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THE TESTING OF MODEL PLANT *RAPHANUS SATIVUS* GERMINATION AND PHYTOMASS PRODUCTION ON OIL-COMPOSTS

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ABSTRACT

In industrialized society, large amounts of oily sediments from contaminated sites as well as oily sludge from industrial processes need to be treated in sustainable way. Nowadays biological treatment is becoming more important. The purpose of biotreatment is to decrease the concentration of organic pollutants (e.g. oil) in soil or compost by mineralizing hazardous chemicals into harmless compounds such as carbon dioxide or some other gas or inorganic substance, water, and cell material. Whereas hydrocarbons are generally well degradable, some organic compounds (e.g. PAH) are less degradable; and some (heavy metals) can not be degraded. However, resistant compounds can be transformed through sorption, methylation, and complexation, and change in valence state, which affect mobility and bioavailability. The use of oil-compost depends on legislative limits, and response of vegetation. Oil-content may have a negative effect on plant root system even in low concentrations. Heavy metals may inhibit the growth, but in the other hand, the plants are also known in uptaking heavy metals and other contaminants, known as phytoremediation. This may create a situation, where vegetation cover acts as additional treatment system for matured oil-composts. The objective of this study was to examine the effect of hydrocarbon residues in different *substances* (compost and soil mixtures) on soil model plants (*Raphanus sativus*) germination and phytomass production. The germination study demonstrated that the plants germination and biomass production was highly dependent on compost decomposition degree, nutrient content and biological properties of soil. On less matured compost, the germination and growth was suppressed. The phytomass production experiment showed that plants in oil compost had decreased height, taproot mass and above ground phytomass. The application of complex mineral fertilizers increased the volume of abovementioned parameters.

KEYWORDS

Phytoremediation; Oily compost; Radish; Germination; Phytomass production

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1 INTRODUCTION

Petroleum hydrocarbon continues to be used as the main source of energy and hence an important global environmental pollutant. Soil contamination from leaking storage tanks is one of major problems today. Apart from accidental environmental contamination with petroleum hydrocarbons associated to oil production and shipping operations, vast amounts of oily sludge generated in refineries from oil separation systems and accumulation of waste oily materials in crude oil storage tank bottoms pose great problems because of the expensive treatment and disposal methods.

Nowadays biological treatment of hydrocarbon-contaminated soil, sludge and sediments is becoming more important. The purpose of biotreatment is to decrease the concentration of organic pollutants (e.g. oil) in soil or compost by mineralizing hazardous chemicals into harmless compounds such as carbon dioxide or some other gas or inorganic substance, water, and cell material [1, 2, 3, 4]. Composting has proved to be an extremely versatile biological remediation process that can be adapted to treat a wide variety of organic contaminants. Whereas hydrocarbons are generally well degradable, some organic compounds (e.g. polycyclic aromatic hydrocarbons, PAH) are less degradable; and some (heavy metals) can not be degraded [4]. Traditionally cleaned soils and oil-compost are disposed of in landfills, or incinerated. Unlike applications of household waste composting, oil-compost is often considered as no value-added product [5]. This statement, however, highly depends on contamination pattern, intended use, and often timeframe. Natural attenuation after active phase of composting is often ignored. Resistant compounds in oil-compost can be transformed through sorption, methylation, and complexation, and change in valence state, which affect mobility and bioavailability. The use of oil-compost depends on legislative limits, and response of vegetation. Oil-content may have a negative effect on plant root system even in low concentrations. Heavy metals may inhibit the growth, but in the other hand, the plants are also known in uptaking heavy metals and other contaminants, known as phytoremediation. This may create a situation, where vegetation cover acts as additional treatment system for matured oil-composts. In evaluation of the quality of oil-compost, analytical methods should be combined with toxicity tests with plants. Long and short term tests should be used.

In the following study, a series of tests were done to examine the effect of hydrocarbon residues in different substances (compost and soil mixtures) on germination and phytomass production of soil microorganisms and plants. The radish (Raphanus sativus) plants were studied as a short-term model plant in the basis of the interaction of vegetation, soil organisms and biological degradation of contaminants. The main objective of this study was to create basis for development of an environmentally sound and cost-effective method for evaluating suitability of treated oil-compost as vegetation substrate. For that purpose the knowledge of different compost substrates and plants interactions were studied.

