REAL-WORLD PHYTOREMEDIATION DATA ON AMARANTH AND EXPERIENCE FROM A TWO-YEAR FIELD TRIAL IN THE PHY2CLIMATE PROJECT

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ABSTRACT: The "Phy2Climate" project aims to achieve clean biofuel production and phytoremediation solutions from contaminated lands worldwide. A site contaminated with petroleum hydrocarbons, previously used as an oil storage base during the Soviet era, was selected for the project. Due to accidental spills and past mishandling, the soil at the site remains contaminated with petroleum hydrocarbons levels up to 22 times above the limit values. The primary phytoremediation method for petroleum-contaminated soil is rhizodegradation, which involves stimulating the population of organic-degrading microorganisms through the plant rhizosphere. Therefore, the contaminated soil was treated with organic and mineral fertilizers to support plant growth and microbiological additives to ensure rhizodegradation. *Amaranthus caudatus* was cultivated on a prepared plot for two consecutive years. The main parameters used to assess phytoremediation efficiency were biomass output and changes in petroleum hydrocarbon concentration in the soil.

Keywords: phytoremediation, energy crops, soil contamination, organic pollutants, amaranth.

1 INTRODUCTION

Phytoremediation is recognized as an environmentally friendly and cost-effective method for treating contaminated soil. Recently, there has been a surge in large-scale phytoremediation projects, with a critical milestone being the shift from pot experiments to extensive field research in real-world conditions.

The "Phy2Climate" project aims to produce clean biofuels and provide phytoremediation solutions for contaminated lands globally. Integrating phytoremediation with energy plants maximizes environmental, economic, and social benefits. Energy plants, when used for biofuel production, add economic incentives to phytoremediation projects by converting biomass into biofuels, making the process sustainable. This dual approach addresses both environmental and energy challenges: phytoremediation cleans contaminated soil, while energy plants generate renewable energy, offering dual benefits (Figure 1).

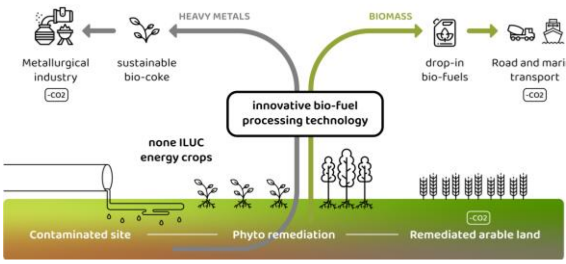

Figure 1: Principal scheme of "Phy2Climate" project [3]

Utilizing contaminated lands for energy crops avoids competition with agricultural land needed for food production, ensuring efficient land use without compromising food security. The high biomass yields and deep root systems of energy plants enhance long-term phytoremediation, maintaining soil health and fertility. Productive use of biomass, such as biofuels, syngas, or biochar, reduces waste and adds value. Growing energy plants for biofuel reduces greenhouse gas emissions and improves soil quality through biochar, further benefiting the environment. Additionally, revenue from biofuel production can offset phytoremediation costs, making the process financially feasible for stakeholders [1].

Choosing the right energy plant species for phytoremediation involves several considerations to ensure effective soil remediation and optimal biomass production for biofuels. Key factors include the plant's tolerance to contaminants, its capacity to uptake and accumulate pollutants, and its ability to produce high biomass yields. Fast-growing species with deep root systems and effective rhizosphere interactions are preferred as they enhance the phytoremediation process. Additionally, the economic value and biofuel potential of the plant, along with market demand for its biomass, play crucial roles in its selection. The plant must also be adaptable to the local environment, climate, and soil conditions, requiring minimal inputs. Regulatory and ecological considerations are also important, ensuring that the plant is non-invasive and complies with local regulations [2].

Plants such as miscanthus (*Miscanthus x giganteus*), switchgrass (*Panicum virgatum*), kenaf (*Hibiscus cannabinus*), industrial hemp (*Cannabis sativa*), and sunflower (*Helianthus annuus*) are considered top species for their phytoremediation capacity and economic viability in biofuel production. These species are chosen for their high efficiency in phytoremediation, economic benefits, and sustainability in biofuel generation.

