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Integrated chemo-phyto-ecological process for the treatment of polymetal contamination in landfill sites and the consequent soil recovery

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ABSTRACT

Non-hazardous chemical waste and municipal solid waste (MSW) are typically disposed in landfill sites. Therefore, the soils in these sites are heavily contaminated by hazardous polymetallic substances, accompanied by biocenosis disruption. Analyses of a landfill soil in Moscow showed that the concentration of metals (As, Cr3+, Zn, Cu, Ni, and Co) exceeded the established standards, and bacteria were the dominant microorganisms in the soil. A combination of sodium salts of gibberellic acid and ammonium salt of orthochlorophenyl acetic acid showed a positive effect on soil phytoremediation. These findings lay a foundation for the application of chelate-assisted and chemo-microbe-assisted phytoextraction processes in MSW soil remediation.

1. Introduction

Approximately, 100 billion tons of municipal solid waste (MSW) and industrial waste have accumulated in landfill sites in Russia, covering a total area of around 4 million hectares (Berseneva, 2016). The soil beneath the MSW-disposal sites are characterised by contaminant accumulation and biocenosis disruption (Kjeldsen et al., 2010; Lou et al., 2009; Ye et al., 2019). The remediation processes of soil formation in these lands and their rational management require complex measures, and considerable time and money (Berseneva, 2016). Therefore, studies have focused on the development of novel energy-efficient remediation processes for landfills and the improvement of available technologies (Ye et al., 2019).

Polymetals are one of the main sources of pollution in MSW-disposal sites (Fait et al., 2018; Petruzzelli et al., 2018; Ye et al., 2019). Heavy metal ions pass through landfill filters and reach the soil layers, resulting in high concentrations of heavy metal compounds in landfill sites, especially in areas of new soil formation and near the shore of sanitary protection zones (Lutsevich, 2014). Indeed, heavy metals were reportedly the most hazardous soil contaminant in MSW sites, according to surveys in Russia (Moscow, Saint Petersburg, Middle Ural, Kazan, and Volgograd) and other countries (Fait et al., 2018; Pozza et al., 2019). The remediation and restoration of MSW soils are challenging owing to the large area, heterogeneous concentration profile of pollutants in the surface layers, and variations in the composition and maximum metal ion concentrations in each MSW site. Consequently, soil remediation with conventional physico-chemical processes such as soil flushing, solidification and stabilisation, and electrokinetic recultivation is expensive (Ye et al., 2019). Moreover, the low efficiency and incompatibility of these methods with the soil structure and fertility level have been reported (Koptsik, 2014). In this milieu, we aimed to develop a new chemo-phyto-remediation process to improve the efficiency of soil remediation in MSW sites. To this end, previously, we examined chelate-assisted and microbe-assisted phyto-extraction (Robinson et al., 2015; Franchi and Petruzzelli, 2017; DalCorso et al., 2019) and explored the possibility of successfully combining them for environmental-assisted phyto-extraction

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Abbreviations: MSW, municipal solid waste; EDTA, ethylenediaminetetraacetic acid; HEDP, hydroxyethylidine diphosphonic acid; PGPR, plant growth-promoting rhizobacteria; CFU, colony-forming units; TLV, threshold limit value.

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to achieve efficient and economically feasible recovery of MSW soils. Furthermore, we examined the compatibility of these processes with other biologically active components (Cassina et al., 2012;Sun et al., 2013; Aderholt et al., 2017).

Natural phyto-extraction is a slow process under normal agronomic conditions (Evangelou et al., 2007; Robinson et al., 2015). Extensive research has been conducted to develop novel methods for accelerating the remediation process by improving the availability of heavy metals to plants (Epelde et al., 2008; Arabi et al., 2017). Meers et al. (2008) reviewed potential soil chelators that could increase the uptake of heavy metals. Studies in this field have mostly focused on nitric ligand complexes of amino-polycarbonic acids (e.g. ethylenediaminetetraacetic acid [EDTA], diethylenetriamine pentanoic acid, ethyl glyoxylate, and ethylenediamine-*N*,N′-disuccinic acid). However, compounds of organophosphorus complexes such as bisphosphonates have not been considered as potential additives and reagents for the phyto-extraction of heavy metals.

The Institute of Chemical Reagents and Special Purity Chemicals of the National Research Center 'Kurchatovsky Institute' developed a new technology using biphosphonic complex derivatives. Complex derivatives are expected to have a substantial effect on contaminated soils based on the following assumptions.

1 The best-known bisphosphonate compound is hydroxyethylidine diphosphonic acid (HEDP), which forms soluble complexes with several heavy metals (Mn, Cr, Fe, Co, Cd, Cu, Zn, and Ni) over a wide pH range (from 1 to 10). This is a key property, as complex formation does not occur at a high EDTA concentration.
2 Bisphosphonates show distinct biological activities. For example, HEDP is used as a medical substance, which is characterised by stereochemistry and the mutual influence of phosphonic fragments. As an analogue of natural pyrophosphates, HEDP is involved in more than 60 biochemical cellular reactions by regulating ionic calcium and phosphorus exchange. Thus, HEDP has the ability to stabilise cell membranes by interacting with the ligands of membrane proteins. Furthermore, HEDP has the ability to suppress the activity of enzymes that degrade the membrane components.
3 Organophosphorus is considerably less toxic to living systems and organisms than carboxyl-containing complexes, providing a scientific basis for the application of a microbe-assisted phyto-extraction process. During recent years, several studies have focused on strengthening the process of heavy metal phyto-extraction involving plant growth-promoting rhizobacteria (Rajkumar et al., 2010; Franchi and Petruzzelli, 2017; Guarino and Rosaria., 2017; DalCorso et al., 2019).

