

## Herbaceous energy crops for cleaning of soils contaminated by petroleum hydrocarbons

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There is a possibility to create a sustainable method of biomass growth in mid-low contaminated sites soil system using phytoremedial methods in biomass engineering. Main aim of the research was to assess herbaceous plants abilities of clean the soil contaminated by oil hydrocarbons. Results show, that the proper selection of herbaceous energy crops can reach high level of sites treatment at pollution concentration levels from 223 mg/kg to 594 mg/kg of oil hydrocarbons. The soil pollution was reduced by 1.6 – 3.2 times using 3 selected plants. In all cases, the test showed that the soil volume of oil products was lower or slightly higher than threshold value of permissible maximum (200 mg/kg) soil pollution.

*Plants endurance limit has not been reached, visual oil hydrocarbons toxicity effects were not observed. Oil hydrocarbons have significant impact on plant roots and aerial parts lengths and biomass accumulation of both roots and above ground parts. E-2 case accumulated significantly more biomass in contaminated soil than in control group. Other cases showed minor and insignificant differences between the control and experimental groups.*

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### Introduction

In most European Union countries, the national energy strategy emphasizes the importance of biomass and it's development opportunities. The biomass for energy needs increases rapidly, so during the last decades, more and more attention is given in the field of agricultural research, on developing new energy crops for biomass (Kryževičienė et al., 2005; ASU, 2014). Biomass, biofuels and biogas are produced from various common and uncommon herbaceous species due to the introduction of new technologies (ASU, 2014). Herbaceous plant biomass is a great alternative to complement such resources as biomass from forests, agricultural products biomass like straw or peat (Kryževičienė et al., 2005).

Studies conducted in USA and Europe indicates that traditional herbal plants, compared with the short rotation trees, are superior, because of its biomass that can be used in the next year after sowing (Kryževičienė et al., 2005). In general, the herbaceous plants are resistant to adverse weather conditions and various diseases, also can produce high biomass yields. In addition, perennial herbaceous plants can be consistent and can give yield without annual over seeding. Hilly land is protected from erosion and maintains soil fertility and reduces the greenhouse effect as well (Kryževičienė et al., 2005; Sendžikienė et al., 2012).

In order to avoid competition with traditional agriculture and make more efficient use of the country's bio-energy potential, herbaceous energy crops can be cultivated in abandoned land (Tripathi et al., 2016). Combining a variety of scientific disciplines, we can achieve not only energetic goals but also it will further enhance the environmental effect (Pandey et al., 2016). Applying phytoremedial biomass engineering methods, we can easily create the system of anthropogenic pollution damaged areas reconstruction.

Data shows that the soil and groundwater pollution levels exceed the limit of oil hydrocarbons. There are approx. 5000 sites contaminated with hydrocarbons which make up about 40 % of all known sites where could be soil and groundwater pollution in Lithuania (Januševičiūtė et al., 2015). Self-

purification is very limited, slow, some of the cases is not possible, and without purposeful human intervention xenobiotic compounds in the environment is not decontaminated effectively (Kalėdienė, 2009). Traditional soil treatment methods require a lot of resources: large labour resources, energy and time. Thus effective environmental remediation technology is economically attractive (Rubežius, Venslauskas, 2015).

One of these new, low-cost technologies are phyto-cleaning technology, which adapt the unique abilities of plants to absorb contaminants, promoting their degradation or transformation which are used for final soil clean-up. While cleaning of oil-polluted land, soil is mainly stimulated by the phyto- and rhizo- degradation, in order to break down pollutants. Herbal plants with a well-developed root system improves aeration, soil is enriched with biologically active substances, which stimulate the activity of microorganisms (Januševičiūtė et al., 2015). It was found, that use of the plants of microbes and fungi activity in the rhizosphere increases from 10 to 100 times (Ali et al., 2013). The most efficient of these plants cleaners are: alfalfa (*Medicago sativa*), white clover (*Trifolium repens L.*), red clover (*Trifolium pratense*), galega (*Galega orientalis*), switchgrass (*Panicum virgatum*), meadow fescue (*Festuca pratensis*) and other.

