration (charged chemical movement), electro-osmosis (movement to fluid), electrophoresis (charged particle movement), and electrolysis (chemical reactions due to an electric field).

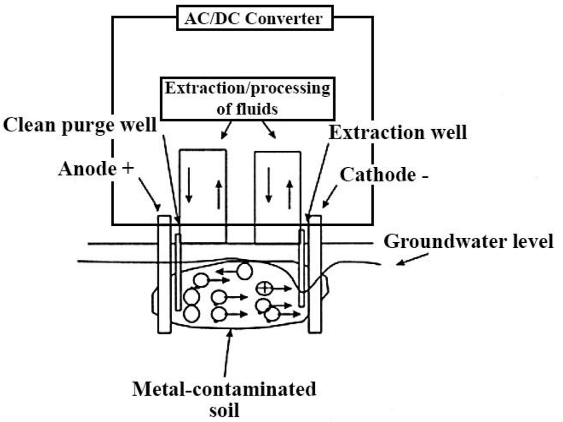

Figure 6 – Electrokinetic process for soil remediation (buffer solutions are added and removed by purge and extraction wells [25])

Improving soil aeration conditions also contributes to the biodegradation of soil contamination by blowing soils and groundwater with air at different pressures in combination with the introduction of nutrients through holes with air or spraying micro-particles of nutrient solutions.

A number of bioventilation screens are installed in the unsaturated zone. Air is blown in using a ventilator, and decomposition of the contamination is stimulated. Usually, a number of passive "air-emission screens" are located at appropriate distances depending on the characteristics of the contamination. Bioventilation stimulates biodegradation by blowing in the air, unlike soil vapor extraction, where contamination components are sucked out of the soil. The location of the contamination is significant. For example, this method should be considered where contamination is located under or close to a building (Figure 7).

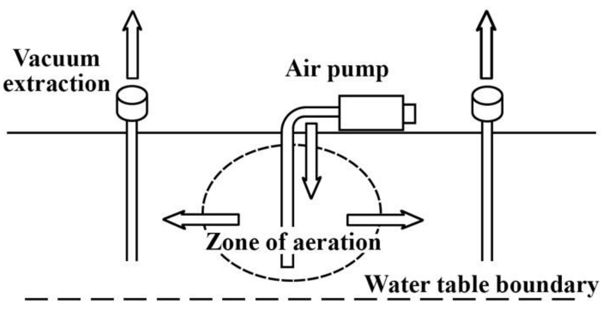

Figure 7 – Typical soil bioventilation system [26]

4.2 Development of a complex biotechnological solution for soil remediation

Previous studies [27] at the laboratory of Sumy State University modeled the mechanisms of influence of biogenic composite material based on sludge and phosphogypsum on soil protective functions and described the dynamics of soil microbial biome in the system "biotic component – biogenic product (digestate)– toxicant (HM)" with the stimulating effect of the biocomposite. This requires further determination of the ecological and biochemical aspects of the impact of organo-mineral bio-composite on the development of natural soil microorganisms and the process of formation of favorable biochemical conditions for the restoration of disturbed lands.

A clear and structured construction of the technological scheme of the movement of material flows is required to implement complex biotechnology of soil remediation.

The main organizational requirements for the biotechnology scheme include the following:

- ease of execution and automation of equipment;
- the expediency of placing equipment under the direction of material flows;
- selection of the optimal mode of operation of technological equipment - continuous or periodic;
- carrying out processes in constant conditions;
- greenhouse gas emissions from biological treatment systems (Figure 8);
- selection of the final product of processing.

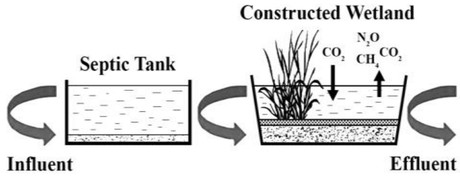

Figure 8 – Greenhouse gas emissions from biological treatment systems

A technological scheme of aerobic plants for soil biodetoxification was developed (Figure 9).

