Taylor & Francis
Taylor & Francis Group

JOURNAL OF ENVIRONMENTAL ENGINEERING AND LANDSCAPE MANAGEMENT
ISSN 1648–6897 print/ISSN 1822-4199 online
2011 Volume 19(4): 316–325
http://dx.doi.org/10.3846/16486897.2011.634056

USAGE OF PLANTS FOR FAT-POLLUTED SOIL TREATMENT

Vaiva Kačkytė[1], Saulius Grigiškis[2], Dainius Paliulis[3], Jolanta Aikaitė-Stanaitienė[4]
[1, 3, 4]*Department of Environmental Protection, Vilnius Gediminas Technical University, Saulėtekio al. 11, LT-10223 Vilnius, Lithuania*
[1, 2, 4]*Joint Stock Company Biocentras, Graičiūno g. 10, LT-02241 Vilnius, Lithuania*
E-mails: [1]vaivakackyte@gmail.lt (corresponding author); [3]dainius@vgtu.lt; [2, 4]biocentras@biocentras.lt

Submitted 15 Mar. 2010; accepted 27 Oct. 2010

Abstract. Following the complex technology for greasy waste utilization, which is under development, two stages of fat degradation – biodegradation and phytoremediation – were applied for treatment of fat-polluted soil. Biodegradation was used in the first stage, and phytoremediation was applied for degradation of residual fat and final restoration of soil structure. The latter technology was used to evaluate the ability of three following species of herbaceous plants to degrade fat in soil: red clover (*Trifolium pratense* L.), meadow fescue (*Festuca pratensis* Huds.), and hybrid ryegrass (*Lolium hybridum* Hausskn.). The evaluation after the stages of phytoremediation showed that hybrid ryegrass was the most effective in fat degradation in soils with high initial fat concentrations (100 g/kg and 55.1 g/kg), i.e. 53% and 67% respectively. With lower initial fat contents in soil (up to 12.7 g/kg), the best ability in fat degradation was determined in hybrid ryegrass and red clover, i.e. 76%. Application of stages of biodegradation and phytoremediation for treatment of soil polluted with greasy contaminants helped to reduce fat contents by 99% with the initial fat concentration of 138.9 g/kg, and 90% with 222.2 g/kg.

Keywords: greasy waste, bioremediation, phytoremediation, red clover (*Trifolium pratense* L.), hybrid ryegrass (*Lolium hybridum* Hausskn), meadow fescue (*Festuca pratensis* Huds.).

1. Introduction

Continuous global expansion of urbanization, growth of meat, fish and other industry, and increase in production capacities results in growing amounts of greasy waste of vegetal and animal origin. Following environmental requirements of the European Union, landfills reduce the amount of accepted biodegradable waste continuously every year (European Union... 2006; Baltrėnas, Kvasauskas 2008), thus this leads to the issue of greasy waste treatment.

Thermal, physicochemical, and biological methods are usually used for treatment of soil polluted with organic contaminants. The most economically effective are the methods of biological treatment: biodegradation, aeration, bioremediation (composting, biorecovery, biostimulation, etc.). One of the cheapest and the most effective methods of soil treatment is bioremediation. This is a natural process used globally to degrade harmful organic and inorganic substances. Technological processes of bioremediation are based on application of plants (phytoremediation), microorganisms (remediation by microorganisms), and animals (zooremediation), which use environment-contained contaminants as a substrate to maintain their own growth and metabolism (Gupta *et al.* 2003; Kalėdienė 2009).

One of the soil-contained contaminant treatment methods used in bioremediation is composting. During the process of composting, microorganisms degrade organic waste in soil and the final good quality product – compost – is obtained (Haug 2000). Phytoremediation is usually applied for small amounts of organic or inorganic compounds in soil and water; treatment of contaminated wastewater and accumulation of biogenic substances; and restoration of soil structure after application of other treatment methods (Liužinas, Paunksnytė 2008). This contamination treatment method is considered as an alternative to physical remediation, which is destructive to the environment (Gupta *et al.* 2003).

Method of phytoremediation used for improvement of soil properties and its structure restoration is economical and effective as it requires less maintenance than conventional treatment methods (Landmeyer 2001; Trapp, Karlson 2008; Jankevičius, Liužinas 2003). Effectiveness of phytoremediation depends on physical and chemical properties of contaminants, their concentration in soil, and species of plants used (Gao, Zhu 2003). This method is the final stage of degradation of organic (e.g. fat) and inorganic contaminants.

For soil restoration, the following taller cultivated or wild plants can be used for phytoremediation: trees – wild pine (*Pinus sylvestris* L.), eastern poplar (*Populus deltoides* W. Bartramex Marshall), willow (*Salix* sp.); spiked grasses – red fescue (*Festuca rubra* L.), smooth meadow-grass (*Poa pratensis* L.), perennial ryegrass (*Lolium perrene* L.), cereal rye (*Secale cereale*); pulse – white clover

(*Trifolium repens* L.), medick (*Medicago* sp.), and etc. Treatment of soils polluted with fat or other organic substances mostly uses pulse and spiked plants as they feature well-evolved root system (Hou *et al.* 2001; Pichtel, Liskanen 2001). Diffuse roots improve ground aeration, increase content of nitrogen and biologically active substances that accelerate degradation of organic contaminants (Jankevičius, Liužinas 2003).

According to the mechanism of contaminant removal from soil, phytoremediation is divided into rhyzodegradation, phytostabilisation, phytodegradation, and phytotranspiration/phytoaccumulation. Depending on contaminant type and environmental conditions, treatment processes can take place simultaneously or separately.

Development of phytoremediation technology needs an appropriate choice of plants. Selection of plants requires consideration of environmental conditions, contaminant uptake capacity, tolerance to drought or flooding, pH, salinity of soil and groundwater, root length, growth rate, and rates of water consumption and evaporation. Fat removal depends on soil granulometric composition, contaminant origin, peculiarities of plant root system, and concentration of microorganisms in the rhizosphere. Contaminant degradation accelerates when there is higher microorganism concentration in rhizosphere and plants have dense root systems (Chang, Carapcioglu 1998; Corgie *et al.* 2004; Hou *et al.* 2001).

Experimental research conducted in the laboratories of the Joint Stock Company Biocentras following the Optimised Complex Technology for Grease Waste Utilization (EUROENVIRON DEGREAS E!3726) project of the EUREKA programme resulted in the development of soil treatment technology (Fig. 1) using two stages of biological treatment: biodegradation and phytoremediation. First treatment stage included degradation of sample greasy waste using Grizinas, a bacterial preparation developed by the JSC Biocentras. Grizinas is a highly effective in fat degradation in both water and soil. The duration of biodegradation stages was 6 months. Results of the research showed that usage of cultures of fat degrading microorganisms resulted in effectiveness of fat degradation of 75–91% in soils with high fat concentrations (222.2 to 138.9 g/kg), and up to 96% with low concentrations (55.6 g/kg) (Aikaitė-Stanaitienė *et al.* 2010). The subsequent stage of treatment – phytoremediation – is used for degrading residual fat and improvement of soil quality; depending on the season and the initial fat concentration in soil, its duration averages 4 to 8 months. The outcome of this two-stage treatment is purified soil, which can be used as compost for improvement of quality of poor soils, cultivation of energy forest, or as fertilizer in fields.