In addition to these species, several other energy plants are popular in phytoremediation for their effectiveness in cleaning contaminated soils and producing biomass for biofuels. For example, species like rapeseed (*Brassica rapa*), sorghum (*Sorghum bicolor*), quinoa (*Chenopodium quinoa*), and Jerusalem artichoke (*Helianthus tuberosus*) were selected for field trials in the "Phy2Climate" project [3]. However, this paper focuses on amaranth (*Amaranthus caudatus*) for its specific properties and benefits in phytoremediation and biofuel production.

Amaranth, commonly known as love-lies-bleeding, is considered effective for phytoremediation. It is suitable for this purpose for several reasons. Firstly, high biomass production is one of its significant advantages. Amaranth produces a substantial amount of biomass, which is beneficial for phytoextraction as it can accumulate a large amount of contaminants. Secondly, it has a deep root system. This allows the plant to access and uptake contaminants from deeper soil layers, enhancing its effectiveness in remediating polluted soils. Thirdly, the plant has a fast growth rate. It grows quickly, enabling it to start remediating soil contaminants relatively soon after planting. Fourthly, amaranth exhibits tolerance to contaminants. It is known for its ability to tolerate various contaminants, including heavy metals and organic pollutants, making it suitable for use in a variety of contaminated sites.

Additionally, the plant shows versatility in different climates. It can be grown in diverse climatic conditions, including those found in regions like Lithuania, making it a flexible option for phytoremediation projects. Finally, the plant has rhizodegradation potential. It stimulates the population of organic-degrading microorganisms in the rhizosphere, enhancing the breakdown of organic pollutants in the soil [4-10].

The objective of this research was to investigate the growth performance of amaranthus on soil contaminated with organic pollutants, quantify the biomass production, assess its capability to degrade these contaminants, and compare the results with previously conducted pot experiments. The study also aims to share both successful outcomes and challenges to provide comprehensive knowledge about the feasibility and limitations of cultivating amaranth under real-world conditions.

2 METHODS

2.1 Site description

The contaminated site is in Siauliai, a city in mid-Lithuania, and is affected by petroleum hydrocarbons (TPH). This area was used as an oil base during the Soviet era and was abandoned after 1990, with the last oil tanks being removed in 2009. Since then, the site has remained unused. A primary eco-geological survey conducted in 2014 revealed TPH concentrations in soil samples ranging from 1.566 mg/kg to 17760 mg/kg [11], which exceed Lithuanian and EU legal limits by up to 22 times [12].

Currently, the former oil base is within Siauliai's water intake sanitary protection zone, a city with approximately 104000 residents as of 2023. Industrial activities in this zone are prohibited to safeguard the water supply from chemical, biological, or physical contamination. The nearest artesian well is about 55 meters from the site, and the groundwater table lies at a depth of 1.1 to 2.2 meters. To date, no remediation efforts have been undertaken at the site.

2.2 Characterization of the contaminated soil

The initial eco-geological survey [11] of the site was conducted in 2014 as part of the State plan to identify contaminated areas throughout Lithuania. At the Siauliai site, the survey included both groundwater and soil sampling. The sampling points were strategically placed to reflect the eco-geological conditions in the main transit areas for potential contamination by oil products, heavy metals, and sediments.

In 2021, soil characterization under the "Phy2Climate" project was conducted taking into consideration the former sampling campaign in 2014. Sampling boreholes were created using a stainless-steel hand auger. Joint soil samples were taken at four different depths (0-20 cm, 20-40 cm, 60-80 cm, and 60-100 cm). A total of 3 joint samples were prepared and transported to certified laboratories for further analysis, including pH, electrical conductivity, total solids, organic matter, concentration of TPH, mobile N, mobile P, mobile K, total C, Mg, and microbial biomass.

2.3 Field trial

In March 2021, the experimental site was cleared of trees, bushes, cement blocks, and other debris, with large holes filled using an excavator. By March 2022, deep tillage was performed to level the soil and shred remaining roots, followed by harrowing in April to aerate and further loosen the soil.