### Research methods and conditions

The study was conducted in accordance with the „Organic research regulation“ (2008), LAND 9-2009 and „Ground water monitoring methodological recommendations“ (1999) provided by the requirements and recommendations.

*Test object geographic - technogenic characteristic.* Testing object (old oil base) is in Kaunas region municipality, Raudondvaris town, Didvyrių Street. During 2010 Lithuanian Geological Service (LGT) investigation of „Užterštų teritorijų poveikio vertinimas“, survey conducted and showed that the test object, as a primer and ground water, is contaminated with petroleum products. Concentration of oil products exceeded the established permitted limit values (200 mg/kg) in soil and the pre-set maximum concentration of oil products, with this value, exceeded 72.6 times. Also it was found the soil pollution, which exceeds the limits multicyclic aromatic hydrocarbons (chrysene, benzo (b) fluoranthene, benzo (a) pyrene) (Grotas, 2010).

*Soil preparation.* Depending on the extent of pollution and geological and hydrological characteristics in the test area, there were distinguished four plots, 1 m<sup>2</sup> for each selected plant species. The rest of the territory remained as a control. The soil is cultivated by hand: loosen to a depth of 20-25 cm, by removing bulky waste (bricks, stones and etc.). Later the soil was homogenized. According to the recommendations of an effective agriculture technologies in line of the scientific research results and theoretical microorganisms nutrients needed, to ensure effective oil hydrocarbons conversion, every area fertilized with mineral fertilizers. Research plots re-fertilized after 38 days.

*The selection of plants.* Plants were selected according to soil structure and soil type, pH, moisture content, the contamination source (chemical composition), the ability to assimilate pollutants. These plants are low-maintenance and low-demanding for special growing conditions. Choice of 3 different herbal plant species: E-1 - poaceae plant family; E-2 - amaranthus plant family; E-3 - poaceae plant family.

E-1 - A perennial herb, forage, is very fertile, resistant to drought and frost, 0,6-1,5 m tall, poaceae family plant. The root system is very strong, and therefore forms a very hard turf. The plant is a little demanding on soil, fertilizer and herbicide use, resistant to leaf fungal diseases. This plant is used for biogas, second-generation bio-ethanol production and direct burn (Vilkonis, 2008; Jakiene et al. 2013).

E-2 - adventitious, annual herbaceous, decorative, edible, 20-120 cm tall, amaranthus family plant. It is grown as field crops, farms and cities. Grows well in poorer soil, well withstand drought and heat, almost not attack by pests. It has high heavy metals and petroleum hydrocarbons assimilative properties. From this plant can be produced high quality silage (Vilkonis, 2008; Jakiene et al. 2013).

E-3 - perennial grasses, decorative, poaceae family plant. Grows between 0,5 and 1,5 m in height, while the roots penetrate to a depth of 3 meters. In Lithuania this plant is rare. For eligibility for poor soil and climatic conditions, rapid growth and low demand for plant-care products are considered to be good candidates for biofuel and bio-ethanol production (Vilkonis, 2008, Lithuanian Energy Institute, 2011).

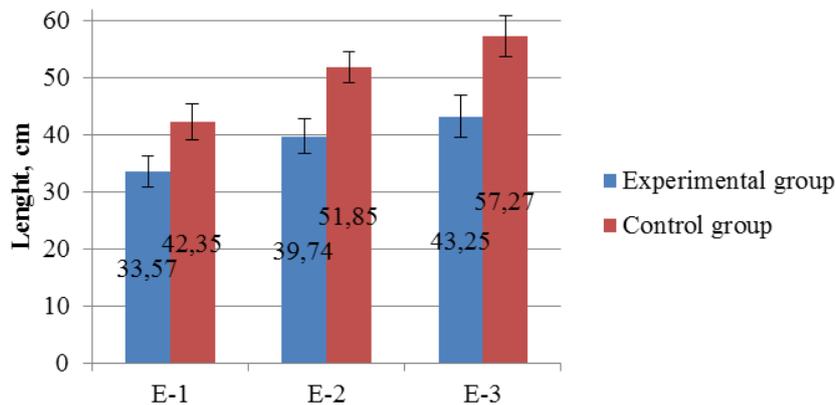
*Soil pollution.* Soil samples were taken after the 131-day of plant growth exposure. The envelope sampling principle was used: in 10 points on test area and 25 evenly distributed points on the research sub-areas. The aforementioned areas were evenly distributed in 25 points samples, in order to compare pollution levels. Soil analyses were performed in accordance with the standards of LAND 89-2010.