The objective of this paper is to determine capability of red clover (*Trifolium pratense* L.), hybrid ryegrass (*Lolium hybridum* Hausskn.), and meadow fescue (*Festuca pratensis* Huds.) to degrade finally the residual fat after stages of biodegradation, and restore soil structure.

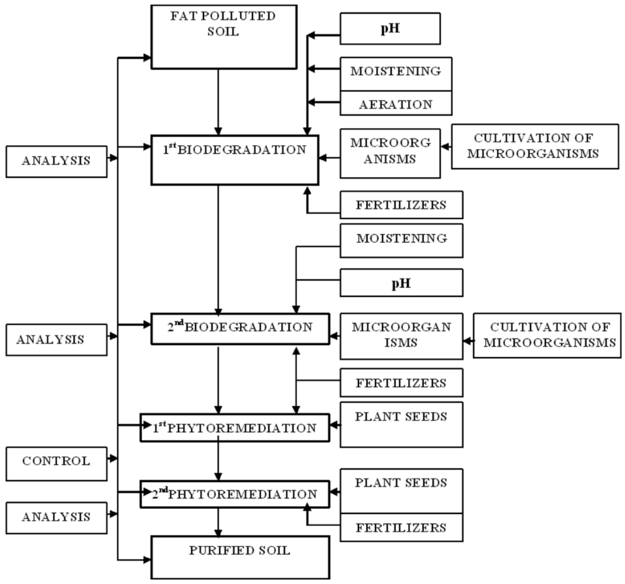

Fig. 1. Technological scheme of fat utilization

2. Research methods

After completing biodegradation stages according to the technological scheme for greasy waste utilization (Fig. 1), possibilities of application of phytoremediation for degradation of residual greasy waste and improvement of soil quality was researched.

For the biodegradation analysis, the following most common fats (according to the composition of fatty acids) were found in waste chosen as a sample substrate: pork fat, beef tallow and vegetable oil, all mixed at the ratio of 1:1:1. The following initial concentrations of sample greasy waste was chosen: 55.6 g/kg, 138.9 g/kg, control 138.9 g/kg (further referred to as C) and 222.2 g/kg, which decreased respectively to 2.5 g/kg, 12.7 g/kg, 100.0 g/kg (C), and 55.1 g/kg at the end of biodegradation stages. No treatment with biopreparation for greasy waste degradation was applied for control sample (C).

For phytoremediation, the soil was obtained after the stages of biodegradation. Residual fat concentrations in soil determined at the end of biodegradation stages were used as a starting point for stages of phytoremediation. Fat-uncontaminated soil with determined initial fat concentration of 0 g/kg (further referred to as C1) and soil with residual fat concentration of 100 g/kg after the stages of biodegradation (further referred to as C2) were chosen for control purposes. These control samples were designed to evaluate growth and development of plants in soils with initial fat content of 0 g/kg and 100 g/kg. Other initial technological parameters were also determined: C/N ratio, pH, an amount of microorganisms, and contents of ammonium nitrogen and orthophosphate phosphorus. Sample mixtures placed into 20-litre vessels prepared for phytoremediation (three vessels of each sample).

Following the data provided in literature, three species of herbaceous **plants** were chosen for phytoremediation:

- Red clover (*Trifolium pratense* L.) – a herbaceous plant in the leguminous (*Fabaceae*) family, clover (*Trifolium*) genus; height 15 to 40 cm.
- Hybrid ryegrass (*Lolium hybridum* Hausskn.) – belongs to the family of true grasses (*Poaceae*), ryegrass (*Lolium*) genus; valuable perennial dwarf forage grass.
- Meadow fescue (*Festuca pratensis* Huds.) – a plant that belongs to the family of true grasses (*Poaceae*), fescue (*Festuca*) genus (Agrolitpa 2006).

Germination capacity of seeds of red clover, hybrid ryegrass, and meadow fescue was determined. Analysis of seed germination capacity was performed under germination-favourable environment conditions (constant temperature of 22 °C). Filtering paper was used as germination medium. It was moisturised with water, which conformed to the following requirements: low content of organic-mineral impurities, pH range from 6.0 to 7.5. Germination took place in watertight vessels, in a lit place, and under constant temperature. Two samples of 400 seeds each were separated randomly from well-mixed batches of clean seeds of each plant species and then divided into smaller subsamples of 100 seeds each. On the moist medium, seeds were evenly distributed maintaining equal distance between them in order to prevent influence of adjacent seeds on development of growing sprouts and to ensure an even germination of seeds. The number of germinated seeds was calculated and percentages were estimated (Order of the Minister... 2003).

After germination capacity was determined, each soil sample had 100 seeds of every plant species sowed. Plants were cultivated outdoors during the period from April to August. Soil moisture content and development of plants were monitored.

At the end of the first stage of phytoremediation, after 9 weeks from the sowing, plants were pulled up by the roots and weighted; height of plants and root length were measured. Soil analysis was also performed and the following values were determined: pH, an amount of microorganisms (CFU/g), residual fat content (g/kg), contents of ammonium nitrogen and orthophosphate phosphorus, and C/N ratio.

At the beginning of the second stage of phytoremediation, the soil was fertilised and the same plants were sown. The duration of this stage also amounted to 9 weeks. At the end of this stage, plants were pulled up by their roots and weighted; height of plants and root length were measured; pH, the amount of micro organisms (CFU/g), residual fat content (g/kg), contents of ammonium nitrogen and orthophosphate phosphorus (mg/kg), and C/N ratio were determined; and soil quality was estimated.

The Kjeldahl method (LAND 84-2006) was used for determination of total content of nitrogen in soil samples.

The total carbon content was estimated by oxidising soil samples in hot solution of potassium dichromate.

Moisture content was determined by drying of 10 g of soil sample in a drying oven at 105 °C± 2 °C for 40 to 48 hours until invariable weight (Alef, Nannipieri 1995).

pH value in samples was measured using InoLab 720 pH meter (Germany) working with the mixture of analyzed soil and 1 M potassium chloride solution mixed at the ratio of 1:2.5 (LST ISO 10390:2005).

Fat concentration was determined using the method of Soxhlet (Alef and Nannipieri 1995).

The spectrophotometric method (LAND 38-2000, LAND 58-2003) was used for determination of contents of ammonium nitrogen and orthophosphate phosphorus. Optical density was measured using the Shimadzu UV-1601 spectrophotometer (Japan).

3. Research results

The analysis results showed that germination capacity of seeds of red clover, hybrid ryegrass, and meadow fescue reached up to 95–97%. The analysis of two different samples of each of the plant species showed similar results, which stayed within the range of permitted deviation (4–6%), therefore, seeds of all chosen plants were suitable for further research.

Changes of fat concentration in soil were determined at the end of the first (after 9 weeks) and the second (after 18 weeks) stages of phytoremediation. The reduced fat content was determined in all samples after each stage of phytoremediation (Figs 2 and 3).