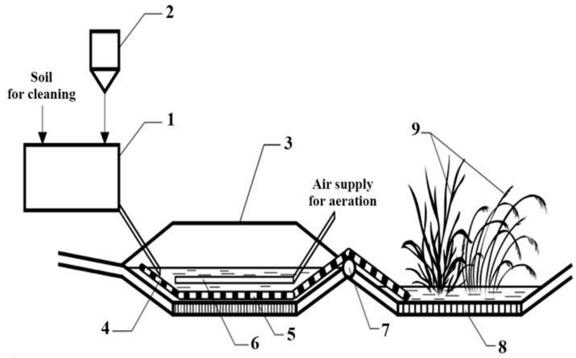

Figure 9 – Principle biotechnological scheme of soil detoxification: 1 – hopper for mixing the crushed soil and biocomposite; 2 – batches for biocomposite supply; 3 – bioreactor; 4 - scraper conveyor; 5 - isolated bottom; 6 – aeration system; 7 – electric drive; 8 – platform; 9 – phytoremediation plants

The technological scheme works as follows. The crushed soil is contaminated with heavy metals, and the biocomposite fed from the dispenser enters the hopper for mixing. This mixture enters the bioreactor via a pipeline, made in a trench with an insulated bottom and a scraper conveyor, through which the soil is moved.

An aeration system is arranged above the conveyor, which air is supplied to the bioreactor to improve the aeration conditions. The bioreactor is equipped with an electric drive and is covered on top with a sealed opaque polymer coating.

Pre-cleaned soil with a scraper conveyor is fed to a particular site, where it is planted plants – remediates (9) for further cleaning, monitoring, and control of the content of pollutants.

Thus, cleaning the soil of heavy metals and restoring its fertility due to improved conditions for the development of soil microbiota is proposed in a two-stage process:

1) aerobic tillage together with the biocomposite;
2) stage of phytoremediation for purification.

5 Conclusions

The peculiarities of the processes of the heavy metal in the soil and the relationship with other components of the ecosystem are studied, and the factors on which the indicators of heavy metal adsorption depend are identified.

The existing methods of soil cleaning from heavy metals are reviewed, and it is substantiated that the restoration of technogenic contaminated soil with biological methods is the most promising area that is developing dynamically and is considered environmentally friendly.

There are two groups of biological methods of soil purification, including methods using microorganisms and plant bioaccumulation and/or redistribution of pollutants in the soil while simultaneously affecting the biological and inert components of the soil. Features of bioaugmentation, the advantages, and disadvantages of this method are considered, and the influence of effectors, mainly chelated compounds, on the degradation of soil pollutants. Some of their features are determined based on a review of studies by foreign and domestic scientists.

A review of technological solutions for intensification of soil bioremediation processes, which will reduce the duration of treatment, using bioreactors, including fixed-layer reactors and suspension with stirring, analyzed methods, and technological means of activating the microflora, improving bioremediation processes improving aeration conditions by blowing the soil with air). Accordingly, a biotechnological scheme of soil detoxification has been developed and described, which has two stages: aerobic tillage and the biocomposite based on digestate, and stage of phytoremediation for purification and control of the content of toxicants in the soil.

6 Acknowledgments

This research was carried out as a planned joint Ukrainian-Czech R&D project “Bioenergy innovations in waste recycling and natural resource management” (2021–2022).

