Phy2Climate | A global approach for recovery of arable land through improved phytoremediation coupled with advanced liquid biofuel production and climate friendly copper smelting process.
Combined clean biofuel production and phytoremediation solutions from contaminated lands worldwide.
H2020-LC-SC3-2020-RES-RIA | LC-SC3-RES-37-2020 | GRANT AGREEMENT NUMBER: 101006912

A global approach for recovery of arable land through improved phytoremediation coupled with advanced liquid biofuel production and climate friendly copper smelting process

Deliverable 2.3: Annual report on phytoremediation performance and monitoring [M24]; update [M30]

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presented by Phy2Climate project consortium

N°	Short	Beneficiary	Role
1	ITS	ITS Foerderberatung GmbH	CO
2	Fraunhofer	Fraunhofer Gesellschaft	BEN
3	AUR	Aurubis AG	BEN
4	LEITAT	LEITAT Technological Center	BEN
	CUJ	Central University of Jharkand	IL3P
5	IFVCNS	Institute of Field and Vegetable Crops	BEN
6	SUT	Silesian University of Technology	BEN
7	ETA	ETA Florence Renewable Energies	BEN
8	UHA	University Hasselt	BEN
9	INTA	National Institute of Agricultural Technology Argentina	BEN
10	UNSPMF	University of Novi Sad - Faculty of Sciences	BEN
11	BVA	Biovala	BEN
12	PWMCVV	Public Water Management Company Vode Vojvodine	BEN
13	LTC	Litoclean SL	BEN
14	CLH	Compania Logistica de Hidrocarburos S.A.	BEN
15	PUW	Pro Umwelt	BEN
16	UCB	Umwelttechnologie Cluster Bayern	BEN

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Document Type		
PRO	Technical/economic progress report (internal work package reports indicating work status)	
DEL	Technical reports identified as deliverables in the Description of Work	**X**
MoM	Minutes of Meeting	
MAN	Procedures and user manuals	
WOR	Working document, issued as preparatory documents to a Technical report	

INF	Information and Notes	

Dissemination Level		
PU	Public	**X**
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RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	
CON	Confidential, only for members of the Consortium	

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29, 30	30/05/2023	Discussion added	Nora Matanzas Valtuille
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1. EXECUTIVE SUMMARY

This annual report is intended to review the phytoremediation performance two years after the start of the Phy2Climate project [M24]. In Spain, Serbia and Lithuania, the first cycle of field trials was completed before December 2022 and the results are discussed in the first version of the Deliverable. Due to different climatic conditions, the field tests in Argentina were only halfway through during the preparation of the Deliverable, so the results from Argentinian pilot site are included in this updated version [M30].

This deliverable provides information about establishment of the field trials, observations from plant and weather monitoring, phytoremediation performance, and encountered problems. Although each pilot site has its own characteristics (type of soil, type of contaminant, plant species, amendments, climatic conditions, etc.), the 4 pilot sites have assessed their progress by evaluating changes in soil parameters and by assessing the biomass output. The biomass output was/is of critical importance in order to reach Milestone MS3 *to deliver first batch of biomass for biofuel production*, to ensure the advancement of the project. The Deliverable provides details on harvesting and biomass preparation campaign.

2. INTRODUCTION

The 4 Pilot Sites in Argentina, Lithuania, Serbia, and Spain have different sources of contamination such as heavy metals and metalloids, petroleum hydrocarbons and polycyclic aromatic hydrocarbons, and Fe, Na, K in excess concentration. Hence different phytoremediation strategies, developing different phytoremediation mechanisms, were applied to remediate these sites. Prior to starting phytoremediation on the contaminated sites, pot trials were carried out by each pilot sites leader with the aim to determine the best phytoremediation strategy to be applied in the specific contaminated site. Field trials were implemented in the second year of the Phy2Climate based on the results from the pot-experiments. The implementation of field trials includes landscape and soil preparation activities, seeding and planting, setting up monitoring programme, harvesting and pelletizing. As it was defined in the Harmonized plan, a certain set of soil parameters has to analysed by every pilot site leader before and after the field trials to evaluate phytoremediation performance and to enable representable comparison of phytoremediation strategies [D2.4].