2 MATERIAL AND METHODS

2.1 Characterization of Substrate

Three compost mixtures from various full-scale and pilot scale biodegradation studies were used. The composts were mixed with agricultural soil in various ratios.

Compost I. From 2001 to 2004, full scale remediation works took place at oily leachate pond at Laguja landfill, Estonia [6]. Compost mixture was prepared by removing of highly contaminated bottom sediments at the pond, mixing of these oily sediments with well decomposed peat, shredded bark, and fine sawdust. After 24 months of passive windrow composting, the oil content was reduced to 3 400 mg kg^{-1}, and in 2005 it was used for covering of the landfill. Some of this compost was used during the current vegetation test. The agrochemical parameters of this compost are presented in the Table 1, (C I).

Compost II. Oily flotation-flocculation sludge from water treatment plant of the refinery was mixed with shredded woodchips and municipal household compost, and composted in 1 m^3 plastic experimental containers during biotreatment research. After one year, the compost had reached the agrochemical parameters as presented in Table 1, (C II).

Compost III. The compost mixture was obtained from Epler & Lorenz Ltd in Estonia. The company recycles oily wastes and remediates contaminated soils. The compost mixture, (C III, Table 1) was obtained while composting of residues of heavy heating oil, mixed with peat and horse manure. The maturation period was 1.5 years.

Table 1. The agrochemical characteristics of the composts.

Soil characteristic	Compost I (C I)	Compost II (C II)	Compost III (C III)
pH_{KCl}	7.5	6.8	6.8
N, %	0.9	3.0	0.4
C, %	nd	22.8	7.0
P, mg kg^{-1}	9.7	12.9	15.6
K, mg kg^{-1}	15.5	95.2	66.7
Ca, mg kg^{-1}	932.2	986.5	437.9
Mg, mg kg^{-1}	64.0	85.8	74.5
C/N ratio	nd	7.6	17.5
Ca/Mg ratio	14.6	11.5	5.9
N/P ratio	9.3	23.3	2.6

nd- not determined

Soil. The soil was obtained from the experimental area of IOSDV - International organic nitrogen long-term-experiment (58°23'N and 26°44'E). The soil type is accordingly to WRB soil classification *Stagnic Luvisol* with soil profile: A-Ew-Bt-C. The *Stagnic Luvisol* is one of the most important soil types in South Estonia region. The soil comprises clay 8.5%, silt 32.1% and sand 59.4%. The average field agrochemical parameters are N 0.09%, C 0.99%, C/N rate is 11/1 pH_{KCl} is 6.3.

Laboratory analyses. The soil samples were analysed for organic C according to the Tjurin method [7]. For the determination of total N the Kjeldahl method was used [8]. Available P, K, Ca and Mg were analysed according to Mehlich-3 method [9]. For the characterization of the microbial biomass activity a respiration rate was determined [10]. The pH was measured from the soil suspension with 1M KCl (1:2.5 w/w) [8]. The content of petroleum hydrocarbons, measured as non-polar fraction was determined in the dry solids (DS) by infrared spectrometry in the laboratory of Tartu Environmental Research Ltd (Tartu, Estonia), according to the Finnish and Swedish standard methods [11,12]. STATISTICA 7 [13] was used for the statistical analysis and standard deviation (± SD).

2.2 The experimental set up

The germination

The phytotron experiment was established at the Estonian Agricultural University (EAU) in 2005. In germination experiment Composts I and II were used. Three replications with radish were planted in matured oil-composts, mixed in various ratios with agricultural soil (Table 2). The containers were kept in phytotron under the average temperature 20.4°C and with relative humidity 91%. The radish cultivar Novired seeds were sown to the depth 1cm. Novired was used as model plant since it is not sensitive to day length. The experiment was finished while the true leaves on model plants started to appear.

Table 2. The soil-to-compost ratios used in germination test.