The amaranth (*Amaranthus caudatus*, variety Raudonukai) was seeded by hand in the designated 310 m² parcel, with approximately 580 grams of seeds (18 kg/ha). After seeding, the surface was lightly raked. Control parcel, consisting of fresh and non-contaminated sandy loam, were established adjacent to the contaminated site. The sandy loam's granulometric composition matched that of the contaminated soil. The clean soil was placed in a raised bed approximately 0.5 meters high. The control parcel received about 7 kg of compost but no additional fertilizers.

In mid-June 2022, a bacterial additive consisting of various *Bacillus spp*. and *Pseudomonas spp*. strains was applied to the contaminated site. Approximately 100 kg (dry weight) of the additive was mixed with lukewarm water in a 9 m³ tank, along with meat and bone meal (MBM) to activate the bacteria. The mixture was aerated and then poured onto the soil of the contaminated site, while the control parcels did not receive this treatment.

Prior to seeding, the soil in the amaranth parcel was fertilized with 420 kg of compost (13.5 t/ha) and 25 kg of mineral fertilizer (NPK(S) 12-11-18 - 8S) per parcel (0.8 t/ha).

In mid-October 2022, amaranth was harvested at the end of its blooming phase (phenological stage BBCH 69), when the plants began losing their first leaves. The aboveground biomass was harvested using disc trimmers, cut into swaths, left to pre-dry in the field for two days, and then transported to drying facilities. The drying process for the amaranth biomass took place in a hay-shed facility, utilizing atmospheric air. The biomass was dried for about one month, from mid-October to mid-November, to prepare it for further processing.

Soil preparation for amaranth in the spring of 2023 included power harrowing, followed by the removal of stones and small debris using a special raking tool. A second power harrowing was performed after debris removal. Post-fertilization, the soil was leveled with a towed leveler. The soil was fertilized with NPK(S) 12-11-18 - 8S at 25 kg/parcel (0.80 t/ha) and $(NH_4)_2SO_4$ at 8 kg/parcel (0.26 t/ha). Seeding was done using a "Gardena" seeder with 600 g of seeds per parcel, followed by manual raking. Weed control was managed by applying the herbicide "Barbarian Biograde 360" at a rate of 0.174 L/parcel (2 L/ha) before seeding. Additionally, white goosefoot (*Chenopodium album*) weeds were manually pulled out to prevent competition with the amaranth plants.

In October 2023, the amaranth plants were harvested following the same procedures as in 2022. The aboveground biomass was cut using disc trimmers, pre-dried in the field, and then transported to drying facilities.

The biomass was dried for one month using atmospheric air.

Both years, plant monitoring was conducted every 10-14 days in three replicates within different 1 m² sub-plots. The following parameters were evaluated: germination rate, soil cover with plants, plant density, luxuriance (lushness of the plants), stem height, and root length. Data from these assessments provided insights into plant health and growth dynamics throughout the season. Weather conditions were monitored through the Lithuanian Hydrometeorological Service Station, which provided hourly data sets every 10 days, including air temperature, air humidity, precipitation amounts, sunny hours, average wind speed, and wind direction.

2.4 Evaluation of phytoremediation process

Phytoremediation performance was assessed by examining changes in soil parameters, including general soil characteristics and contaminants, biomass output, and associated costs.

Inorganic contaminants like heavy metals are absorbed by plants through their root systems and can either accumulate in the root zone or be transported to aboveground parts. Organic contaminants, which are lipophilic and hydrophobic, typically are not absorbed by plants unless they come into direct contact with the plant in liquid or vapor form from the atmosphere [13]. Therefore, traditional calculations of translocation and bioaccumulation factors used for heavy metals are not applicable for TPH.

Based on the expenses required to manage a 310 m² phytoremediation field trial, the cost for administrating one hectare was estimated using the database for sustainable agriculture available at https://www.ktbl.de/ [14].