*Measurement of plants morphometric parameters.* After an exposure of 84 days (E1) and 134 days (E-2, E-3) the measured roots and ground parts length and the green biomass weight were of randomly selected 15 plants. Measurements of morphometric parameters were taken of Aleksandras Stulginskis University biogas laboratory using a ruler and Kern EG 4200-2NM (readout 0,01 g) scales.

## Results and Discussion

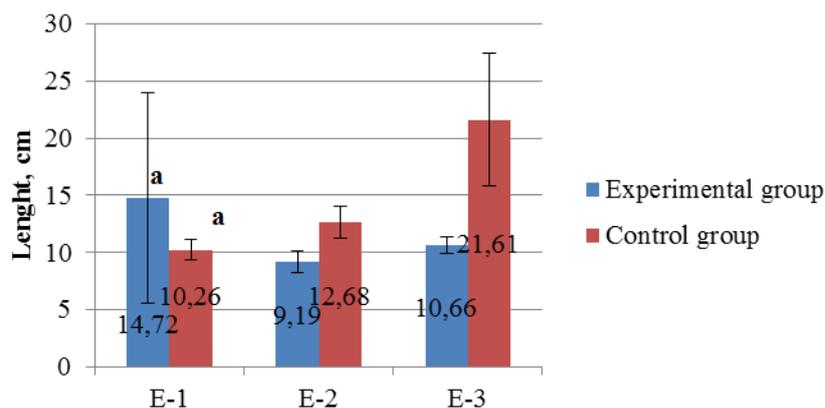
Studies in E-4 have failed (the beginning of visually-coming vegetation E-4 plants, for disease (Fusarium), or bad growing conditions, were completely destroyed) and study data of plant are not available for further examination.

Figure 1 represents aerial parts lengths averages of cultivated plants. The figure shows that all types of plant control groups were higher than experimental groups of plants. Differences between control and experimental groups were statistically significant ( $p < 0.05$ ). For every plant species it was found that the average of E-1 control group is nearly 1.3 times higher than the experimental group of plants. The same disparities were collected from all evaluations E-2 and E-3 plants.



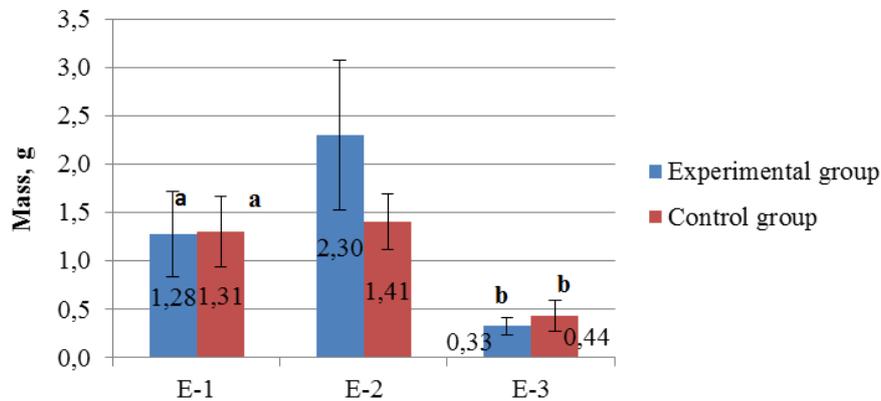
**Fig. 1.** The length of overhead.

Figure 2 represents averages of cultivated plant root lengths. Contrary to the assessment of the length of overhead, the results are more varied. It was found that the E-1 experimental group of the root mean length was 1.4 times higher than the control group, but this difference was not statistically significant ( $p > 0.05$ ). Mean differences in the control group E-2 and E-3 are 1.4 times and 2.0 times higher respectively, than those in the experimental group. The differences between the control and experimental groups were statistically significant ( $p < 0.05$ ).