3. PHYTOREMEDIATION PERFORMACE IN FIELD TRIALS

3.1 Objectives

The pilot site validation is going to be measured according to Key Performance Indicators shown in Table 6 in Phy2Climate proposal. The objective of each pilot site is to produce >40kg (dry weight) of energy crops per growing season and remediate the contaminated sites in a rate that results in <20 years for complete site remediation and its transition as arable land.

3.2 Description of landscape and soil preparation, and seeding campaign

Pilot site preparation activities, including terrain delimitation, area division into control and experimental parcels, soil ploughing and levelling, installation of irrigation equipment, if applicable were carried out by each pilot site leader. The soil preparation as well as the seeding were programmed according to the local weather conditions and agricultural practices in each pilot site, as well as the seeding seasons for each energy crop, considering that pilots are being made in both south and north hemispheres. The fertiliser programmes were carried out according to the specific energy crop cycle and soil conditions, including frequency, nutrient dose, and application type (broadcasting, ferti-irrigation, foliar application).

3.3 Description of monitoring means

The plant growth was carefully monitored through logs and sampling programmes every 10-14 days, during the growing season. Plant monitoring included such parameters as germination, soil cover by vegetation, plant height, plant density, luxuriant of the plants and species composition (if applicable) in parcels.

Weather conditions monitoring was performed by each pilot site directly on-site or by a national weather station. Weather monitoring included parameters that are listed in Table 3.1. However, for the interpretation of the obtained results, mainly air temperature and precipitation were used.

Table 3.1. Weather monitoring data means

Parameter	Unit
Precipitation	mm
Air temperature	°C
Wind speed and direction	m/s
Humidity	%
Light regime	hours of light/dark mode

3.4 Description of harvesting campaign and biomass processing

Pilot sites harvesting campaigns were held according to plant species in each pilot site. The biomass harvesting and the collection of all plant materials (at least 40 kg dry weight for each season, each variety) and processing (drying and pelletizing) was performed with the protocols and frequency described in D2.1 by the partners involved in each pilot site and readied to be sent to FRA for WP3. These are the tasks corresponding to Milestone MS3 *to deliver first batch of biomass for biofuel production.*

The harvested energy crops from the phytoremediation sites after processing will be/were shipped to the biorefinery in form of oil seeds and/or bulk biomass. On one hand, the oilseeds will be used for biodiesel production through the well-known transesterification reaction. On the other hand, the bulk biomass will be fed to the Thermo-Catalytic Reforming (TCR).

3.5 Description of phytoremediation performance M12-M24

Phytoremediation performance was evaluated in two aspects: i) changes in the soil parameters, including general soil parameters and contaminants, and ii) biomass output, which is of critical importance not only within the Phy2Climate framework, but also to make phytoremediation commercially available.

Soil parameters obtained after the harvest were compared with the initial characterisation performed in different soil depths at the beginning of the Phy2Climate project. To evaluate the effect of phytoremediation, translocation factor for heavy metals, and phytoremediation potential for organic contaminants were calculated. Finally, biomass output was evaluated and compared to the expectations calculated based on the pot experiments from 2021.

3.6 Assessment of Soil quality index

WP2 participants have made an extensive literature review on Soil quality index (SQI) and have preselected different soil quality indexes. Among those, the "Soil quality index for agricultural areas under different levels of anthropopressure" proposed by Klimkowicz-Pawlas et al. in 2019[1] was selected. This SQI was selected because it aims at indicating the agricultural quality of soil and because all the main contaminants of Phy2Climate pilot sites (TPH, PAH, heavy metals) are included in the parameters considered to estimate it.

The authors established a minimum dataset (Table 3.2) through a principal component analysis performed using seventeen different soil parameters. This dataset is dependent on the level of anthropopressure.

Table 3.2 Minimum data set of the SQI selected for different levels of anthropopressure

LOW ANTHROPOPRESSURE	HIGH ANTHROPOPRESSURE
Total nitrogen content *(g/kg)*	Humins *(g/kg)*
Potential of *nitrification (µg* NO_2^-*/g dw h)*	Zn *(mg/kg)*
Sand content *(%)*	Basal microbial respiration *(µg* CO_2 */ g dw h)*
Dehydrogenase activity *(µg TPF/g dw)*	pH
	Silt *(%)*

According to what is established in Klimkowicz-Pawlas et al (2019), pilot site leaders evaluated to which anthropopressure level they belong and included corresponding parameters in the monitoring (sampling) plan.