References

1. Intergovernmental Forum on Chemical Safety Global Partnerships for Chemical Safety Contributing to the 2020 Goal. (2008). *Review of the problem of environmental pollution with cadmium, lead and mercury in Russia and Ukraine.* Retrieved from https://www.who.int/ifcs/documents/forums/forum6/eco_accord_ru.pdf.
2. Zhang, J., Cao, X., Yao, Z., Lin, Q., Yan, B., Cui, X., He, Z., Yang, X., Wang, C.-H., Chen, G. (2021). Phytoremediation of Cd-contaminated farmland soil via various Sedum alfredii-oilseed rape cropping systems: Efficiency comparison and cost-benefit analysis. *Journal of Hazardous Materials,* Vol. 419, 126489, doi: https://doi.org/10.1016/j.jhazmat.2021.126489.
3. Oladoye, P. O., Olowe, O. M., Asemoloye, M. D. (2021). Phytoremediation technology and food security impacts of heavy metal contaminated soils: A review. *Chemosphere,* 132555, doi: https://doi.org/10.1016/j.chemosphere.2021.132555.
4. Chernysh, Y. Y., Plyatsuk, L. D. (2018). Binding of heavy metals in the soil complex at the introduction biocomposite based on sewage sludge and phosphogypsum. *Young Scientist,* Vol. 1 (53). Retrieved from http://molodyvcheny.in.ua/files/journal/2018/1/103.pdf.
5. Pathak, P., Bhattacharya, D. (2021). Phytoextraction of heavy metals by weeds: Physiological and molecular intervention. *Handbook of Bioremediation. Physiological, Molecular and Biotechnological Interventions,* pp. 49–59, doi: https://doi.org/10.1016/B978-0-12-819382-2.00004-1.
6. Nedjimi, B. (2021). Phytoremediation: a sustainable environmental technology for heavy metals decontamination. *SN Applied Sciences,* Vol. 3, 286, doi: https://doi.org/10.1007/s42452-021-04301-4.
7. Biodegradation of hydrocarbons as a solution to the problem of oil pollution. Retrieved from http://eco.com.ua/content/biodegradaciya-vuglevodniv-yak-vyrishennya-problemy-naftovogo-zabrudnennya.
8. Nikovska, H. M., Ulberh, Z. R. (2003). *Method for removal of heavy metal and radionuclids from soil.* Patent of Ukraine, No. 58557.
9. Xinhui, D., Ping, T., Mengqi, M., Liang, M., Yunjun, Y., Zhenxing, W. (2021). The Study of Conditions for Bioleaching Heavy Metals from Polluted Soil and Metabonomics in Penicillium chrysogenum. *Journal of Biobased Materials and Bioenergy,* Vol. 15(1), pp. 117–124(8), doi: https://doi.org/10.1166/jbmb.2021.2023.