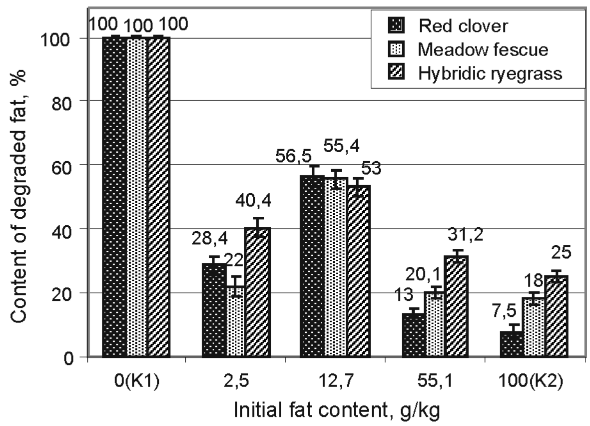

Fig. 2. Rate of fat degradation at the end of the first stage of phytoremediation

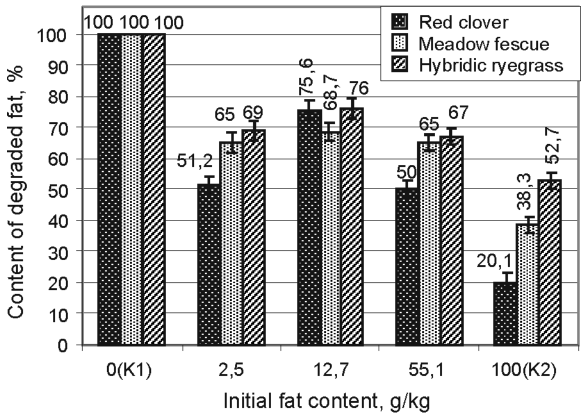

Fig. 3. Rate of fat degradation at the end of the second stage of phytoremediation

At the end of the first stage of phytoremediation, the best degradation of fat was determined in soil with initial fat content of 12.7 g/kg (Fig. 2). This tendency was observed using all of the plant species analysed. Effectiveness of fat degradation in soil with cultivated red clover reached 56.5%, meadow fescue – 55.4%, and hybrid ryegrass – 53%.

With the initial fat concentration in soil of 100 g/kg (C2), fat degradation was slow in all three samples. Cultivation of red clover resulted in the lowest fat degradation rate of 7.5%; it was slightly higher with meadow fescue – 18%; and hybrid ryegrass showed the highest result of 25%.

At the end of the second stage of phytoremediation, with initial fat concentration in soil of 100 g/kg (C2), the effectiveness of fat degradation with cultivated hybrid ryegrass reached 52.7%, meadow fescue – 38.3%, and red clover – 20.1% (Fig. 3). With the initial fat content of 55.1 g/kg, hybrid ryegrass was the most effective in fat degradation – 67%, meadow fescue – 65%, and red clover – 50%. Similar fat degradation rate (around 76%) was determined using red clover and hybrid ryegrass when the initial fat concentration in soil was 12.7 g/kg. In soils with low initial fat contents (2.5 g/kg), it was determined that the most significant reduction of fat amounted to 69% and was obtained by cultivating hybrid ryegrass.

Comparison of fat content reduction in all samples showed that the most significant fat degradation was in samples with initial fat concentration of 12.7 g/kg. It is obvious that plants naturalise better in soils polluted with less fat. Fat degradation rate was lower in soils with initial fat concentration of 2.5 g/kg rather than in soils with 12.7 g/kg. In case fat concentration in soil is very low, biodegradation of organic contaminants becomes slower during phytoremediation as plant roots are not capable of reaching all the fat contained in soil. J. Juhanson and his co-authors (2007) also observed similar results.

At the end of the first and second stages of phytoremediation, experimental analysis was conducted and C/N ratio of soil was determined (Figs 4 to 6).

Soil-contained organic carbon maintains ground structure, improves the physical environment for roots to penetrate better into the soil, and the nitrogen is required for protein synthesis (the European Communities 2009). Lack of nitrogen in soil results in rotting and endothermic processes, which feature the release of unpleasant odours into the environment.

C/N ratio is one of the key characteristics of soil. Therefore, monitoring of it is essential during the complete process of phytoremediation. According to the literature data provided by Haug (2000), the appropriate C/N ratio in soil must be 20/1–40/1 and the optimal – 25/1–30/1.

Cultivating red clover (Fig. 4), the highest C/N ratio of 59/1 was determined in soil with initial fat concentration of 100 g/kg (C2). Analogous results of 58/1 were obtained with concentration of 2.5 g/kg. However, at the end of the second stage of phytoremediation, this ratio decreased to 16/1. The appropriate C/N ratio (20/1 to 40/1) was obtained in soils with initial fat concentration of 12.7 g/kg and 55.1 g/kg and reached up to 23/1 and 30/1, respectively.

At the end of the second stage of phytoremediation, cultivation of hybrid ryegrass in soils with initial fat concentration of 12.7 g/kg resulted in optimal C/N ratio of 27/1 (Fig. 5).