The SQI during the 3.5 years campaign will be calculated by assigning a weighted value to each of the parameters calculated and then integrated into SQI. Once the SQI is determined, the classification criteria will be the one established in Table 3.3.

Table 3.3. Classification criteria of the soil quality for the minimum data set

Indicator	**Soil quality grade**				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**	**Grade 5**
	Very high	High	Moderate	Low	Very low
SQI	>0.6	0.55-0.6	0.45-0.54	0.38-0.44	<0.38

3.7 Summary

The global goal to achieve 40 kg of dry biomass from each pilot site has been successfully achieved in Spain, Serbia, and Lithuania by the end of 2022. Whereas, in Argentina, due to different climatic conditions, the first harvest was successfully achieved at the beginning of 2023. All pilot sites reported on promising results regarding soil decontamination as well. Furthermore, an excessive literature review was done towards defining a useful tool to evaluate efficiency of phytoremediation – soil quality index. Despite, minor practical issues that arose during this year, no major drawbacks were faced.

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[1] Klimkowicz-Pawlas, A., Ukalska-Jaruga, A., & Smreczak, B. in 2019, Soil quality index for agricultural areas under different levels of anthropopressure. International Agrophysics, 33(4), 455-462.

4. FIELD TRIALS ON THE SPANISH PILOT SITE

4.1 Landscape preparation

The area where in-situ phytoremediation strategy was planned to be implemented consists of an unpaved area of 800 m^2, located in the southern part of EXOLUM company (see Figure 4-1).

Figure 4-1. Unpaved area of 800 m^2 located in the southern part of EXOLUM company, in Tarragona city (Catalonia region, Spain), where in-situ phytoremediation strategy is implemented

Given that the soil contamination has been mainly found at a depth of 2 – 4 m (from the initial site characterization campaign), an excavation was found to be necessary to prepare the experimental subparcel (600 m^2 area), to relocate TPH contamination to the upper layers of the soil, which is expected to increase pollution bioavailability for the plant species and, thus, the effectiveness of the phytoremediation pilot test.

Hence, an excavation vessel of 600 m^2 and 3 – 4 m deep has been defined and, approximately, 2000 m^3 of soil have been carefully excavated, aiming to keep the unaffected soil layers apart from the polluted ones (see Figure 4-2). Once finalized, the excavation hole has been refilled, firstly, with the non-polluted soil, and secondly, with the polluted layers. Between both layers, a geotextile fabric of PEAD (high density polyethylene) has been installed, to prevent contaminant leaching to non-impacted soil layers.

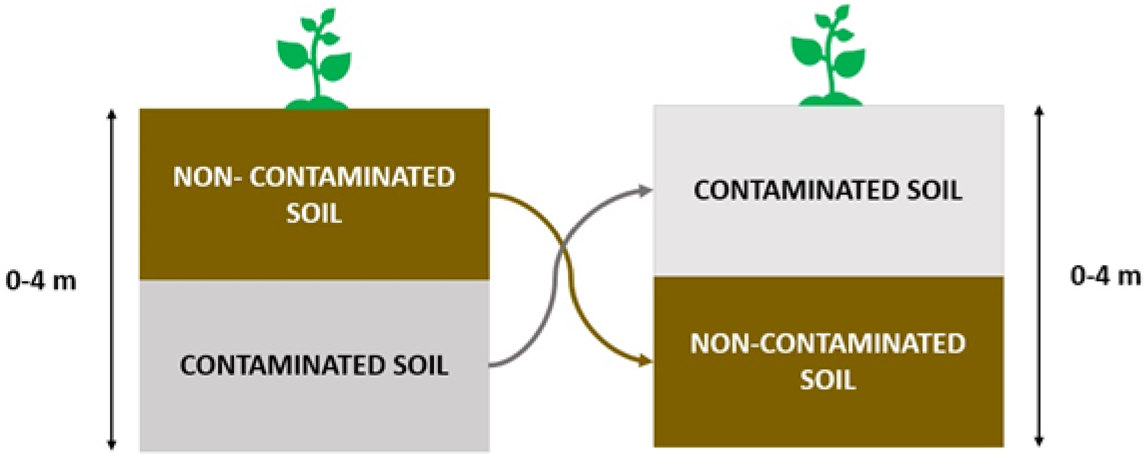
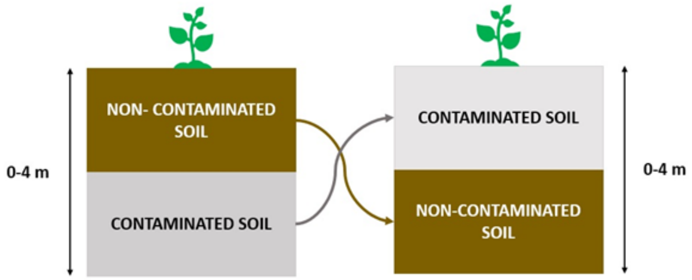