Biomass production is key for the success of phyto-remediation technology. Indirect stimulation of heavy metal phyto-extraction via the actions of microorganisms is associated with the production of biologically active components such as phytohormones (e.g. auxins, gibberellins, and cytokinins), certain enzymes that directly affect the growth and development of plants, and organic acids and other classes of compounds. A recent review reported that bacterial indole 3-acetic acid auxin, which promotes plant growth-promoting rhizobacteria (PGPR), facilitates the adaptation of host plants to metal-contaminated sites, causing physiological changes in plant cell metabolism under metal stress and inducing resistance in plants to heavy metals at high concentrations (DalCorso et al., 2019). A chemo-microbe-assisted process has been proposed for effective phyto-remediation; furthermore, a positive effect of the combination of an EDTA-chelating agent and bacterial inoculum on phyto-remediation has been demonstrated under laboratory conditions with polymetallic soil samples (Hadi et al., 2010; Cassina et al., 2012; Aderholt et al., 2017).

Despite these encouraging laboratory results *in vitro* (Rajkumar et al., 2010; Tak et al., 2013), studies on chemo-microbe-assisted phyto-remediation under real-world conditions are limited (Hamidpour et al., 2019), because of the laborious and time-consuming task of isolation and culture of several microorganisms in MSW soil samples. In addition, for the practical application of chemo-microbe-assisted phyto-extraction in the remediation of polygons, it is important to assess the resistance of the inoculum to stress factors, such as soil pH, natural colonisation capacity, interaction with the indigenous soil microbiome, nutrient deficiency, toxicity level, polymetallic contamination conditions, temperature, and climate variability (Franchi and Petruzzelli, 2017; Ye et al., 2019). Although these factors pose challenges for chemo-microbe-assisted phyto-extraction with effective PGPR strains, they should not deter efforts to develop novel combined processes for the remediation of MSW soil by exploiting the natural mechanism of close interaction between microbiota and plants.

A recent study showed that the simple washing of metal-contaminated soils with EDTA solution, with a high reagent concentration, had a minimal effect on microbial respiration in acidic soils (Kaurin and Lestan, 2018). Plant root exudates also provide essential nutrients for the survival of the rhizospheric microbial community. Root selection is crucial in determining microbial composition and diversity (Rajkumar et al., 2010; Tak et al., 2013; Franchi and Petruzzelli, 2017; Vandenkoornhuyse et al., 2015). Accordingly, we proposed an ecological approach for the restoration of MSW landfill sites based on the concept of 'complex partial substitution therapy', involving the simultaneous promotion of heavy metal ion absorption, photosynthetic plant activities, and biomass growth by combining chelation with stable iron complexes and hormone addition. Thus, indirect stimulation and modulation of indigenous microbiota along with the restoration of soil functions are expected to occur. In addition, some studies on the effects of combination of chemical corrections and hormone addition on heavy metal phyto-extraction have shown positive results (Aderholt et al., 2017).

The aim of the present study was to explore the possibility of applying an integrated phyto-ecological process in the remediation and restoration of polymetal-contaminated soils in MSW sites. Specifically, we tested the effects of chemical additives comprising salts of HEDP–K_2HEDP for chelate-assisted phyto-extraction, ethylenediamine-*N*,N′-o-oxyphenyl-*N*,N′-diacetic acid for the correction of iron deficiency, and gibberellins to enhance plant biomass growth and development. We further included natural minerals with a high silicon content (diatomite, bentonite) and humic preparations as additives that can generally stabilise the soil–biotic complex. The efficiency of the chemo-phyto-extraction processes for soil remediation with the proposed complex and the effects of the active additives on the microbiota of the MSW site under model conditions were evaluated. Specifically, we examined the auxin-like activity of model soil containing sodium salts of gibberellic acids and an ammonium salt of orthochloro phenylacetic acid in the phyto-ecoextraction of heavy metals using three battery plants.

2. Materials and methods

2.1. Study site

This study was conducted in the MSW polygon in Moscow, Russia, with a total area of approximately 37 ha (Fig. 1); the polygon was established in the mid-1970s in an old clay quarry. The capacity of the waste layer reaches up to 54 m. Specifically, the landfill is located in the Klinsk-Smolensk upland (a part of the Smolensk-

Fig. 1. Landfill location.

Moscow upland), which is formed by a moraine, covered by clover leaves, and the relief is broken up by deeply mangled river valleys.

The climatic conditions of the area are as follows: average annual air temperature of +4 °C (range −40 °C to 36 °C), average annual precipitation of 600 mm, average wind speed of 3–4 m/s, and snow cover height of 42 cm. The plant composition of the landfill comprises camomile (*Matricaria chamomilla*), common mugwort (*Artemisia* sp.), clover (*Trifolium pratense*), burdock (*Arctium* sp.), and bindweed (*Convolvulus arvensis*).

2.2. Pollutant content analysis

2.2.1. Soil samples

Soil samples to detect the main pollutants were collected on 27 June 2019. On the sampling day, the humidity was 80 % with light rain. The top soil is sandy–clay, which is a low-fertility soil type. The total number of sampling points in three replicate sites was five, with a sampling depth of 0.15–0.25 m. All sampling points were located in the same site. The collected soil samples were placed in plastic containers for transport to the laboratory.