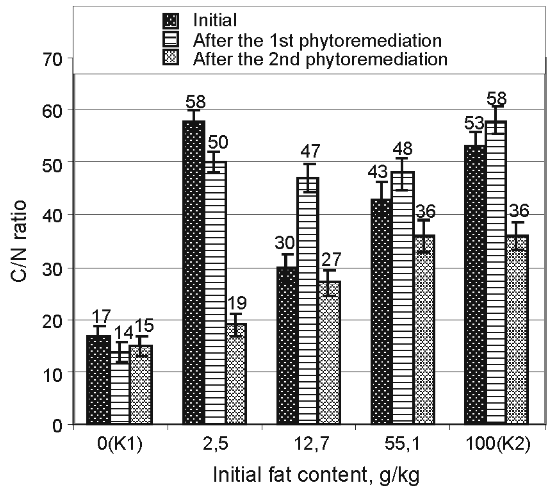

Fig. 5. Evaluation of C/N ratio in soil with hybrid ryegrass

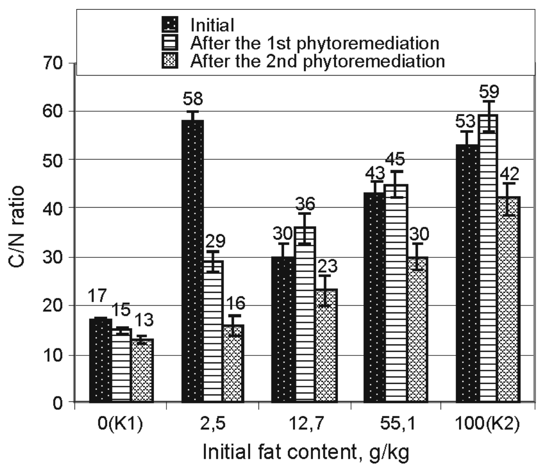

Fig. 4. Evaluation of C/N ratio in soil with red clover

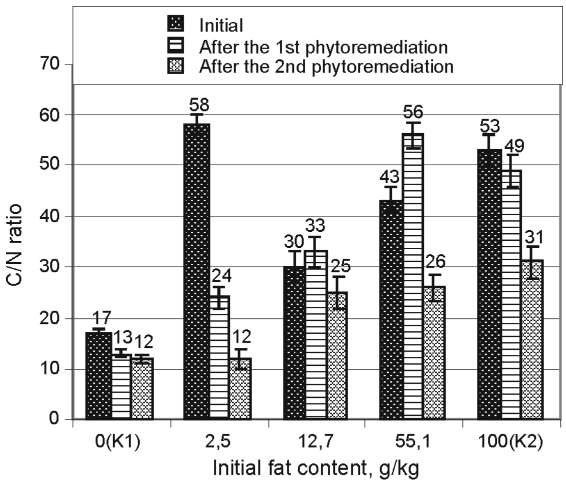

Fig. 6. Evaluation of C/N ratio in soil with meadow fescue

Cultivation of hybrid ryegrass in soil with initial fat concentration of 2.5 g/kg resulted in C/N ratio of 19/1, and with 100 g/kg (C2) – 36/1 (Fig. 5). Fat is the main source of carbon; therefore, the reduction of fat concentration in soil results in respectively lower C/N ratio.

At the end of both stages of phytoremediation, cultivation of meadow fescue in soil with initial fat concentration of 12.7 g/kg resulted in optimal C/N ratio of 25/1 (Fig. 6). The appropriate C/N ratio was determined in soil with initial fat content of 55.1 g/kg and 100 g/kg (C2) and reached up to 26/1 and 31/1, respectively.

At the end of the second stage of phytoremediation, a comparison of C/N ratios showed that appropriate C/N ratio (19/1) in soil with initial fat concentration of 2.5 g/kg obtained by cultivation of hybrid ryegrass. In case of initial fat concentration in soil of 12.7 g/kg, optimal C/N ratio of 25/1 and 27/1 was obtained with meadow fescue and hybrid ryegrass, respectively. Cultivation of red clover resulted in C/N ratio of 30/1 in soils with higher initial fat concentration (55.1 g/kg); with meadow fescue, the ratio was 26/1.

At the end of the first (after 9 weeks) and the second (after 18 weeks) stages of phytoremediation, other parameters, such as pH, contents of ammonium nitrogen and orthophosphate phosphorus, and amount of microorganisms was also determined (Tables 1 to 3).

Table 1. Influence of red clover on soil parameters during phytoremediation

Week	Initial fat concentration, g/kg	Parameters researched			
		pH	NH_4^+-N, mg/kg	PO_4^{3-}-P, mg/kg	Amount of microorganisms, CFU/g
0	0(C1)	6.15	402.3	289.4	$1.9\cdot10^5$
	2.5	6.10	385.7	264.9	$8.4\cdot10^5$
	12.7	6.29	62.5	20.2	$3.6\cdot10^5$
	55.1	5.87	174.4	18.3	$4.2\cdot10^5$
	100(C2)	5.32	237.9	45.7	$3.7\cdot10^5$
9	0(C1)	6.31	55.8	4.5	$1.9\cdot10^5$
	2.5	6.23	23.3	3.7	$1.1\cdot10^6$
	12.7	6.51	42.3	2.0	$4.2\cdot10^5$
	55.1	6.25	69.9	3.0	$4.8\cdot10^5$
	100(C2)	6.23	74.7	3.1	$6.0\cdot10^5$
18	0(C1)	6.80	77.1	2.5	$2.0\cdot10^5$
	2.5	6.70	31.9	1.9	$2.0\cdot10^6$
	12.7	6.94	66.4	7.0	$5.4\cdot10^5$
	55.1	6.7	105.6	2.7	$5.0\cdot10^5$
	100(C2)	6.81	93.3	2.2	$7.0\cdot10^5$

Comparison of pH results of soil samples with the initial values (Tables 1 to 3) showed the insignificant increase of pH in all samples at the end of the first stage of phytoremediation; the value of pH has reached its optimal level (pH 6.5–7.0) at the end of the second stage of phytoremediation. According to Haug (2000), the appropriate value of soil pH must range from 5.5 to 9.0 and the optimal – from 6.5 to 8.0.

The analysis of the soil with red clover showed the decrease of contents of ammonium nitrogen and orthophosphate phosphorus at the end of the first stage of phytoremediation (Table 1). The results show the reduction of contents of ammonium nitrogen and orthophosphate phosphorus, as these elements are sources of easily absorbed nutrients for such plants and are necessary for the formation of their root systems and maintenance of growth.

During stages of phytoremediation, the total amount of microorganisms in soil with red clover was evaluated (Table 1). The amount of microorganisms in soil is essential as higher concentration of microorganisms in the rhizosphere results in acceleration of fat degradation. It was determined that the amount of microorganisms has been increasing insignificantly or stayed stable in all soil samples.

Similar results were obtained during the analysis of soil with meadow fescue and hybrid ryegrass (Tables 2 to 3).

All soil samples with hybrid ryegrass showed higher pH than other plant species (Table 3).

Table 2. Influence of meadow fescue on soil parameters during phytoremediation

Week	Initial fat concentration, g/kg	Parameters researched			
		pH	NH_4^+-N, mg/kg	PO_4^{3-}-P, mg/kg	Amount of microorganisms, CFU/g
0	0(C1)	6.15	402.3	289.4	$1.9\cdot10^5$
	2.5	6.10	385.7	264.9	$8.5\cdot10^5$
	12.7	6.29	62.5	20.2	$3.6\cdot10^5$
	55.1	5.87	174.4	18.3	$4.2\cdot10^5$
	100(C2)	5.32	237.9	45.7	$3.7\cdot10^5$
9	0(C1)	6.28	49.8	6.2	$1.9\cdot10^5$
	2.5	6.11	17.7	5.9	$9.8\cdot10^5$
	12.7	6.53	36.3	1.7	$6.8\cdot10^5$
	55.1	6.18	79.9	4.6	$5.5\cdot10^5$
	100(C2)	6.24	75.6	4.4	$7.6\cdot10^5$
18	0(C1)	6.79	65.9	2.8	$2.1\cdot10^5$
	2.5	6.65	20.5	2.0	$2.5\cdot10^6$
	12.7	6.89	19.9	5.8	$2.8\cdot10^6$
	55.1	6.63	92.5	6.9	$5.7\cdot10^5$
	100(C2)	6.84	102.4	7.7	$9.0\cdot10^5$

Table 3. The influence of hybrid ryegrass on soil parameters during phytoremediation

Week	Initial fat concentration, g/kg	Parameters researched			
		pH	NH_4^+-N, mg/kg	PO_4^{3-}-P, mg/kg	Amount of microorganisms, CFU/g
0	0(C1)	6.15	402.3	289.4	$1.9\cdot10^5$
	2.5	6.10	385.7	264.9	$8.5\cdot10^5$
	12.7	6.29	62.5	20.2	$3.6\cdot10^5$
	55.1	5.87	174.4	18.3	$4.2\cdot10^5$
	100(C2)	5.32	237.9	45.7	$3.7\cdot10^5$
9	0(C1)	6.81	45.8	7.1	$2.0\cdot10^5$
	2.5	6.32	15.5	4.6	$1.0\cdot10^6$
	12.7	6.61	38.1	4.4	$3.1\cdot10^5$
	55.1	6.28	72.7	2.0	$5.6\cdot10^5$
	100(C2)	5.94	69.9	4.9	$5.6\cdot10^5$
18	0(C1)	7.01	57.1	2.5	$2.0\cdot10^5$
	2.5	6.80	16.2	1.8	$2.3\cdot10^6$
	12.7	6.84	56.0	7.8	$3.2\cdot10^5$
	55.1	6.8	90.3	2.3	$6.1\cdot10^5$
	100(C2)	6.94	85.1	5.2	$6.1\cdot10^5$

The amount of microorganisms in soil samples showed an insignificant increase or remained stable (Tables 2 to 3). The highest amount of microorganisms was determined in soil with the lowest fat concentration (2.5 g/kg). This can be explained by the fact of improved aeration, sufficient moisture content, better-developed plant root system, and etc. Low fat content in soil ensures better growth-favourable conditions for microorganisms.