Figure 4-2. Schematic view of the excavation performed to relocate TPH contamination to the upper layers of the soil

The excavation-related works have been carried out with a 30-ton hydraulic crawler excavator.

For the control subparcel, no excavation was needed since the non-contaminated soil is already located in the surface. However, some land moving manoeuvres have been carried out to ensure that soil structure and compaction was comparable in both parcels. The works carried out to prepare the pilot site are summarized in the following table:

Table 4.1 Overview about the main activities during the site preparation

 	Excavation to relocate TPH contamination to the upper layers of the soil to increase pollution bioavailability for the plant species
	Relocation of TPH contamination to the upper layers of the soil to increase pollution bioavailability for the plant species

	Installation of a geotextile fabric of PEAD (high density polyethylene) to prevent contaminant leaching to non-impacted soil layers

4.2 Soil preparation and seeding campaign

Pilot area was already flat hence no major levelling works were carried out while soil ploughing was performed mechanically. Debris removal was not necessary.

The 800 m^2 area was divided into an experimental area of about 600 m^2 and a control area of about 200 m^2. To facilitate the sampling and monitoring, the 600 m^2 experimental area has been divided into 4 parcels (E1, E2, E3, E4) each one divided into 4 more sub parcels except for parcel E3 divided into 2 sub parcels. The 200 m^2 control area was divided into 2 parcels, each divided into 2 sub parcels (see Figure 4-3).

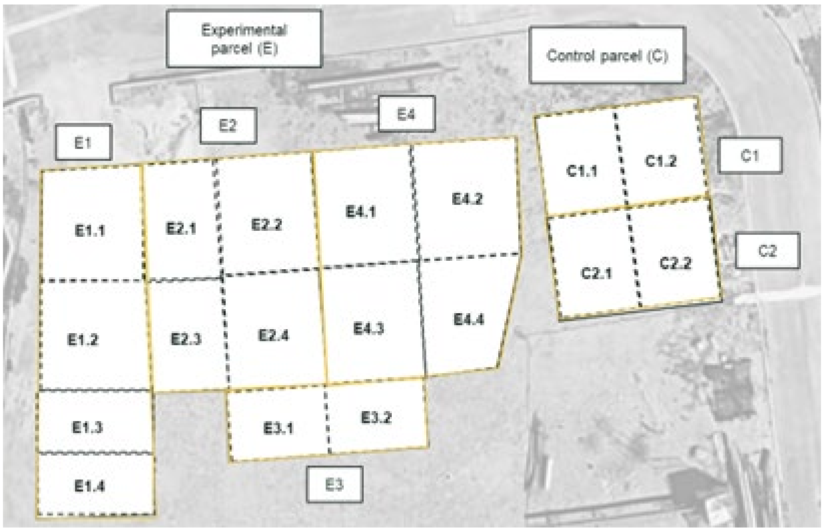


Figure 4-3. Area division at the Spanish site

Based on the results of the initial site characterization and on the results of preliminary pot tests, the following specific phytoremediation strategy was established: Rotation of *Sorghum sp.* and *Brassica napus*, to avoid bare soil during the 3.5 years campaign (being a summer and a winter specie respectively) and exploit the synergies between different species. As amendments the mix of compost, biochar and PGPR was used.