2.2.2. Chemical analysis of soil samples

All soil samples were dried at 105 °C and passed through a nylon sieve with 2-mm mesh to remove large particles. Chemical measurements were made in the Laboratory of Institute for Chemical Reagents and High Purity Chemical Substances, Moscow, Russia, by atomic emission spectrometry with inductively bound plasma (iCAP 6300 duo; Intertech Corporation, Moscow, Russia) and mass spectrometry with inductively bound plasma (Elan DRC PerkinElmer; Find Lab, Moscow, Russia). The level of 13 metals (Ca, Mg, Cd, Zn, Cr, Pb, Ni, Co, Mn, Cu, Fe, Hg, and As) was determined. The pH of the samples was determined using the potentiometric method.

2.3. Soil microbiological analysis

2.3.1. Soil samples

Soil samples for microbiological assessment were collected on 25 July 2019. On the sampling day, the humidity was 60 %. Five sampling points with three replicates were established. Similar to soil sampling for the chemical pollutant analysis, all ground sampling points were located in the same site. The sampling depth was 0.15–0.25 m, and the samples were collected from the permeability area in accordance with aseptic requirements and placed in sterile bags.

2.3.2. Isolates of microbial groups

Soil samples were examined using the Koch method. The residual moisture content in the soil samples ranged from 8.5%–15.7%, which was determined by drying individual samples in an oven at 102 °C to a constant weight. The total microbial count was determined as the number of colony-forming units (CFU) per gram of soil sample by dry weight, considering the variation in moisture content. We focused on two functional groups of microorganisms, bacteria and yeast. Bacteria were cultured in L-Broth medium and yeast in Reader medium.

The L-broth medium comprised the following: Hottinger medium, 125.0 cm^3/dm^3; baking yeast hydrolysate, 5.0 g/l; ammonium sulphate [$(NH4)_2SO_4{\cdot}4H_2O$], 3.0 g/l; sodium chloride (NaCl), 5.5 g/l; single-substituted potassium phosphorous acid (K_2HPO_4), 1.5 g/l; double-substituted potassium phosphorous acid (KH_2PO_4), 0.6 g/l; calcium chloride ($CaCl_2$), 0.01 g/l; glucose, 10.0 g/l; and agar-agar, 5 g/l. After preparation, the pH of the medium was set to 6.5—7.0. Rieder medium comprised the following: ammonium sulphate, 3 g/l; magnesium sulphate, 0.7 g/l; calcium nitrate, 0.04 g/l; sodium chloride, 0.5 g/l; potassium dihydrophosphate, 1.0 g/l; and potassium hydrophosphate, 0.1 g/l. To the medium, 2% sugar and the following crystalline vitamins (mg/mL) were also added: inosite, 5 g/l; biotin, 0.0001 g/l; pantotenic acid, 0.25 g/l; thiamine, 1.0 g/l; pyridoxin, 0.25 g/l; and nicotine acid, 0.5 g/l. After preparation, the pH of the medium was set to 6.6. The microorganisms were manually counted by direct electron microscopy. The isolates were detected using a Biomed 5 optical microscope equipped with a Levenhuk C310 NG camera. The cell morphology of the isolates (structure, inclusions, flagellates, spores) was also analysed.

2.4. Production of mixed microbial cultures

Mixed cultures of the dominant microorganisms were obtained by accumulative culture, involving incubation of inoculum in liquid nutrient medium with continuous shaking. The experiments were carried out in sterile 100-ml conical bulbs, in which 40 mL of selective growth medium and samples of pre-dried soil as the inoculum (10 % mass) were randomly added. To the resultant system, 10 mL of mixed bacterial and yeast cultures composed of the allocated isolates was added. The samples were incubated in a thermostat (Lovibond TC 255 S) at 37 °C and 30 °C for the bacterial and yeast mixed cultures, respectively, and continuously mixed at 200 rpm. The organisms were cultured for 2 days; after settling in fresh nutrient medium, the samples were sown on solid media (L-broth and Reader media for bacteria and yeast, respectively).

2.5. Application of K_2HEDP

An aqueous solution of the double-substituted potassium salt of HEDP (K_2HEDP) with a mass content of the target component of 28.3 % was provided by the Laboratory of Institute for Chemical Reagents and High Purity Chemical Substances of NRC Kurchatovsky Institute. The effects of the aqueous solution of K_2HEDP on the growth and activity of the mixed cultures were investigated by adding it to liquid nutrient medium at various concentrations (1.0×10^{-10}, 1.0×10^{-5}, 1.0×10^{-1}, and 1.0 g/l).

2.6. Application of a stabilising soil–biotic complex of the mineral additive diatomite

Diatomite powder (NDP-D-700; Quant, Russia) was used as the mineral additive. Diatomites are natural materials with a significant mobile silicon content, which is beneficial for the optimisation of soil properties (Kozlov et al., 2017). The mean particle dispersion was 50–80 μm. An aqueous suspension of the diatomite powder was added to the liquid nutrient medium for culturing microor-

Table 1
Characteristics of NDP-D diatomite powder.

Characteristics of NDP-D	Value
Humidity, %	2.5−3.0
Volumetric weight, kg/m^3	550–800
Density, kg/m^3	2160
Porosity, %	72.3
Specific surface area, 10^3 cm^2/g	2.0–5.0
Average particle size, μm	50–80
pH (10 % suspension)	7–8
Chemical composition, wt%	
SiO_2	84–87
Al_2O_3	5.5–6.0
Fe_2O_3	2.5–3.0
CaO	01.17
Loss on ignition, % mass	5

ganisms at 0.5 % of the general composition. The characteristics of the diatomite powder are shown in Table 1.