10. Nguyen, T. H., Won, S., Ha, M.-G., Nguyen, D. D., Kang, H. Y. (2021). Bioleaching for environmental remediation of toxic metals and metalloids: A review on soils, sediments, and mine tailings. *Chemosphere,* Vol. 282, 131108, doi: https://doi.org/10.1016/j.chemosphere.2021.131108.
11. Shtyka, O. S. Assessment of alternative remediation technologies of soils polluted with heavy metals. Retrieved from http://tes.igns.gov.ua/materials/5n/Shtyka.pdf.
12. Shweta, N., Samatha, S., Keshavkant, S. (2021). Mechanisms, types, effectors, and methods of bioremediation: The universal solution. *Microbial Ecology of Wastewater Treatment Plants,* pp. 41–72, doi: https://doi.org/10.1016/B978-0-12-822503-5.00010-2.
13. Raffa, C. M., Chiampo, F., Shanthakumar, S. (2021). Remediation of Metal/Metalloid-Polluted Soils: A Short Review. *Applied Sciences*, Vol. 11(9), doi: https://doi.org/10.3390/app11094134.
14. Akbari, S., Abdurahman, N. H., Yunus, R. M., Fayaz, F., Alara, O. R. (2018). Biosurfactants - a new frontier for social and environmental safety: A review. *Biotechnology Research and Innovation,* Vol. 2(1), pp. 81–90, doi: https://doi.org/10.1016/j.biori.2018.09.001.
15. Violante, A., Caporale, A. G. (2015). Biogeochemical processes at soil-root interface. *Journal of soil science and plant nutrition,* Vol. 15(2), pp. 422–448, doi: http://dx.doi.org/10.4067/S0718-95162015005000038.
16. Dobrovolsky, V. V. (2004). The role of soil organic matter in the migration of heavy metals. *Nature,* Vol. 7, pp. 35–39. Retrieved from http://vivovoco.astronet.ru/VV/JOURNAL/NATURE/07_04/35-39-0704.PDF.
17. Adejumo S.A., Togun A.O., Adediran J.A., Ogundiran M.B. (2011) Field Assessment of Progressive Remediation of Soil Contaminated with Lead-Acid Battery Waste in Response to Compost Application. Pedologist, pp. 182–193.
18. Balseiro-Romero, M., Monterroso, C., Kidd, P. S., Lu-Chau, T. A., Gkorezis, P., Vangronsveld, J., Casares, J. J. (2018). Modelling the ex situ bioremediation of diesel-contaminated soil in a slurry bioreactor using a hydrocarbon-degrading inoculant. *Journal of Environmental Management,* Vol. 246, pp. 840–848, doi: https://doi.org/10.1016/j.jenvman.2019.06.034.
19. Han, Zuozhen, Dan Li, Hui Zhao, Huaxiao Yan, and Peiyuan Li. (2017). Precipitation of Carbonate Minerals Induced by the Halophilic Chromohalobacter Israelensis under High Salt Concentrations: Implications for Natural Environments. Minerals, Vol. 7(6), 95, https://doi.org/10.3390/min7060095
20. Li, J., Yang, H., Tong, L., Sand, W. (2021). Some Aspects of Industrial Heap Bioleaching Technology: From Basics to Practice. *Mineral Processing and Extractive Metallurgy Review*, doi: https://doi.org/10.1080/08827508.2021.1893720.
21. Yanin, E. P. (2014). Remediation of territories contaminated with chemical elements: general approaches, legal aspects, main methods (Foreign experience). *Environmental and natural resource issues,* Vol. 3, pp. 3–105. Retrieved from http://www.nparso.ru/images/docs/Remediation_of_the_territories.pdf.
22. Rosestolato, D., Bagatin, R., Ferro, S. (2015). Electrokinetic remediation of soils polluted by heavy metals (mercury in particular). *Chemical Engineering Journalthis,* Vol. 264, pp. 16–23, doi: https://doi.org/10.1016/j.cej.2014.11.074.
23. Hazrati, S., Farahbakhsh, M., Cerdà, A., Heydarpoor, G. (2021). Functionalization of ultrasound enhanced sewage sludge-derived biochar: Physicochemical improvement and its effects on soil enzyme activities and heavy metals availability. *Chemosphere,* Vol. 269, 128767, doi: https://doi.org/10.1016/j.chemosphere.2020.128767.
24. Cameselle, C., Gouvei, S., Akretche, D. E., Belhadj, B. (2013). *Advances in Electrokinetic Remediation for the Removal of Organic Contaminants in Soils*. Organic Pollutants - Monitoring, Risk and Treatment, M. Nageeb Rashed, IntechOpen, doi: 10.5772/54334.
25. Jankaitë, A., Vasarevièius, S. (2005). Remediation technologies for soils contaminated with heavy metals. *Journal of Environmental Engineering and Landscape Management*, Vol. 13(2), pp. 109–113, doi: 10.3846/16486897.2005.9636854.
26. Guidelines on remediation of contaminated sites. Retrieved from https://www2.mst.dk/udgiv/publications/2002/87-7972-280-6/html/kap09_eng.htm.
27. Chernysh Y., Ablieieva I., Makarekno N., Plyatsuk L., Trunova I., Burla O. (2021) Investigation of the directions of using a hybrid composition bioproduct for detoxification of a soil ecosystem contaminated with heavy metals and oil products. Biodiversity & Environment, Vol. 13(1), pp. 80–94.