3 RESULTS AND DISCUSSION

3.1 Plant development and biomass output

In 2022, amaranth germination was poor in contaminated soil, with significant delays compared to clean soil. Both experienced weak germination, primarily due to dry conditions after sowing and poor-quality seed material, which became apparent later in the year. Soil cover by amaranth was low, reaching only 30% in clean soil and 48% in contaminated soil, though the latter figure was inflated by the presence of weeds. Actual soil cover by amaranth was much lower. Plant density was low, reflecting poor soil coverage and low luxuriance scores. Amaranth struggled in both soil types, with luxuriance lingering around 3 due to the subpar seed quality. Maximum plant height was not achieved in either soil, with similar height development observed.

Biomass output results in 2022 were disappointing. Based on pot experiments from 2021, it was estimated that amaranth could produce about 27.12 t/ha of dry biomass [15]. However, field trials showed only 1.38 t/ha of dry biomass, far below expectations. The poor results can be attributed to prolonged dry conditions, which slowed seed germination and allowed weeds, which are typically more resistant to unfavorable weather conditions, to overshadow the amaranth plants. Additionally, the poor quality of seeding material significantly impacted the growth and development of the plants. In comparison, the highest recorded dry above-ground biomass yield for amaranth in Lithuania [16] was 9.5 t/ha, achieved in 1998 under optimal conditions.

In 2023, significant improvements were observed after changing the seed supplier. Amaranth germinated similarly in both soil types, initially delayed by lack of moisture but improving significantly by late May to early June. Germination rates reached 75% in clean soil and 100% in contaminated soil. The improved seed quality led to better growth, with soil cover in contaminated soil reaching 85%, compared to only 30% the previous year. However, coverage in control soil remained poor at 1%, likely due to adverse growing conditions in spring. Despite low plant density and luxuriance in control soil, both parameters improved in contaminated soil, with plant density reaching 8 points and luxuriance 7 points. Amaranth plants in contaminated soil were significantly taller, reaching 0.9 meters before harvest, compared to those in clean soil.

Biomass yield increased significantly in 2023, with amaranth producing 11.10 t/ha of dry biomass, an eightfold increase from the previous year. This improvement was attributed to better agronomic practices, elimination of previous mistakes, and the use of plant care products to address weed problems. Additionally, the selection of a new seed supplier and independent germination testing contributed to the improved results. The overall success highlights the importance of high-quality seeds and effective agronomic strategies in achieving optimal phytoremediation outcomes. The comparison of our 2023 yield with the historical maximum yield of 9.5 t/ha indicates that our strategies not only improved the biomass output but also exceeded the best previously recorded yields in Lithuania.

3.2 Changes in soil parameters

The obtained results suggest overall improvements in soil quality. After the first-year field trial, total solids decreased from 99.5%-99.7% to 86.8%-94.7%, and organic matter increased from 2.03%-3.06% to 2.6%-4.85%, suggesting improved soil structure and fertility. Stable pH levels and increased electrical conductivity, peaking at 21.8 mS/m, indicate changes in soil chemistry due to microbial activity and amendments. Total carbon (C) concentrations initially ranged from 2.3% to 2.9%, and after the first-year field trial and fertilization, carbon levels increased to 3.26%-4.00%. During the first year of field trials, mobile nitrogen (N) concentrations increased significantly across all depths, with the 0-20 cm depth rising from 0.61 mg/kg to 636 mg/kg and the 20-40 cm depth increasing from 1.63 mg/kg to 1033 mg/kg. In contrast, mobile phosphorus (P) levels showed a decreasing trend, with the 0-20 cm depth dropping from 321 mg/kg to 22.1 mg/kg and the 20-40 cm depth decreasing from 309 mg/kg to 38.0 mg/kg. Mobile potassium (K) levels also exhibited a decreasing trend, with the 0-20 cm depth reducing from 1334 mg/kg to 119 mg/kg and the 20-40 cm depth declining from 1332 mg/kg to 158 mg/kg. By the second year, mobile N levels remained elevated, with the 0-20 cm depth at 545 mg/kg and the 20-40 cm depth at 386 mg/kg. Mobile P levels showed some stabilization, with the 0-20 cm at 19.1 mg/kg and the 20-40 cm at 19.1 mg/kg, while mobile K levels demonstrated a slight recovery, with the 0-20 cm at 242 mg/kg and the 20-40 cm at 215 mg/kg. At the 60-100 cm depth, mobile N increased significantly from 9.1 mg/kg to 1840 mg/kg, while mobile P and K decreased from 427 mg/kg to 56.4 mg/kg and from 2081 mg/kg to 154 mg/kg, respectively. These changes indicate that while the application of fertilizers and soil amendments significantly increased mobile N, enhancing nutrient availability, the levels of mobile P and K showed a decreasing or mixed trend, highlighting the need for ongoing soil management to maintain soil fertility and health.