2.7. Combination of diatomite powder and K_2HEDP

We further tested the effects of the combination of NDP-D (0.5 %) and K_2HEDP (1×10^{-5} g/l). The microorganisms were cultivated in a liquid nutrient medium for hydrocarbon-oxidising microorganisms, containing heavy metals (1 mL of heavy metal solution per 100 mL of medium) and oil as a carbon source under stress (1 mL of oil per 100 mL of medium).

2.8. Model vegetation

Model experiments were carried out in accordance with the ISO 22030:2005 standard “Soil quality. Biological methods. Chronic toxicity in higher plants” using standard laboratory equipment, phytolamps, scales with an accuracy of 0.1 mg, universal soil (pH 5.8–6.2), and a set of plastic vegetation vessels for seed planting. The following chemical compounds were used to simulate the soil composition in the MSW landfill site at the worst concentrations determined in the landfill soil analysis: 51 mg/kg $ZnSO_4$, 21.7 mg/kg $CuSO_4$, 14.2 mg/kg $CuSO_4{\cdot}7H_2O$, 59 mg/kg $K_2Cr_2O_7$, 12.4 mg/kg $Pb(CH_3COO)_2$, and 28.5 mg/kg $NiSO_4{\cdot}7H_2O$. Each substance was weighed against the tipping board and dissolved in distilled water.

Table 3
Microbial content in soil samples (expressed on a dry weight basis).

Soil sample	Microbial count (CFU/g)	
	Bacterial count	Yeast count
1	130.0 × 10^5	–
2	11.6 × 10^5	–
3	49.0 × 10^5	1.8 × 10^5
4	52.9 × 10^5	0.8 × 10^5
5	68.9 × 10^5	0.55 × 10^5

The resultant solution was added to a general soil container; the soil was first mixed manually, and then using a laboratory shaker to distribute the substances evenly in the soil. The contaminated soil was air-dried for 2 days and mixed again using the laboratory shaker. The soil was stored at 24 °C—25 °C for 7 days under full field water capacity, and was then filled in 0.1-kg vessels with openings at the bottom to return the irrigation water. We used three model plants for this experiment: white mustard (*Sinapis alba*), brown mustard (*Brassica juncea*), and rye (*Secale cereale*). These species can absorb various heavy metal ions from the soil. Among these, the brown mustard is the most widely used species in phyto-extraction research. Several previous studies have demonstrated the high absorption capacity of brown mustard for Cd, Pb, Ni, Cu, and Zn, and its tolerance to stress conditions (Meers et al., 2008). The potential of white mustard has also been evaluated in some studies, and it has been proposed for use in phyto-remediation (Kos et al., 2003;Evangelou et al., 2008;Postniks, 2009). There has been some interest in evaluating the effectiveness of rye in phyto-remediation, because it is widely distributed in Russia. Rye is capable of germinating at 1 °C—2 °C, develops a large biomass within a short time, is insensitive to the acidic reaction of the soil, and is capable of growing on soils with poor bases. Rye makes better use of autumn and spring moisture reserves, which can be an added advantage for phyto-extraction in MSW landfill sites.

The growth experiments were performed in three replicates. After seeding three times every 7 days, 10 mL of the working solution of the hybrid preparation Pochvovit (trademark number 559,263), which comprises the sodium salt of gibberellic acid GA3 and orthochlorophenyl acetic acids (N-tris [-2-hydroxylatel] of ammonium salt orthochlorophenyl acetic acid with auxin-like properties) was added to the soil. The working solution of Pochvovit

Table 2
Results of the analysis of landfill soil samples.

No.	Parameter	TLV, mg/kg	Sampling point					Concentrations exceeding the TLV	Complex formation with HEDP	ln K complex with HEDP
			1	2	3	4	5			
1	Mn	1500	219	158	36	77	678	–	+	9.16
2	As	2	5.9	4.2	2.6	6.4	10.6	1.3–5.3		
3	Hg	2.1	<0.5	<0.5	<0.5	<0.5	<0.5	–		
4	Pb	32	10.7	12.4	12.0	9.4	12.0	–		
5	Cr (III)	6	15.7	24.4	13.1	23.5	59	2.2–9.8	+	
6	Zn	23	37	48	33	27	51	1.4–2.2	+	10.73
7	Cu	3	9.9	21.7	13.2	15.9	18.9	3.3–7.2	+	12.48
8	Co	5	5.7	3.9	5.7	5.2	14.2	1.1–2.8	+	6.04
9	Ni	4	10.2	9.9	5.7	12.6	28.5	1.4–7.1	+	
10	Cd	2	0.18	0.17	0.07	0.14	0.48	–	+	3.7
11	pH		9.2	9.2	2.5	3.2	8.7			
12	Cation exchange capacity (mg, eq/100 g of soil)		31	22	27	19	44			
13	Mobile phosphorus (P_2O_5), mg/kg		258	190	220	35	239			
14	Mobile potassium (K_2O), mg/kg		203	189	195	79	187			
15	Hydrocarbons, mg/kg		137	144	253	46	174	–		

TLV: Threshold limit value.

Table 4
Micromorphological parameters of the isolated microbial isolates (bacterial and yeast cultures).