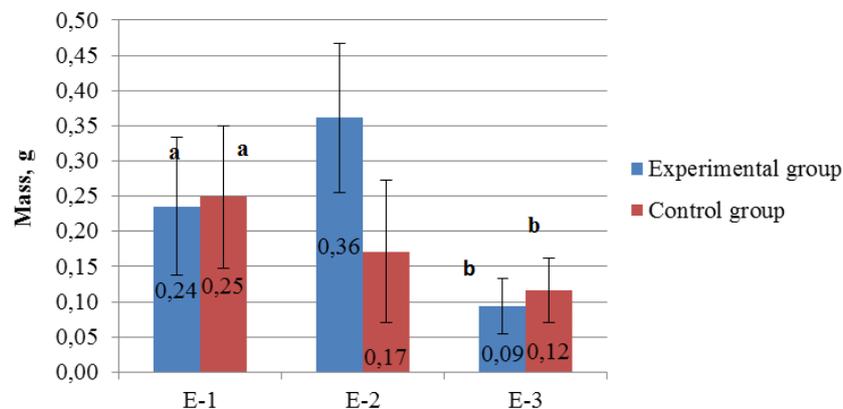
**Fig. 2.** The length of root.

Figure 3 represents the averages of cultivated plant mass. Significant and obvious difference between the control and experimental groups were observed only in E-2 case. The measured average of E-2 in the experimental group was 1.6 times higher than the control group, the difference was statistically significant ( $p < 0.05$ ). Other E-3 and E-1 differences between the control and experimental groups, were small and statistically insignificant ( $p > 0.05$ ).



**Fig. 3.** The mass of part above ground.

Figure 4 represents a cultivated plant root mass. The same as research of the land mass, significant and obvious difference between the control and experimental groups were observed only in E-2 case. In the experimental E-2 group the average was 2.1 times higher than the control group and the difference was statistically significant ( $p < 0.05$ ). Other E-3 and E-1 cases had small differences between the control and experimental groups and results are statistically insignificant ( $p > 0.05$ ).



**Fig. 4.** The mass of root.

It was estimated that the yield of selected plants ranging from 3.46 t/ha to 5.08 t/ha (Table 1). The highest yield obtained by calculating E-2 biomass and the lowest – E-3.

**Table 1.** Plant yield

Plant type	E-1	E-2	E-3
Biomass yield, t/ha	4.15	5.08	3.46

The data shows, that all tested cases of soil pollution with oil products, was significantly lower than in the soil around the research units (Table 2). The lowest soil pollution with oil products and the greatest phyto-cleaning efficiency determined by using E-2 plants, where soil pollution with oil products was 3.2 times lower than in the control group.

**Table 2.** Concentration of oil products in the soil.

Plant type	E-1	E-2	E-3	Around the Research Units
Concentration of oil products, mg/kg	155	108	213	343

A bit worse, but also promising results were obtained using the E-1 and E-3 plants - soil pollution with oil products decreased by 2.2 and 1.6 times respectively. In all cases the subjects of soil pollution with oil products were lower or slightly higher than the set threshold value of the overall petroleum products - 200 mg/kg.

### Discussion

Second half of summer (2015) was special – in many districts of Lithuania was announced drought. According to the meteorological stations and agrometeorology data, it has been very dry in southern Lithuania and in the central regions of the country. It was found that the temperature of August in many districts was from 2.9 °C to 3.8 °C higher than the standard rate of climate (SRC), and in a larger part of the country, during the month, rain fell only 2-16 mm (3-20 % SRC) ([www.meteo.lt](http://www.meteo.lt)). While selected plants are resistant to drought, but the high temperatures and lack of rainfall had apparently clear impact on plant growth and development. Because of these factors the dry part of plants investigated was of groups E-3 and E-2. Plant group E-1 biomass was taken a month earlier (07.24.2015) therefore the greater effects of drought were avoided and green plant biomass were measured.

Although, when targeted plants suffered from drought, it did not prevent the morphometric study. In assessing the height of the aerial parts showed, that all types of plant control groups were taller than the experimental groups of plants. The highest ground components measured by the E-3, but compared to the control (57.27 cm) and the experimental (43.27 cm) groups of plant.