The increase of C/N ratio in all samples with the initial fat concentrations of 12.7 g/kg, 55.1 g/kg, and 100 g/kg (C2) observed at the end of the first stage of phytoremediation can be related to the reduction of ammonium nitrogen concentrations. An increase of ammonium nitrogen content was observed at the end of the second stage of phytoremediation (Tables 1 to 3).

Morphological analysis of red clover, hybrid ryegrass, and meadow fescue was performed at the end of both stages of phytoremediation. Ten plants from each of the analysis vessels were pulled up by roots randomly; height of plants and root length were measured; plant weight was determined (Figs 7 to 12).

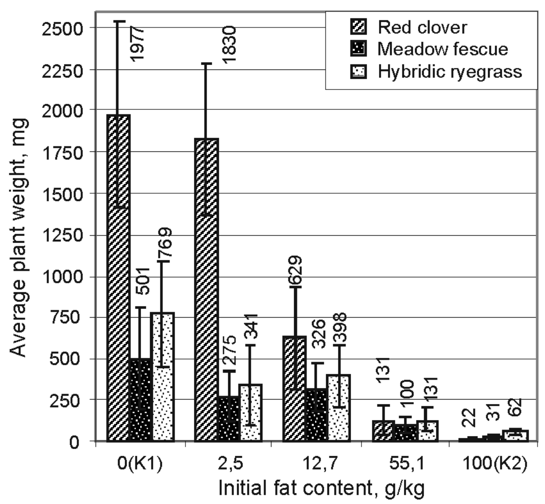

Fig. 7. Average weight of plants after the first stage of phytoremediation

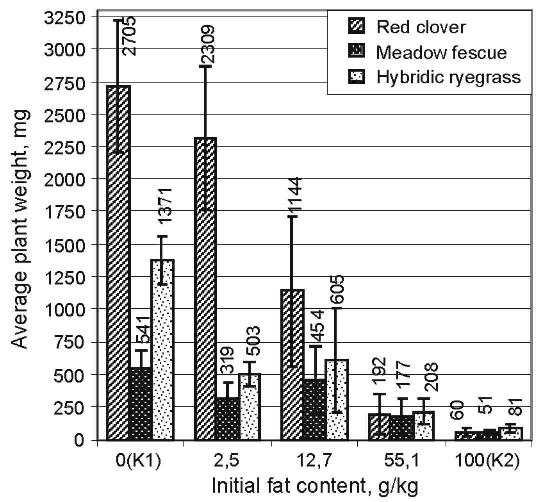

Fig. 8. Average weight of plants after the second stage of phytoremediation

Analysis and comparison of separate plants (Figs 7 and 8) shows that cultivation of plants during the second stage of phytoremediation results in significantly higher average weights than during the first stage.

Red clover was distinguished by the higher average weight of separate plants in soil samples with lower initial fat content (2.5 g/kg and 12.7 g/kg). The highest average weight of plants (2.705 g) was determined in the C1 sample of red clover at the end of the second stage of phytoremediation (Fig. 8). The lower average weight (2.309 g) of separate red clover plants was found in soil samples with initial fat concentration of 12.7 g/kg. In soils with high initial fat concentration (100 g/kg (C2) and 55.1 g/kg), germination of red clover was weak and so was the development. Meanwhile, growth of hybrid ryegrass was good even with higher fat concentrations (55.1 g/kg and 100 g/kg); average weight of separate plants of hybrid ryegrass reached 0.208 g and 0.081 g, respectively.

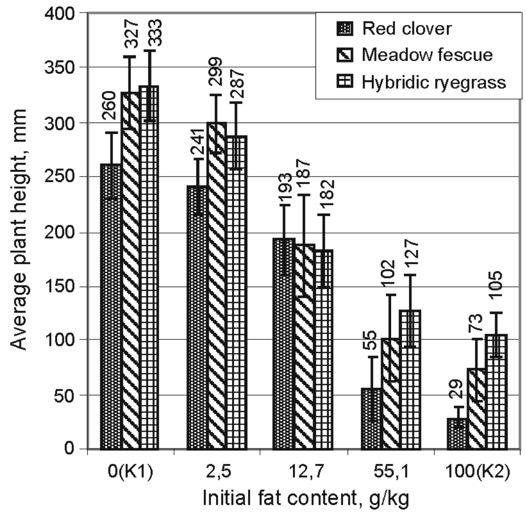

Fig. 9. Average height of plants after the first stage of phytoremediation

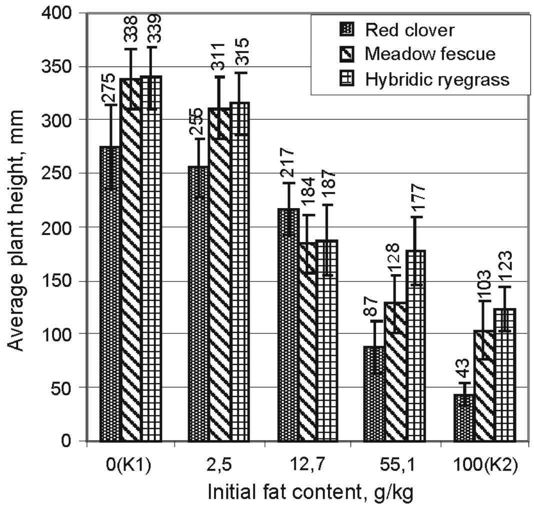

Fig. 10. Average height of plants after the second stage of phytoremediation

Measurement of plant height showed different data distribution among plant species (Figs 9 to 10). On the increase of initial fat concentration in soil, reduction of average plant height was observed for all plants during both stages of phytoremediation.

At the end of the first stage of phytoremediation, it was determined that hybrid ryegrass has been growing better than other plants (Fig. 9) in soils with high initial fat concentrations (100 g/kg (C2) and 55.1 g/kg); average heights of these plants were 105 mm and 127 mm respectively. In soils with the same initial fat concentrations, meadow fescue showed an average growth (73 mm and 102 mm), and the smallest height values were of red clover, amounting to 29 mm and 55 mm.