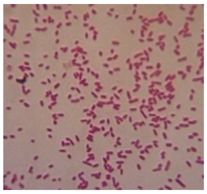
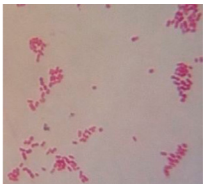
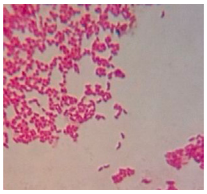
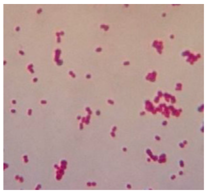
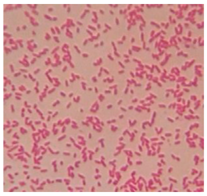
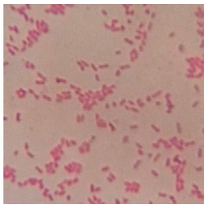
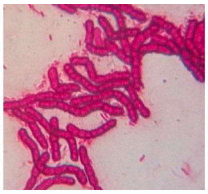
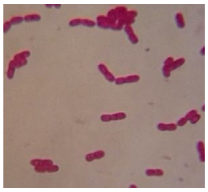
No.	Type of microorganism	Morphology	Colony colour	Form
1	Bacteria	Colonies are circular, conical, flat, and homogeneous	Light cream with yellowish shade	
2	Bacteria	Colonies are circular, strongly convex, droplet-like, with a smooth edge, and homogeneous structure	Light cream	
3	Bacteria	Colonies are circular, conical, flat, and homogeneous	Light cream	
4	Bacteria	Colonies are circular, conical, flat, and homogeneous	Light cream	
5	Bacteria	Colonies are circular, conical, flat, and homogeneous	Light cream	
6	Bacteria	Colonies are circular, conical, flat, and homogeneous	Yellowish	
7	Bacteria	Colonies are circular, conical, flat, and homogeneous	Bright yellow	
8	Bacteria	Colonies are circular, conical, flat, and homogeneous	Yellow	

Table 4 (*Continued*)

No.	Type of microorganism	Morphology	Colony colour	Form
9	Yeast	Colonies are circular, conical, flat, and homogeneous	Light cream	
10	Yeast	Colonies are circular, convex, bulky, and finely grained	Light cream	
11	Yeast	Colonies are circular, convex, with a smooth edge, and homogeneous	Light cream	

was prepared in a conical bulb by adding 100 mL of distilled water to 2 mL of the stock concentrated solution. The morphometric parameters of the plants (length of the shoot, cm) were measured for 20 days of growth using a ruler.

2.9. *Statistical analysis*

Data are presented as arithmetic mean and standard deviation or coefficient of variation, and the data were statistically compared between groups using Fisher's test with Microsoft Office Excel 2007 software.

3. Results and discussion

3.1. *Content of pollutants in soil samples from the landfill*

Table 2 shows polymetallic contamination of the soil in the test site, with heavy metals as the main source of pollution.

The results are consistent with those of previous studies (Fait et al., 2018; Petruzzelli et al., 2018). The concentration of As, Cr (III), Zn, Cu, Ni, and Co in the samples exceeded the threshold limit values (TLVs) by 2–10 times. As the soil samples were collected from the surface layer, it is reasonable to expect that the heavy metal content would be significantly higher in the deeper layers of the landfill and the crust due to element migration. Notably, the pH of the soil samples was significantly higher or lower than the neutral value, ranging from 9.2 to 2.5. Table 2 also shows the metals that formed complexes with HEDP and the stability constants that characterise the strength of these chelate compounds. The average and minimum values of the stability constants of the HEDP complexes with several metals were positive, indicating their potential to convert to their chelate forms with the bioligands of plants when absorbed.

3.2. *Effects of K_2HEDP and diatomite on microbiota of the MSW range*

Considering the moisture content of the soil samples, the total microbial count (CFU/g soil dry weight) was determined, and the results showed that bacteria were the dominant microorganisms in all samples (Table 3).

After inoculating the soil samples using the Koch method, 11 dominant microbial isolates were obtained and identified based on culture in selective nutrient media. Table 4 shows the micromorphological parameters of the isolated microbial cultures (bacterial and yeast cultures) that are resilient to heavy metals.

The resulting mixed cultures were cultured in appropriate liquid nutrient media with different concentrations of K_2HEDP and diatomite mineral additives. Thereafter, the microorganisms were counted by plating samples on solid nutrient media using the Koch method and compared with the counts obtained under the control conditions (no amendment). As shown in Fig. 2, the introduction of a dimerised potassium salt of HEDP at the selected concentrations had a negligible effect on microorganism growth.

The application of the mineral diatomite (Fig. 3) had similar weak effects on microorganism growth. However, there was a difference in the growth of bacteria and yeast; a slight stimulating effect was observed in the mixture of yeast isolates, whereas a slight suppressive effect was observed in the mixture of bacterial cultures.

Fig. 4 shows the effects of the mixed composition of K_2HEDP and NDP-D-700, and the results indicated that the introduction of chelating and mineral additives had no significant effect on the growth of microorganisms; rather, the effect was neutral at the tested concentrations of K_2HEDP and NDP-D-700.

Variations in the total microbial count can be attributed to an error while performing the Koch method and expected sorption of microorganisms on the surface of the mineral diatomite. The microbial community has been shown to be highly resistant to heavy metals, and they can damage organic substances. In chelate-assisted phyto-extraction process, the chelators are typically applied at working concentrations of 0.5–10 ml/kg soil. These values are comparable with those applied in our study to test the effects of K_2HEDP at different concentrations on the state of microbial functional groups in a native landfill soil. Thus, the treatment of heavy metal-contaminated landfill soils with HEDP salts may not substantially disturb the soil cycles. Accordingly, the focus should be on determining the most effective working concentrations of the agents for the absorption of metal ions by plants.