Initially, microbial biomass ranged from 4.1 x 10^4 CFU/g to 1.0 x 10^6 CFU/g. After the first-year field trial, microbial biomass increased significantly, reaching up to 3.0 x 10^7 CFU/g at 20-40 cm depth. Following the second-year field trial, microbial biomass remained elevated, with levels ranging 1.75 x 10^6 CFU/g on average, indicating sustained microbial activity and improved soil health. Microbial biomass represents 1-5% of the soil's organic carbon and is crucial for nutrient cycling and soil health. Higher microbial biomass indicates better soil fertility and structure [17]. The significant increase and sustained elevated levels (1.75 x 10^6 CFU/g) after the second-year trial suggest that the application of bacterial additives was effective in enhancing soil microbial activity and overall soil health through improved nutrient cycling and soil structure.

3.3 Soil decontamination

Petroleum hydrocarbons in the soil are classified into lighter fractions (C6-C10) and heavier fractions (C10-C40). During the initial characterization of the contaminated soil, lighter fractions of petroleum hydrocarbons (C6-C10) were undetectable, likely due to their volatile nature, which causes them to evaporate or degrade quickly under environmental conditions [18]. In contrast, the heavier total petroleum hydrocarbons (TPH) fractions (C10-C40) were present in significant concentrations. Initially, the TPH concentrations ranged from 245 mg/kg to 1029 mg/kg across various soil depths (Figure 2).

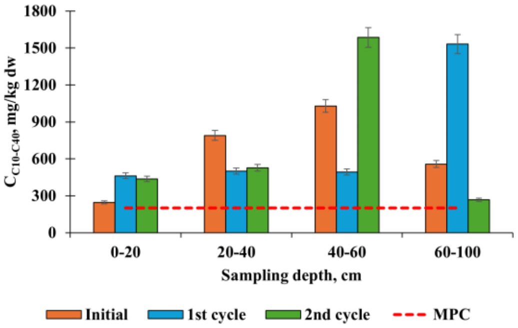

Figure 2: Concentration changes of heavy fractions of petroleum hydrocarbons (C10-C40) across soil depths over two years of field trials

After the first-year field trial, the TPH concentrations showed a reduction, with values such as 462 mg/kg and 500 mg/kg at shallower depths (0-40 cm), but increased to 1532 mg/kg at deeper levels (60-100 cm). This reduction suggests some degree of degradation or bioremediation, potentially facilitated by the microbial activity enhanced through soil amendments and bacterial additives. Following the second-year field trial, the TPH concentrations varied, with noticeable reductions at certain depths, indicating ongoing bioremediation and enhanced biochemical processes in soil.

In Lithuania, the maximum permissible concentration (MPC) of TPH for sensitive soils, such as those in the Siauliai water intake sanitary protection zone, is 200 mg/kg. This threshold is critical for ensuring the protection of water resources and maintaining soil health in ecologically sensitive areas. The presence of TPH above this limit in the initial characterization underscores the need for effective remediation strategies. Heavier TPH fractions are more persistent in the environment due to their complex structure and lower volatility, which makes them more resistant to natural degradation processes. These hydrocarbons can adhere to soil particles, reducing their bioavailability but also making them more challenging to remove. Bioremediation efforts, including the addition of microbial consortia such as *Bacillus spp*. and *Pseudomonas spp*., have shown promise in breaking down these compounds, enhancing microbial activity, and ultimately improving soil quality. The substantial increase and sustained high levels of microbial biomass after the second-year trial indicate that the application of bacterial additives effectively enhanced soil microbial activity. This improvement contributed to overall soil health by promoting better nutrient cycling and soil structure. Additionally, soil works such as power harrowing carried out in both 2022 and 2023, and deep tillage performed in 2022, likely contributed to the upturning and redistribution of soil layers. These practices could have facilitated the movement of TPH from deeper layers to the surface, enhancing the exposure of contaminants to microbial degradation. These findings highlight the challenge of remediating soils contaminated with heavier petroleum hydrocarbons and underscore the importance of sustained bioremediation efforts to reduce their environmental impact [19].