To achieve the Objective 1.1 of 40 kg of biomass production per season, 20 plants/m^2 will be seeded with a spacing of 50x50 cm^2. The number of seeds to be planted and seeding rate will be determined depending on the characterization of the seed stock and their germination results before each sowing season.

The seeding of both *Sorghum sp.* (realized on May 4th, 2022) and of *Brassica napus* (realized on September 8th, 2022) has been performed manually.

It is important to notice that it has been decided to focus the *Brassica napus* seeding to only the most contaminated sub parcels (most conservatory solution) that resulted to be E1.1, E2.1, E2.2, E4.1, E4.2 (by the soil characterization of the site before and after *Sorghum sp.* campaign) and consequently the control parcels were also reduced to two (C1.1 and C1.2). The new configuration of the pilot site (5 experimental parcels: E1.1, E2.1, E2.2, E4.1, E4.2 and 2 control parcels: C1.1, C1.2) will be applied for the rest of the field campaign.

Concerning the amendments application, to determine the amount of compost, the amount of N needed to reach the biomass production goal of 3000 kg/ha has been calculated. It has been decided to focus only on the nutrients for which the highest deficiencies have been detected during pot tests, such N. Moreover, the amount of the compost to be added has been calculated considering that the soil of the site has very low values of nutrients so these must all be provided by the added compost. The amount of biochar was calculated as 20% volume of the total amount of compost. Compost and biochar have been added and mixed directly on field. Concerning PGPR the fertilizer program (with different applications during the 3 months of the phytoremediation strategy) provided by the supplier has been followed.

Considering the nitrogen deficiencies in most important growth phases of both *Sorghum sp.* and *Brassica napus* detected during the pot tests, natural bio stimulants were added to the fertilizer program.

During the *Sorghum sp.* campaign, the bio-stimulants have been tested only in some experimental and control parcels so to compare results and determine their efficiency (see Figure 4-4). Biostimulants will not be applied during *Brassica napus*.

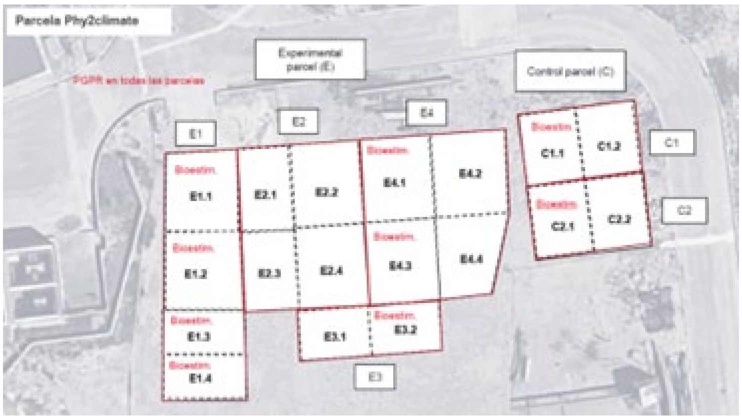


Figure 4-4. Scheme of bio-stimulants application in the Spanish pilot site during *Sorghum sp.* first year of field campaign (May 4th 2022, September 2nd 2022)

4.3 Monitoring program

The Spanish pilot site is located within the facilities of Exolum (formerly known as Compañía Logística de Hidrocarburos S.A. (CLH) and a partner in the present project. Thus, the site is a restricted access area with private surveillance and no fences were needed.

To determine the water requirements of the plant species, a meteorological station together with sensors to detect water content, electrical conductivity, and temperature in soil, have been installed at the site to collect meteorological parameters established in the common framework (Table 3.9 of D2.1). The meteorological station specifically records the following parameters: solar radiation (W/cm^2); precipitation (mm), wind speed (m/s), wind direction (degrees), air temperature (ºC), Vapour pressure (hPa), Air pressure (hPa), Relative humidity (%), and daily global solar exposure (MJ/m^2).

The collected data are sent remotely to LEITAT's facilities and introduced into the CROPWAT software, a decision support tool developed by the Land and Water Development Division of FAO for the calculation of crop water requirements and irrigation requirements based on soil, climate, and crop data.