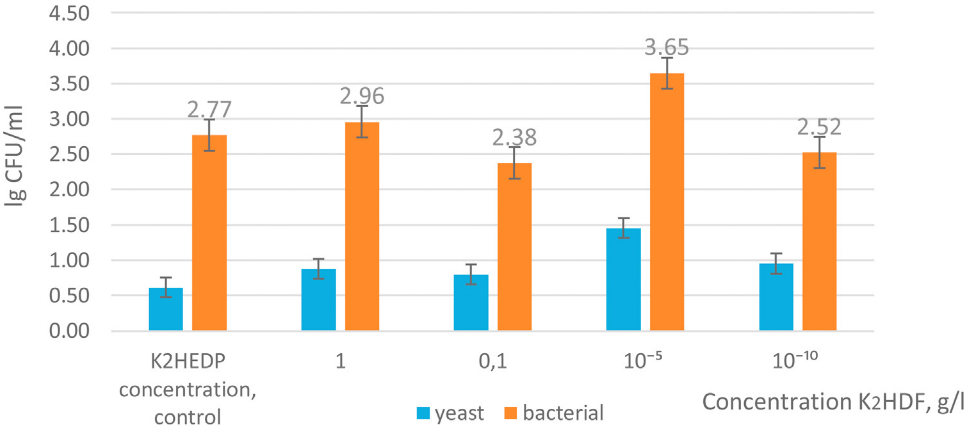


Fig. 2. Growth of the mixed bacterial and yeast isolates in the presence of K_2HEDP.

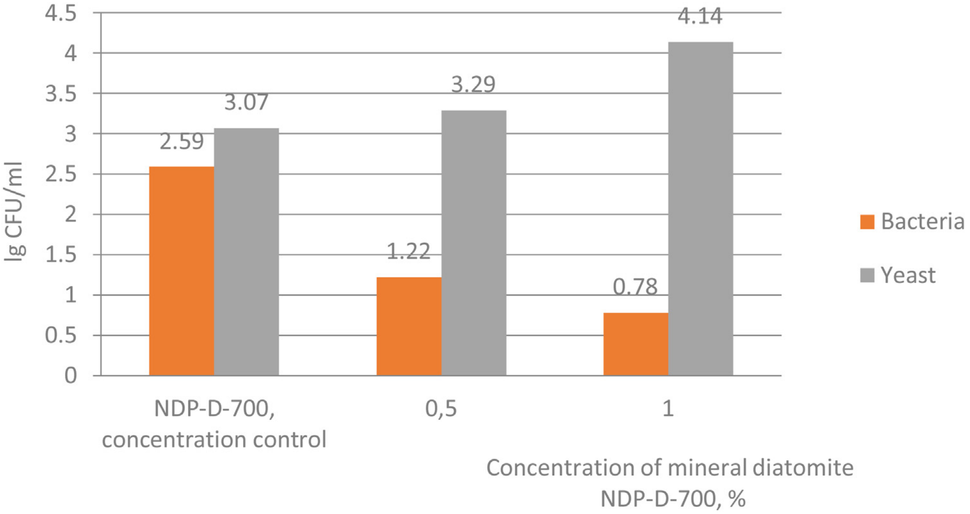


Fig. 3. Growth of the mixed bacterial and yeast isolates in the presence of NDP-D-700 diatomite powder.

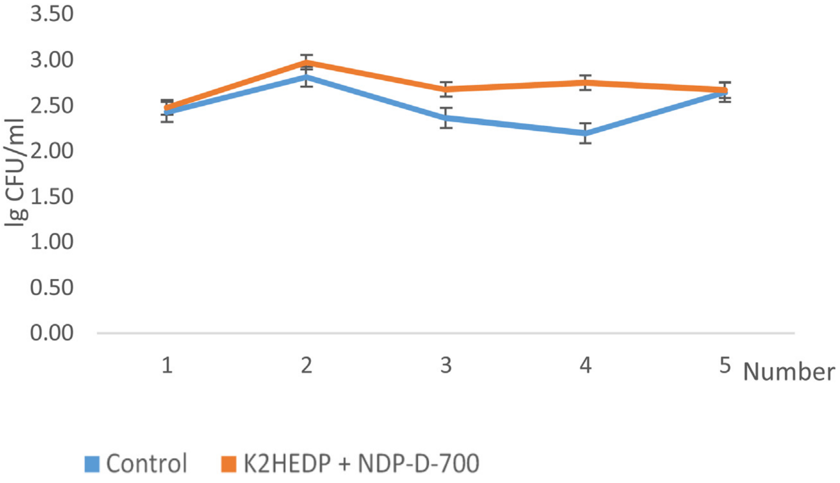


Fig. 4. Effects of the components of a hybrid biopreparation on the growth of mixed culture of hydrocarbon-oxidising microorganisms.

3.3. Vegetation models of soil contamination similar to that in the test site

The average heights of the terrestrial plant species studied are shown in Tables 5–7 and Figs. 5–7.

In November 2019 (Table 5, Fig. 5), more intensive growth of plants was observed in vegetation vessels treated with the hybrid preparation, Pochvovit. The bioadditive had a positive effect on the growth and development of white mustard. In February, the average height of white mustard in vegetation vessels treated with Pochvovit was slightly lower than that in vessels with contaminated soil. The growth of white mustard in the control vessels was slightly delayed compared with that in the other vessels.

Table 5
Heights of the white mustard (*Sinapis alba*) plants, ± 0.05 (cm).