The growth of meadow fescue was also good in soils with low fat concentration. However, initial fat concentration of 12.7 g/kg was more favourable to the growth of red clover as it reached 193 mm.

At the end of the second stage of phytoremediation, analogous tendencies of average plant height and weight distribution was observed. However, plants of all species were heavier and taller. Evaluation of plants of all species (Figs 11 and 12) cultivated in soils with the lowest initial fat concentration (2.5 g/kg) showed that the average root length had practically no difference from the C1 control samples at the end of both the first and the second stages of phytoremediation.

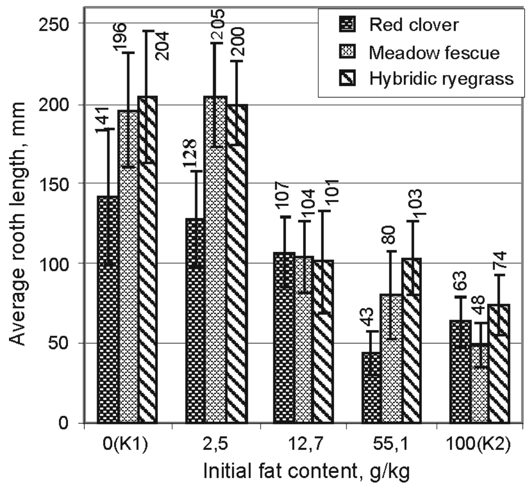

Fig. 11. Average length of plant roots at the end of the first stage of phytoremediation

This leads to a conclusion that low initial fat concentration has no significant influence on the formation of a root system.

During the second stage of phytoremediation, root length of hybrid ryegrass from soils with initial fat concentration ranging from 12.7 g/kg and 100 g/kg (C2) remained almost the same (ranged from 113 mm to 93 mm), and it was still smaller than C1 by around 50% (Fig. 12). Cultivation of meadow fescue showed similar tendency in soils with initial fat concentration of 12.7 g/kg and 55.1 g/kg. The shortest roots were of red clover. For soils with initial fat concentration of 12.7 g/kg, it was similar to the length of hybrid ryegrass, i.e. 115 mm. Comparison of morphological data of plants allows stating that values of average weight, height, and root length of red clover, hybrid ryegrass, and meadow fescue were higher at the end of the second stage of phytoremediation than after the first stage.

This was due to the reduced fat concentration in soil and improved ground characteristics.

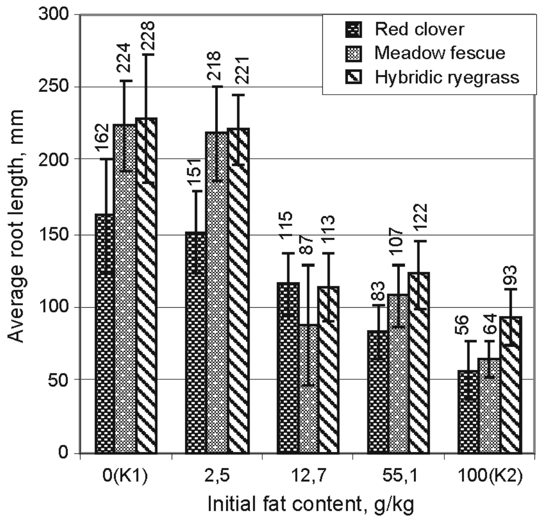

Fig. 12. Average length of plant roots at the end of the first stage of phytoremediation

The research results allow stating that out of the species researched for phytoremediation in soils with higher initial fat waste contents (up to 100 g/kg), hybrid ryegrass and meadow fescue are the most suitable plants. Red clover might only be used in soils with low (up to 10 g/kg) fat concentrations. Morphological parameters can be seen in Figs 13 and 14. The summary of the research results allows stating that this technology is effective and can be used for treatment of greasy waste. Using a combination of stages of biodegradation and phytoremediation, a high degree degradation of greasy waste can be achieved (Table 4).

Table 4. Rate of fat contaminant degradation at the end of phytoremediation stages

Initial fat concentration, g/kg	Content of degraded fat in soil, %			
	After biodegradation	After phytoremediation		
		Hybrid ryegrass	Meadow fescue	Red clover
55.6	96	98.6	98.4	97.8
138.9	91	97.8	97.2	97.8
222.2	75	91.8	91.3	87.6
138.9 (C)	28	66.2	55.4	42.2

The Table 4 shows that application of complex technology for greasy waste utilization helps in achieving the degree of fat degradation of up to 99% by cultivation of hybrid ryegrass, meadow fescue, and red clove in soils with initial fat concentration of 55.6 g/kg and 138.9 g/kg, and up to 90% in soils with high (222.2 g/kg) initial greasy waste concentrations.