No.	Soil/Compost mixture	Ratio, %
M1	C II	100
M2	C I	100
M3	Soil	100
M4	Soil/C I	80:20
M5	Soil/C I	60:40
M6	Soil/C I	40:60
M7	Soil/C I	20:80
M8	Soil/C II	80:20
M9	Soil/C II	60:40
M10	Soil/C II	40:60
M11	Soil/C II	20:80

M is for marking the substrate used for tests.

The phytomass production

The phytomass production was determined by using Compost III. The containers with volume 12 litres were used. Each container was filled with one litre of gravel, followed by the compost mixture. Each container was equipped with a capillary glass tube to remove excessive water from the bottom. The experiment was established in 6 variants in 3 repetitions. 25 seeds were sown to each container. Approximately 100 ml of mineral complex NPK-fertilizer 'nitroammofoska' was applied as a solution 17:7.5:14.

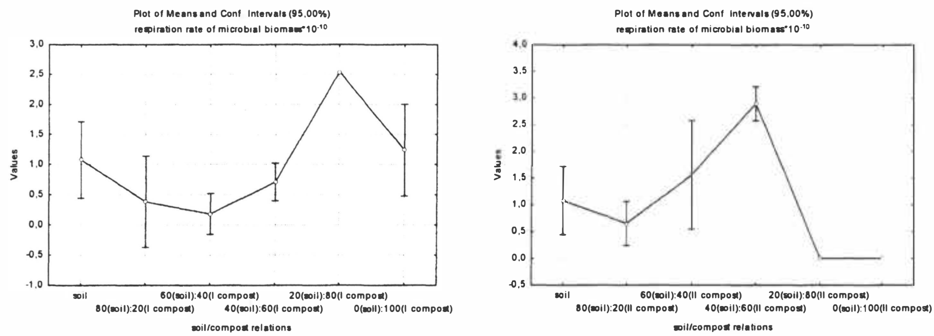
Table 3. The variants for phytomass production test

No.	Soil/Compost III mixture	Ratio, %
M12	Soil	100
M13	Soil	100+NPK
M14	Soil/C III	80:20
M15	Soil/C III	60:40
M16	Soil/C III	80:20+NPK

3 RESULTS AND DISCUSSION

The germination test. The radish plants started to emerge on fourth day after sowing and the first plants were found on variant M1. Following that, the plants started to emerge constantly in all mixtures. The most suitable growth substance for plants revealed to be variant M2 with 100% of C I. In most cases under the equal watering conditions, at the beginning of the experiment, the least plants emerged in the variants where the growth substrate contained mineral soil in higher ratios. The applied composts improved the soil structure and turned it to more suitable germination environment for plants (Table 4).

The development and growth of model plants was suppressed shortly after germination in all mixtures containing C II. The content of P and N in two tested composts were different (analysed after the end of plant growth) and had a significant influence on plant uptake. There was no microbial activity in analysed samples of C II (M1 and M11)(Fig. 1).



A. B.

Figure 1. Respiration rate of microbial biomass (Mean ± SE) in experimental variants. A. Respiration rate in compost mixture I. B. Respiration rate in compost mixture II.

During biodegradation of organic material, microorganisms may affect metal reactivity, mobility and toxicity. It has been suggested that the rhizosphere might also be responsible for accelerated breakdown of organic chemicals by biodegradation [14]. Organic contaminants can undergo biodegradation as a result of the activity of soil microorganisms or abiotic factors [15], giving less toxic, less mobile and less bioavailable products strongly adsorbed onto fine particles, organic matter and in micropores of various soils [16,17]. It is generally known that fine particles and organic matter are mainly responsible for pollutant adsorption on the soil, because hydrocarbons bind strongly to humic substances and to clay minerals [18] but Löser [17] found out that hydrocarbons may be strongly adsorbed even on coarse-grained and organic-free soil soils by microporosity so that they are no longer bioavailable for pollutant-degrading microorganisms and remain after biological remediation.