No.	Control sample			Contaminated sample			Contaminated sample + Pochvovit		
	No. 1	No. 2	No. 3	No. 1	No. 2	No. 3	No. 1	No. 2	No. 3
Date of planting: 16.11.2019									
4	5	4.5	3.5	4	3.5	4.5	5	5	4.5
6	6.5	6.5	6	4.5	4	5	6.5	6.5	6
9	7	6.5	6.5	5.5	4.5	5.5	7	7.5	7
11	7.5	6.8	7	6	5	6	7.5	8	8
13	7.5	6.8	7	6.2	5.2	6	8	8	8.5
16	7.8	7	7.2	6.5	5.5	6.2	8.5	8.5	9
18	8	7	7.5	6.5	6	6.2	9	8.8	9.5
20	8	7	7.5	6.5	6.8	6.4	9	9	10
Date of planting: 26.02.2020									
6	4	2.5	3	6	5.5	6	6	5.5	6
7	4.5	3	3	6.5	6.5	7	6.5	7.5	6.5
9	4.5	3	3.5	6.5	7.5	7.5	7	8	7
13	5	4.5	4	8	8.5	9	7.5	8.5	9
14	5	4.5	4	8	8.5	9	7.5	8.5	9
16	5.5	5	4	9	9	9.5	8	9	9.5
19	5.5	5.25	4.25	9	10	10	8.5	9	9.5

Table 6
Height of brown mustard (*Brassica juncea*), ± 0.05 (cm).

Number of days	Control sample			Contaminated sample			Contaminated sample + Pochvovit		
	No. 1	No. 2	No. 3	No. 1	No. 2	No. 3	No. 1	No. 2	No. 3
Date of planting: 16.11.2019									
4	2.25	3	1.75	2.5	2.5	2.8	2.5	2	3.5
6	3.8	3.5	3	3	3	3	2.8	3	3.2
9	4	3.8	3.2	3.5	3.5	3.5	3.5	3.5	4
11	4	3.8	3.2	3.6	4	3.5	3.8	3.5	4.2
13	4	3.8	3.2	3.8	4.5	3.5	4	3.5	4.5
16	4	4	3.5	4	4.5	3.8	4	4	4.8
18	4	4	3.5	4	4.5	4	4	4	4.8
20	4	4	3.5	4	4.5	4	4	4	4.8
Date of planting: 26.02.2020									
6	–	2.5	2	4	3.5	4	4	4.5	4
7	2	3	2	4.5	4	4	4.5	4.5	5
9	3	4	3	5	4.5	4.5	4.5	5	5.5
13	3	5	–	5.5	5	5	5	5.5	6
14	3	5		5.5	5	5	5	5.5	6
16	4	5		5.5	5	5	5.5	6	6
19	4	5		5.5	5	5	5.5	6	6

Table 7
Seedling height of rye (*Secale cereal*), ± 0.05 (cm).

Number of days	Control sample			Contaminated sample			Contaminated sample + Pochvovit		
	No. 1	No. 2	No. 3	No. 1	No. 2	No. 3	No. 1	No. 2	No. 3
Date of planting: 16.11.2019									
4	6.75	6.25	6.5	8	9	7.6	8	8	9.5
6	15.5	17	16	15	15	12	15	16	17
9	19	18	18	17	17	15	17	17	18
11	20	19	18	17	18	18	20	18	19
13	21	20	19	17	18	18	20	18	19
16	21	21	20	17.5	19	18	21	19	20
18	22	22	21	17.5	19	18	21	19	20
20	22	22	21	17.5	19	18	21	19	20
Date of planting: 26.02.2020									
6	5	6	6	6.5	9	5	7.5	9	4.5
7	7	7	8	7	10	7	9	10	8
9	9	9	11	7.5	10.5	9	10.5	10.5	9
13	9.5	10.5	12	10	11	11	11.5	11	10
14	9.5	10.5	12	10	11	11	11.5	11	10
16	9.5	11	12.5	10.5	11.5	11.5	11	11	10.5
19	9.5	11	12.5	12	11.5	12	12	12	11

The average height of brown mustard (Table 6, Fig. 6) in both November and February was slightly higher in vessels containing Pochvovit than in vessels with contaminated soil.

Both experiments showed the positive effects of polymetals in the test site on the growth of rye (Table 7, Fig. 7). The use of the bioadditive in November had a positive effect on the growth and

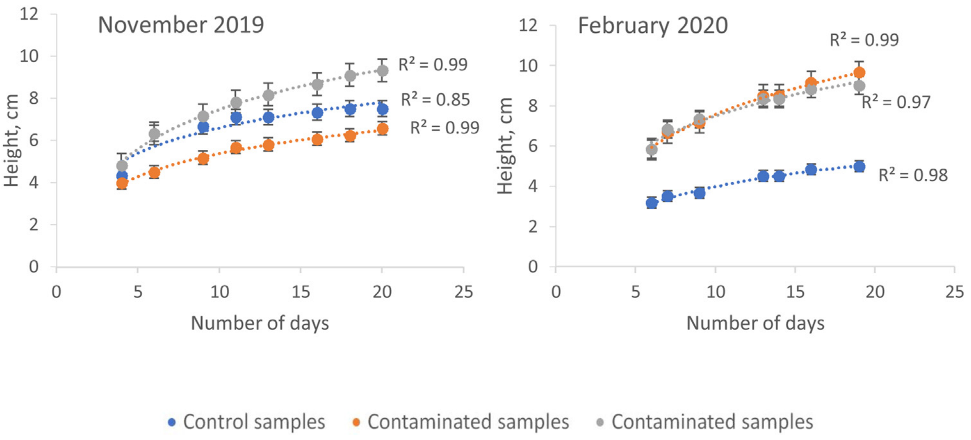


Fig. 5. Average height of white mustard (*Sinapis alba*) over time (days) planted in November 2019 and February 2020.