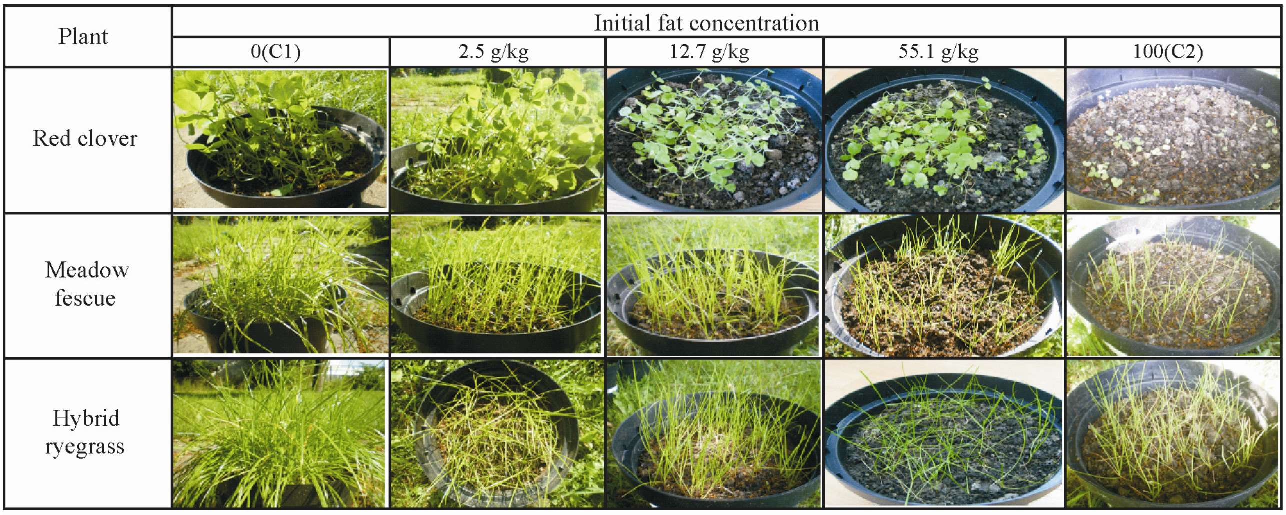

Fig. 13. Plants at the end of the first stage of phytoremediation

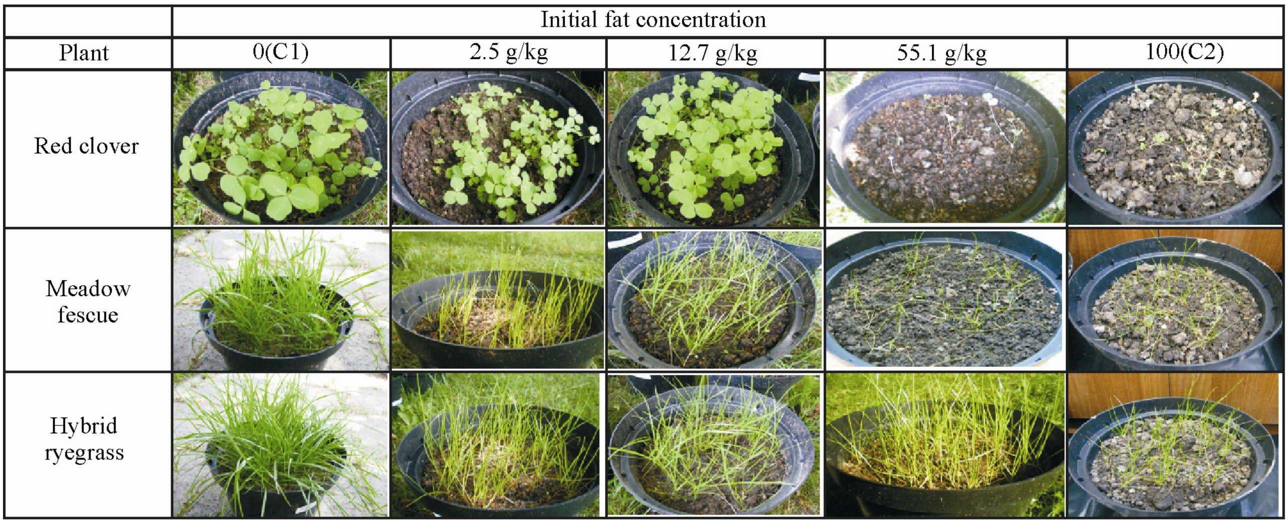

Fig. 14. Plants at the end of the second stage of phytoremediation

In the control soil sample (C) with no biopreparation added, the most effective in fat degradation was hybrid ryegrass with 66.2%. The purified soil can be used for improvement of quality of poor soils.

4. Conclusions

1. It was determined that phytoremediation is a suitable method for treatment of soil with residual fat after the primary stages of biodegradation, and for improvement of soil properties.

2. The research results show that phytoremediation can be applied for treatment of soil with initial fat concentration under 50 g/kg. Plants suitable for treatment of such soil are hybrid ryegrass and meadow fescue. Red clover can be used for phytoremediation in soils with initial fat concentration under 10 g/kg.

3. The research resulted in the most effective fat degradation during cultivation of hybrid ryegrass in soils with initial fat concentration up to 55.1 g/kg; fat concentration in soil reduced by up to 67%.

4. The results of the conducted research allow stating that C/N ratio in soil samples depends on fat concentration in soil. As it was determined at the end of the second stage of phytoremediation, cultivation of hybrid ryegrass and meadow fescue in soil samples with initial fat concentrations ranging from 12.7 g/kg to 100 g/kg resulted in the appropriate C/N ratio (25/1-36/1).

5. It was determined that average weight of separate plants depended on fat concentration in soil. Low initial fat concentrations in soils of 2.5 g/kg and 12.7 g/kg resulted in the highest average weight of red clover amounting to 2.309 g and 1.144 g, slightly lower weight of hybrid ryegrass pieces – 0.503 g and 0.606 g, and finally of meadow fescue – 0.319 g and 0.454 g.

6. Experimental analysis showed that plant height depended on fat concentration in soil. The biggest values of plant height ranging from 123 to 177 mm were of hybrid ryegrass in soils with high initial fat concentration (100 g/kg and 55.1 g/kg).

7. Application of complex technology for greasy waste utilization allows reaching the degree of fat degradation of up to 99%.

References

Agrolitpa: Pašarinių augalų sėklos, prieskoniai. 2006 [online]. [cited 11 January 2010]. Available from Internet: <http://www.agrolitpa.lt/about.php?page_menu=18&page_id=15&lng=LT>.

Aikaitė-Stanaitienė, J.; Grigiškis, S.; Levišauskas, D.; Čipinytė, V.; Baškys, E.; Kačkytė, V. 2010. Development of fatty waste composting technology using bacterial preparation with lipolytic activity, *Journal of Environmental Engineering and Landscape Management* 18(4): 296–305. doi:10.3846/jeelm.2010.34

Alef, K.; Nannipieri, P. 1995. *Methods in applied soil microbiology and biochemistry.* London: Academic Press, Harcourt Brace&Company. 578 p.

Baltrėnas, P.; Kvasauskas, M. 2008. Experimental investigation of biogas production using fatty waste, *Journal of Environmental Engineering and Landscape Management* 16(4): 178–187. doi:10.3846/1648-6897.2008.16.178-187

Chang, Y. Y.; Carapcioglu, M. Y. 1998. Plant – enhanced subsurface bioremediation of nonvolatile hydrocarbons, *Journal of Environmental Engineering* 124(2): 162–169. doi:10.1061/(ASCE)0733-9372(1998)124:2(162)

Corgie, S. C.; Beguiristain, T.; Leyval, C. 2004. Spatial distribution of bacterial communities and phenanthrene degradation in the rhizosphere of *Lolium perenne* L. LIMOS (Laboratoire des Interactions Microorganismes-Mineraux-Matiere Organique dans les Sols), *Applied and Environmental Microbiology* 70(6): 3552–3557. doi:10.1128/AEM.70.6.3552-3557.2004

European Communities. May 2009. Sustainable agriculture and Soil Concervation. Soil degradation processes. Organic matter decline. Fact sheet No. 3.

European Union directive 2006/12/EC on waste (Official Journal L 114, 27/04/2006). 13 p.

Gao, Y. Z.; Zhu, L. Z. 2003. Phytoremediation and its models for organic contaminated soils. Department of Environment Science, Zhejiang University, *Journal of Environ Sci. China* 15(3): 302–310.

Gupta, A. K.; Yunus, M.; Pandey, P. K. 2003. *Bioremediation: Ecotechnology for the present century* [online]. EnviroNews [cited 11 December 2009]. Available from Internet: <http://isebindia.com/01_04/03-04-2.html>.

Haug, R. T. 2000. *The practical handbook of compost engineering*. USA: Lewis publishers. 717 p.

Hou, F. S.; Milke, M. W.; Leung, D. W.; MacPherson, D. J. 2001. Variations in phytoremediation performance with diesel – contaminated soil, *Environmental Technology* 22(2): 215–222. doi:10.1080/09593332208618301

Jankevičius, K.; Liužinas, R. 2003. *Aplinkos biologinis valymas. Grunto valymo technologijos*. Vilnius. 343 p.

Juhanson, J.; Truu, J.; Heinaru, E.; Heinaru, A. 2007. Temporal dynamics of microbial community in soil during phytoremediation field experiment, *Journal of Environmental Engineering and Landscape Management* 15(4): 213–220. doi:10.1080/16486897.2007.9636933

Kalėdienė, L. 2009. *Grunto bioremediacijos mikrobiologiniai tyrimai.* Habilitacijos procedūrai teikiamų mokslo darbų apžvalga. Vilnius. 42 p.