The irrigation through conventional hose has been preferred over other alternatives (for example, a drip irrigation system) because irrigation water will reach the entire topsoil area and, thus, plant roots will be able to grow and extend in all directions. On the contrary, with a drip irrigation system, for instance, roots will tend to grow towards the water source on the surface rather than vertically in depth, which would most probably reduce the phytoremediation capacity, given the distance to TPH soil contamination.

The monitoring of the plants was planned to be carried out every 15 days to evaluate: luxuriant (lushness of the plants), stem height, nutritional deficiencies, and presence of pests.

Soil and energy crop characterization were planned to be carried out before and at the end of each growing season. In the case of Spanish pilot site, the sampling program which was followed, is described in Table 4.9 and 4.10 of D2.1, while the methods for soil characterization and the analysis of the energy crops are described Table 4.11 of D2.1.

4.4 Plant development

The plantation was monitored every 15 days for three months, for which height monitoring and visual evaluation were carried out to prevent problems derived for example from nutritional deficiencies or pests.

After three months it was observed that the specimens established in the control parcels were the most luxuriant and all had panicles (see Figure 4-5), reaching average heights of approximately 134 cm (C1) and 169 cm (C2). In parcel E3 the specimens showed characteristics similar to those of the controls, although approximately half did not have panicles, with an average height of approximately 172 cm. Similar data were observed in E1 in which the average height was approximately 160 cm, although some specimens were small and medium luxuriant.

Figure 4-5. Growth stages, panicles and presence of pests during the first year of field campaign bases on the use of *Sorghum sp.* mixed with compost/biochar/PGPR (May 4th 2022, September 2nd 2022)

The plants that developed on the most polluted parcels (E2 and E4), showed worse growth (see Figure 4-6). Most of the specimens of E2 were small or very small plants with an average height of approximately 84 cm and without panicles, except for the specimens located on E2.4. Better results were obtained for E4, with specimens of all types from very small to luxuriant, and approximately half of them with the presence of panicles, reaching an average height of approximately 140 cm.

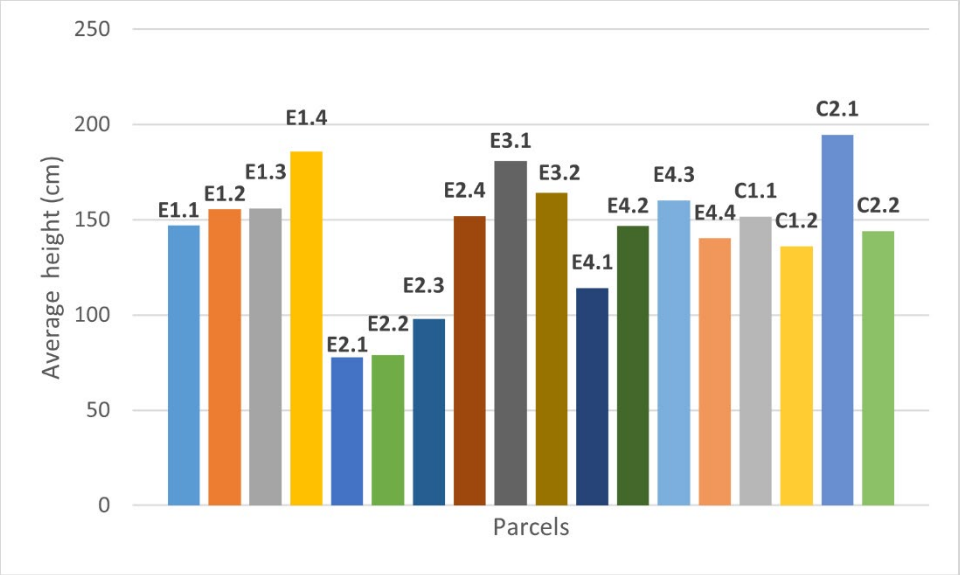


Figure 4-6. Average height of plants at each subparcel of the pilot site, estimated at the end of the first year of the phytoremediation strategy based on the use of *Sorghum sp.* mixed with compost/biochar/PGPR (May 4th 2022 - September 2nd 2022)

Comparing the growth in height of those specimens established in the experimental parcels with those established as a control, it was observed how at the end of the trials the control parcels presented a higher growth in height of approximately between 7% and 20% on average compared to the most contaminated parcels; and a growth in height very similar to the parcels with medium levels of contamination (E1 and E3).