3.4 Cost analysis

To install and run phytoremediation field trials in a pilot site using amaranth, the costs are divided into three main categories (Table I): seeds, agrochemicals, and other consumables; diesel consumption for agricultural machinery; and labor costs for operating the machinery. The total costs for the first year amounted to 17989.99 EUR ha^{-1}, while the second-year costs were EUR 1261.67. The higher costs in the first year were due to initial setup activities such as debris removal, site clearing, deep tillage, and the addition of bacterial additives, which were not required in the subsequent year. Conventional agricultural practices carried out both years included soil harrowing and cultivation, spreading fertilizers and compost, sowing, soil roll-towing, cutting the plants, and pressing them into bales.

Biomass output in 2022 was 1.38 t/ha, while in 2023, it increased significantly to 11.10 t/ha due to improved seed quality. The bacterial additive used was priced at 28 EUR per kg, with approximately 400 kg required per hectare, constituting about 50% of the budget and necessitating careful evaluation. Calculating the cost to produce 1 kg of biomass, in 2022, the cost was approximately 17989.9 EUR / 1380 kg = 13.03 EUR/kg. In 2023, the cost was approximately 1261.7 EUR / 11100 kg = 0.11 EUR/kg. These calculations highlight the cost efficiency improvements alongside the significant increase in biomass yield from 2022 to 2023.

Regarding other expenditures, it needs to be noted that when the size of the phytoremediation field increases and great amounts of biomass are obtained, the need for air-drying and storage also increases. These costs were not included in the calculation presented here. Depending on the conversion technology, the biomass might need additional processing. In this study, the phytoremediation biomass was shredded and pelletized. This was considered as biomass processing cost, thus not included in the phytoremediation cost. Overall, the price to obtain 1 kg of biomass through phytoremediation is considerably higher than through conventional means (e.g., agricultural residues), so the critical point is that the biomass is obtained simultaneously with the soil clean-up and ecosystem restoration processes.

Table I: Costs (EUR ha^{-1}) for establishing and running phytoremediation field trials using amaranth (2022-2023):

	2022	2023
Seeds, agrochemicals and other consumables		
Seeds	35.3	35.2
NPKS fertilizers	800	480
Vermicompost	1989	-
Bacterial additive	11500	-
Diesel consumption		
For agricultural machinery	3464.3	544.9
Labour		
For working with agricultural machinery	201.5	201.5
TOTAL, EUR	17989.9	1261.7

4 CONCLUDING REMARKS

Amaranth's biomass output improved significantly from 1.38 t/ha in 2022 to 11.10 t/ha in 2023 due to better seed quality and agronomic practices, highlighting the importance of high-quality seeds and effective agronomic strategies. Changes in seed supplier and better plant care to address weed problems were crucial. Furthermore, despite the soil being contaminated, the biomass output was comparable to clean soil.

Significant reductions in total petroleum hydrocarbon (TPH) concentrations, especially in the heavier fractions (C10-C40), were observed. Initial TPH levels of 245-1029 mg/kg decreased after two years of field trials, demonstrating effective phytoremediation. The application of bacterial additives and soil amendments enhanced microbial activity, crucial for degrading these contaminants.

Establishing phytoremediation trials for amaranth costs approximately EUR 17989 EUR/ha. In the subsequent years, the costs are significantly reduced to around 1262 EUR/ha due to the elimination of initial setup activities like debris removal, deep tillage and application of bacterial additives shifting the costs on conventional agricultural practices and maintenance.

Overall, growing amaranth is challenging, but with the right agronomic tools, the simultaneous degradation of contaminants and sufficient biomass output is achievable, making it a viable option for both soil clean-up and biofuel production.

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Phy2Climate

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