LAND 38-2000. Vandens kokybė. Amonio kiekio nustatymas. Rankinis spektrometrinis metodas.

LAND 58:2003. Vandens kokybė. Fosforo nustatymas. Spektrometrinis metodas, vartojant amonio molibdatą.

LAND 84-2006. Vandens kokybė. Kjeldalio azoto nustatymas. Mineralizavimo selenu metodas.

Landmeyer, J. E. 2001. Monitoring the effect of poplar trees on petroleum-hydrocarbon and chlorinated-solvent contaminated ground water, *International Journal of Phytoremediation* 3(1): 61–85. doi:10.1080/15226510108500050

Liužinas, R.; Paunksnytė, I. 2008. Biotechnologijos aplinkosaugoje, in *Materials of Environmental Protection Engineering: 11th Conference of Junior Researchers Science – Future of Lithuania, held in Vinius on April 3, 2008*. Vilnius: Technika, 25–35.

LST ISO 10390:2005. Soil quality. Determination of pH. 7 p.

Order of the Minister of Agriculture. October 17, 2003. No. 3D-436. On affirmation of method of seed germinative capacity analysis. Vilnius. 19 p.

Pichtel, J.; Liskanen, P. 2001. Degradation of diesel fuel in rhizosphere soil, *Environmental Engineering Science* 18(3): 145–157. doi:10.1089/109287501750281040

Trapp, S.; Karlson, U. 2008. Aspects of phytoremediation of organic pollutants, *Journal of Soils and Sediments* 1(1): 37–43. doi:10.1007/BF02986468

RIEBALAIS UŽTERŠTO GRUNTO VALYMAS NAUDOJANT AUGALUS

V. Kačkytė, S. Grigiškis, D. Paliulis, J. Aikaitė-Stanaitienė

Santrauka

Gruntui, užterštam riebalinėmis atliekomis, valyti pagal kuriamą kompleksinę riebalinių atliekų utilizavimo technologiją numatyti du riebalų skaidymo etapai – biologinis skaidymas ir fitoremediacija. Pirmuoju etapu taikyta biodegradacija, o likusiems riebalams skaidyti ir grunto struktūrai galutinai atkurti – fitoremediacija, kurios metu įvertinta trijų pasirinktų žolinės augalijos rūšių – raudonųjų dobilų (*Trifolium pratense* L.), tikrųjų eraičinų (*Festuca pratensis* Huds.) ir hibridinių svidrių (*Lolium hybridum* Hausskn.) geba skaidyti riebalus grunte. Po fitoremediacijos etapo nustatyta, kad, esant didelėms pradinėms riebalų koncentracijoms grunte (100 g/kg ir 55,1 g/kg), efektyviausiai riebalus skaidė hibridinės svidrės – atitinkamai 53 % ir 67 %. Esant mažesnėms riebalų koncentracijoms grunte (iki 12,7 g/kg), geriausiai riebalus skaidė hibridinės svidrės ir raudonieji dobilai – 76 %. Riebalais užterštam gruntui valyti etapais taikant biodegradaciją ir fitoremediaciją, riebalų kiekis grunte sumažėjo 99 %, kai pradinė riebalų koncentracija buvo 138,9 g/kg, o kai riebalų koncentracijai 222,2 g/kg – 90 %.

Reikšminiai žodžiai: riebalinės atliekos, biologinis skaidymas, fitoremediacija, raudonieji dobilai (*Trifolium pratens* L.), hibridinės svidrės (*Lolium hybridum* Hausskn), tikrieji eraičinai (*Festuca pratensis* Huds.).

ОЧИСТКА ЗАГРЯЗНЕННЫХ ЖИРАМИ ГРУНТОВ С ИСПОЛЬЗОВАНИЕМ РАСТЕНИЙ

В. Качките, С. Григишкис, Д. Палюлис, Й. Айкайте-Станайтене

Резюме

Согласно создаваемой комплексной технологии утилизации жировых отходов с целью очистки грунта, загрязненного жирами, были применены два этапа для расщепления жиров – биодеградация и фиторемедиация. На первом этапе была применена биодеградация, а для расщепления оставшихся жиров и окончательного восстановления структуры грунта – фиторемедиация, во время которой оценивалась способность трех травянистых видов – клевера лугового (*Trifolium pratense* L.), овсяницы луговой (*Festuca pratensis* Huds.) и плевела гибридного (*Lolium hybridum* Hausskn.) – расщеплять жиры в грунте. После этапа фиторемедиации было установлено, что при высоких заданных начальных концентрациях жиров в грунте (100 и 55,1 г/кг) наиболее эффективно расщеплял жиры плевел гибридный – 53% и 67% соответственно. При более низких начальных концентрациях жиров (до 12,7 г/кг грунта) наиболее высокий процент расщепленных жиров достигался при применении плевела гибридного и клевера лугового – 76%. После применения этапов биодеградации и фиторемедиации для очистки грунта, загрязненного жирами, количество жиров в грунте снизилось на 99% при их начальной концентрации 138,9 г/кг и на 90% при их концентрации 222,2 г/кг грунта.

Ключевые слова: жировые отходы, биоремедиация, фиторемедиация, клевер луговой (*Trifolium pratense* L.), плевел гибридный (*Lolium hybridum* Hausskn), овсяница луговая (*Festuca pratensis* Huds.).

Vaiva KAČKYTĖ. Master's student (environmental protection engineering), Dept of Environmental Protection, Vilnius Gediminas Technical University (VGTU); the JSC „Biocentras“, Vilnius.
Bachelor of Science (applied ecology), ŠU, 2008. Publications: author of 1 research paper. Research interests: ecology, ecological biotechnology, environmental protection.

Saulius GRIGIŠKIS. Dr, Director of the JSC „Biocentras“, Vilnius. Doctor of Technical Science, Moscow Institute of Technology of Food Industry, 1983. First degree in Engineering obtained at Kaunas Polytechnic Institute, 1974. Publications: author of over 50 scientific articles. Conferences: participant of over 40 international and national conferences. Research interests: microbiology, ecological biotechnology, ecology, environmental protection, biological active materials.

Dainius PALIULIS. Dr, Assoc. Prof., Dept of Environmental Protection, Vilnius Gediminas Technical University (VGTU). Saulėtekio al. 11, LT-10223 Vilnius, Lithuania.
Doctor of Technological Sciences (air-cleaning devices), VGTU, 2000. Master of Science, Vilnius University (VU), 1996. Publications: co-author of more than 20 research papers. Research interests: air pollution, chemical pollutants of the environment, environmental chemistry.

Jolanta AIKAITĖ-STANAITIENĖ. Dr, Head of laboratory of chemistry, the JSC „Biocentras, Vilnius. Doctor of Physical Sciences, Vilnius, Institute of Chemistry, 2004. Publications: author of 9 scientific publications. Conferences: participant of over 5 international and national conferences. Research interests: ecology, ecological biotechnology, environmental protection.