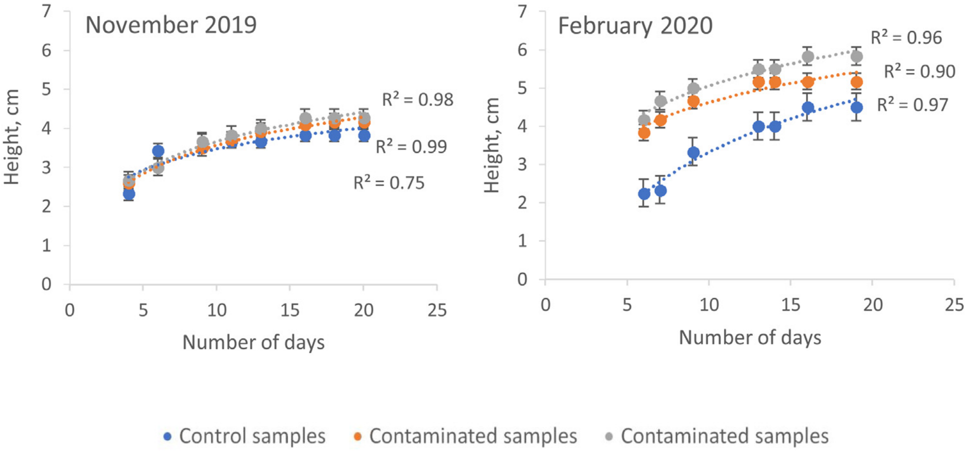


Fig. 6. Average height of brown mustard (*Brassica juncea*) planted in November 2019 and February 2020.

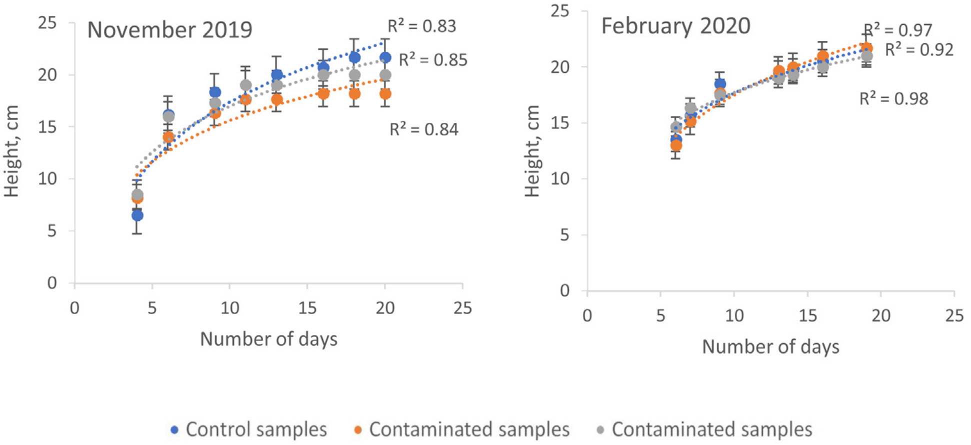


Fig. 7. Average height of rye (*Secale cereale*) planted in November 2019 and February 2020.

Table 8
Average difference in the height of plants among the vegetation vessels.

Plant	Average difference (Δ, cm) in the height of plants between the contaminated and control soils		Average difference (Δ, cm) in soil height between soil additivity and contaminated soil	
	November 2019	February 2020	November 2019	February 2020
White mustard (*Sinapis alba*)	−1.23125	3.76	2.1775	−0.14
Brown mustard (*Brassica juncea*)	0.11375	1.20	0.10125	0.45
Rye (*Secale cereale*)	−1.95375	−0.02	1.43125	−0.05

development of rye. However, in February, there was no significant difference in the height of rye seedlings between the control and contaminated soil vessels (Table 8).

Thus, to increase the growth and plant biomass and improve plant resistance to landfill soil contaminants such as heavy metals, a hybrid biopreparation, comprising an accelerator, a regulator of the biochemical reactions involved in plant cell growth such as gibberellins and HEDP compounds, and several microelements, was added. The results were not always desirable, but positive results were obtained with the most persistent plants, which can be used for phyto-remediation. Based on experiments carried out in different seasons, it can be concluded that the accumulation of heavy metals in plants depends on both season and weather conditions of the year. The uptake of heavy metals by plants is a complex process that depends on many factors, including soil, ecological, and biological properties.

4. Conclusions

The analysis of soil samples in an MSW site in Moscow revealed that the levels of the following heavy metals in the soil exceed their normal levels: As, Cr (III), Zn, Cu, Ni, and Co. Compared with available data for other sites, such polymetallic soil contamination appears to be a common characteristic in previous landfill sites. The microbial community in the soil range mainly comprised bacteria and a few yeasts. Soil treatment with chemical solvents of HEDP did not adversely affect the existing community of microorganisms in the polygon and, consequently, did not affect the soil cycles. The effect of chemical reagents containing HEDP salts of complex preparations on the absorption of metal ions by plants has been experimentally substantiated. Specifically, we demonstrated that the addition of the hybrid preparation Pochvovit, which includes active additives of sodium salt of gibberellic acid GA3 and orthochlorophenyl acetic acids [N-tris(-2-hydroxylethyl) ammonium salt orthochlorophenyl acetic acid], had positive effects on the growth of white mustard, brown mustard, and rye, thus demonstrating its potential for chemo-phyto-extraction in the soil remediation of MSW landfill sites. The next stage of this research will be to select hyper-accumulator plants and determine the most effective working concentration of the chemical components of hybrid preparations that can optimally induce the chemo-phyto-extraction of polymetallic pollutants from landfill soils.

Data statement

As the data were unsuitable to post, survey respondents were assured that raw data would remain confidential and would not be shared.

Data not available or the data that have been used are confidential.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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