Plants, while growing in polluted soil, often have to deal with a lack of nutrients, chemical toxicity and difficult physical conditions. As a result of such conditions is the inhibition of plant growth and biomass accumulation (Gerhardt et al., 2009). It was found that of all tested plant groups experimental biomass were slightly varied (differences not statistically significant ( $p > 0.05$ ) or 1.6 times (E-2 case) were higher than the control groups aerial parts biomass. Therefore, we can assume, while keeping plant biomass morphometric test one of the key factors, that procedure for assessing a plant's ability to assimilate pollutants, selecting fertilizer and organic matter deposit rates, have been properly concluded.

The examined plant green biomass yield of E-1 shows differences between the results obtained in the literature. It was estimated that the E-1 biomass yield of 4.15 t/ha, and the literature indicated that under appropriate conditions it can reach from 12.1 to 13.6 t/ha (1.9-6.2 t/ha dry mass) per year (Tilvikienė et al., 2009). Measured E-2 biomass yield was 5.08 t/ha while the literature indicated 22-37 t/ha of green mass per year (Svirskis, 2003). E-3 plants estimated yield was 3.46 t/ha while the literature indicated yield of 15-25 t/ha of pure plant mass and 7-15 t/ha dry mass per year (Lithuanian Energy Institute, 2011; LAMMC, 2015). On the other hand, the investigation carried out theoretical calculations, based on a single plant harvest results (in the E-1 case they can be 4 cuts) and in E-2 and E-3 cases it was used partially dry, wilted biomass data.

It was found, that the E-2 and E-3 experimental groups plant roots morphometry averages was almost 1.4 times and 2.0 times lower than in the control groups respectively. However the experimental group E-1 root length average was 1.4 times higher than the control group. In the E-1 case soil pollution leads to a vertical root growth and exploration into deeper soil layers. Therefore, E-1 roots began to spread through the most contaminated soil layer (10-25 cm). Other plants roots did not get deeper instead of the roots tended to thicken and spread horizontally to fully occupy little contaminated soil surface. This means, that plants E-3 and E-2, before moving roots into the contaminated zone, tend to grow roots in a clean or slightly contaminated soil layer (Kechavarzi et al., 2007).

Valuable results were found during chemical analysis of soil. In all cases, a positive result of phyto-cleaning efficiency was obtained. Using E-2, E-1, E-3 plants, soil pollution with oil products decreased by 3.2; 2.2 and 1.6 times respectively. In all test cases, oil content in soil was below or slightly above the threshold value of total oil amount of the products, which is 200 mg/kg. Based on these results and previous detailed geochemical evaluation (despite the fact that the pollution by petroleum hydrocarbons in one sample exceeded the threshold value of 70 times), we can conclude that the chosen plants are ideal for cleaning contaminated sites at 223-594 mg/kg of petroleum hydrocarbon contamination.

### Conclusions

1. The chosen plants can be used in oil-polluted soil treatment at 223 to 594 mg/kg of petroleum hydrocarbon concentrations in soil. At these levels endurance limit of the plants (plants death limit) was not reached, visual petroleum hydrocarbon toxicity to plants effects were not observed.
2. The oil hydrocarbons have significant impact on plant roots and aerial parts. In E-2 case, there was significantly accumulated more biomass in contaminated soil than in control group where the soil was clean. In other cases, the differences between the control and experimental groups were minor and insignificant.
3. Using E-2, E-1, E-3 plants the soil pollution with oil products decreased by 3.2; 2.2 and 1.6 times respectively. In all cases, the tests showed that the soil contamination of oil products was lower or slightly higher than the threshold value of the overall petroleum products, which is 200 mg/kg.

### Comments and recommendations

It is advisable to take appropriate weed control measures before the contaminated soil treatment and rehabilitation work by phytoremediation way.

Good soil tillage, used of agricultural technology, necessary to ensure better seed germination and good primary root formation. The contaminated soil surface dressed evenly can be useful to ensure a homogenous root growth and overall decrease of pollutant concentrations. It is necessary to make attention to the above-mentioned claims by making choice of tillage tools and methodology.

In order to maximize phyto-cleaning efficiency and for better results, specific pollutant-degrading microorganisms can be inserted into soil. Also it can be used additional plant growth promoters – rhizobacteria (PGPR), which was created to help plants cope with the stress caused by soil contamination.

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