In our experiment, the comparison of Composts I (M2-M7) and II (M1, M8-M11) showed that in less matured compost (C II, substrates M1 and M11), microbial activity was negligible (Figure 1) even after phytoremediation with plants where the root excretions were secreted. Moreover, the compost inhibited the microbial activity in the mixture with high ratios of compost and soil (M11). Perhaps that is why in the germination emerged equally in early stages of the experiment, but the later the growth and development were significantly inhibited in the variant (M1) with less matured compost (C II).

The phytomass production test. The smallest number of plant was recorded in mineral soil substrate M13 (12 pieces out of 25) (Table 4). In the other hand, the same substrate (M13) produced largest amount of total biomass (125.7 g). The second highest amount of total mass was recorded on substrate M14 (112.2 g).

The highest mass of radish taproots was also grown on mineral soil substrate (M13) where mineral fertilizers were applied (45.9 g). The next highest taproot mass (29.3 g) was recorded if the substrate M 16 was used. In a case where mineral soil was mixed with compost (M14 and M15) the taproot mass was smallest (2.0 and 2.4 g, respectively).

The biggest amount of phytomass was obtained from substrate M13 (75.9 g). The same was found if mineral soil was mixed with compost (M14) and fertilizers were applied. In substrates where only composts were mixed with mineral soil (M14 and M15) the plants phytomass production was smaller (15.7 and 17.3, respectively).

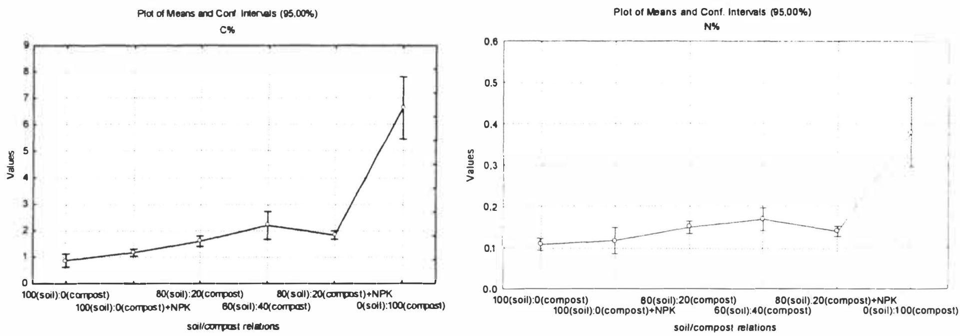
Table 4. Plant mass production.

Variants	Number of plants (pieces)	Total mass (g)	Taproot mass (g)	Phytomass (g)
M12	17 ± 2.3	65.6 ± 4.2	18.0 ± 7.5	42.2 ± 3.7
M13	12 ± 2.1	125.7 ± 38.5	45.9 ± 32.7	75.9 ± 10.4
M14	17 ± 1.0	20.5 ± 6.9	2.0 ± 3.5	15.7 ± 5.2
M15	21 ± 4.0	23.9 ± 4.6	2.4 ± 4.2	17.3 ± 3.3
M16	17 ± 1.5	112.2 ± 38.0	29.3 ± 32.1	75.2 ± 6.0

In the composts usually the content of carbon is sufficient for the microbial activity and plant growth but the content of other nutrients can be limited. Usually the relationship between nitrogen, potassium and phosphorus must be adjusted. According to the suggestions of Permaki [3] the relationship between C:N:P should be 100:10:1. High C/N ratio and the presence of phenolic compounds are typical characteristics of plant wastes [19] with deleterious effects on plant growth. The composting process has been used widely to reduce or eliminate these properties [20] whereas additional nitrogen (N) fertilisation is recommended to compensate for microbial N immobilisation [21].

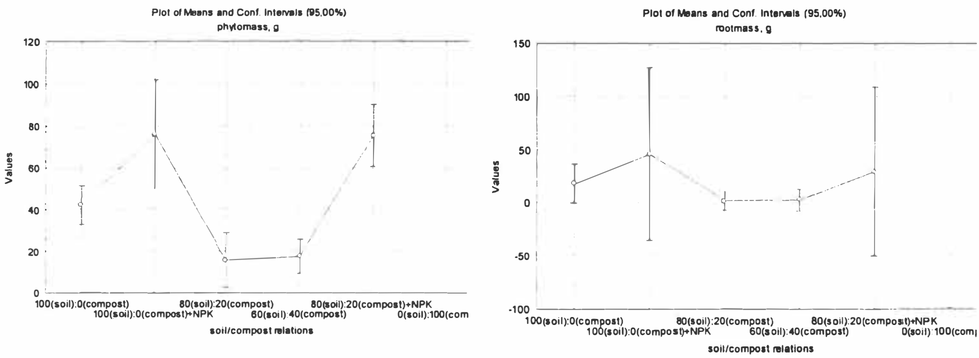
For the optimal growth, the plants require compost with certain physical, chemical, and biological properties [22]. According to Figure 2 (A), the phytomass production was primarily influenced by fertilizer application. Mineral soil parameters were also important to the model plant growth. The later growth conditions were improved by mineral fertilizer application. The development of root mass was influenced by growing conditions. In the growth chamber the light and temperature were apparently not optimal for the radish. Therefore we had large variability between experimental variants (Figure 3 A, B). In some of the variants the taproot did not form, and the same was observed in phytomass production (Table 4).

It has been demonstrated that oil pollution influences the growth of plants in different substrates. The crop yield was reduced by 30% if there was 1.1% oil in soil and plants died when the content of oil increased up to 10.6% [27]. The experiments on determination of the



A. B.

Figure 2. The agrochemical parameters in experimental variants. A. The total content of organic carbon(%). B. The total content of total nitrogen(%).



A. B.

Figure 3. The above ground plant phytomass (g) (A) and taproot mass (g) production (B) on different compost mixtures.

influence of lubricating oil and petroleum-contaminated soil to the spring wheat on clay loam and loamy soil have showed that the plants grow better in soil texture that has higher percentage of clay. The normal productivity of soil was recovered with 5 years after application of 3% of lubricating oil to the clayish soil. In loamy sand, productivity recovered only after 8 years. The oil impact to soil physical properties is following: the soil structure could deteriorate, because the soil aggregates breakdown and the ground disperses. The mechanism is still unclear, because if the biological activity recovers gradually the soil structure.

So far, the treated oil compost has been mainly used for covering landfills. Alternative use has often been confronted by authorities or potential users, whereas green compost is often recognised as biologically rich material for recultivation of waste grounds and industrial areas. Despite of residual contamination in soil compost, this material may also be suitable for various applications. Growing vegetation on treated oily soil has additional values, since plants are well known in uptaking of heavy metals and other contaminants, known as phytoremediation. This may create a situation, where vegetation cover acts as additional treatment system for matured oil-composts. However, depending contamination, on soil type and treatment levels, soil compost may also be unsuitable for vegetation. As agreed by our experiment on the less maturated compost C II, De Song (1980) reported that oil in soil created unsatisfactory conditions for plant growth, probably due to insufficient aeration of the soil [24]. This may be caused by the displacement of air from pore spaces by oil, and an increase in the demand for oxygen brought about by activities of oil-decomposing micro-organisms [25]. Baker [26] reported that oil penetrated and accumulated in plants causing damage to cell membranes and leakage of cell content. Udo and Fayemi (1975) also reported that growth of cereals was affected in oil-polluted soil, growth of plants was generally retarded chlorosis of leaves resulted and the plants were dehydrated. Therefore, is important to improve the composting system and to use different measures also technologies to remove the plants growth inhibiting agents from the composts.

4 CONCLUSIONS

The compost maturation time have significant influence on the plants growth and microbial respiration rate. The soil structure could be improved by addition of composts but the supply of nutrients is important. Application of mineral fertilizers is important as it results with more vigorous plant growth. Our experiment showed that oil compost with longer period of maturation was more suitable growth substrate environment to the plants development. In our future research we will concentrate on plants germination, growth and development on composts with different degree of oil pollution. In case of less maturated oil compost, or in presence of specific hazardous compounds, additional chemical analysis will be required to provide information about interactions of inhibitors, as well as uptake of these compounds, or potential leaching from soil.

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