Below a summary of the first year of *Sorghum sp.* the field campaign that started on May 4th and ended on September 2nd, 2022 can be observed.

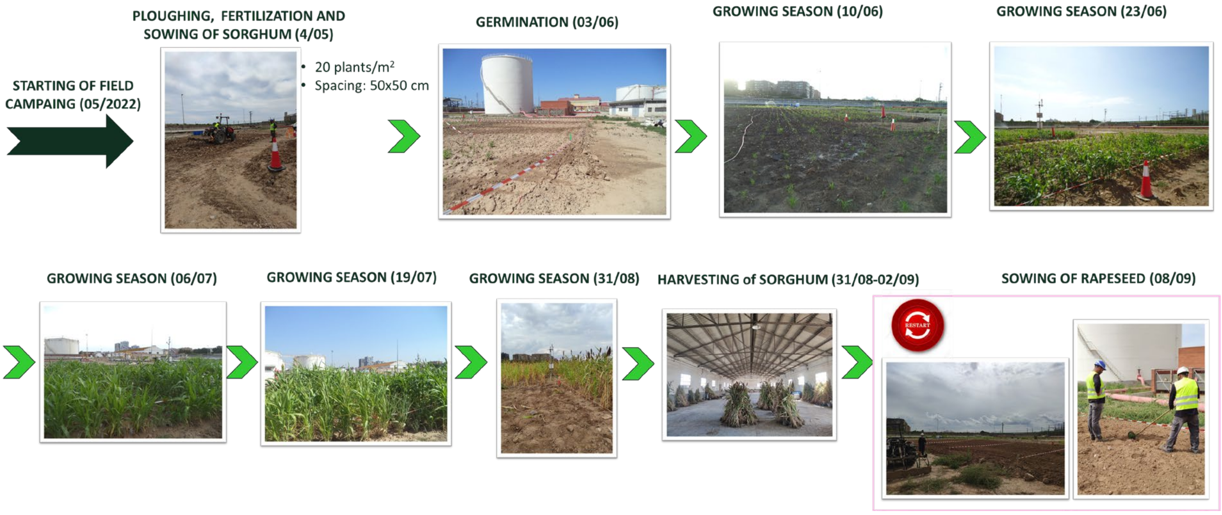


Figure 4-7. *Sorghum sp.* the field campaign that started on May 4th and ended on September 2nd, 2022. The first steps were the site preparation, fertilization and sowing. The following steps were the germination, growth and development of *Sorghum* sp. It was finally harvested in September for drying as shown in the image. The last image corresponds to the sowing of rapeseed.

4.5 Environmental conditions

Concerning the environmental conditions, during the first sowing campaign (from May 4th until September 2nd, 2022) of *Sorghum sp,* the following observations can be made:

- The average temperature detected during the months of May, June, July and August 2022 was about 2-4 ºC higher than the average temperature during the last 10 years;
- The average precipitation detected during the months June and August 2022 decreased to half of the average recorded in the last ten years. During the months May and June 2022, the average precipitation was inside the usual range.

Despite the higher temperatures and lower precipitations, the *Sorghum sp.* grew regularly with no major problems, only the occurrence of pests and specifically aphids and whiteflies, was detected during the second half of July 2022. To fight the plague, phytosanitary products were applied (in total 50 L of water with Tromin Oil (300 ml/100 L) and Bijap (500 ml/100 L)) in mid - August and mid-July 2022.

However, the climatic changes observed during the month of September 2022 and above all the abundant rainfall, have affected the germination of *Brassica napus*, already limited by the high concentration of TPH (as above mentioned the seeding of *Brassica napus* was only performed at the most contaminated subparcels). Replanting strategy was not applied because when rainfall ended the optimal time for sowing the colza was already passed. It is important to notice that some plants of *Sorghum sp.* that were not harvested to test possible re germination, actually started to re germinate, probably due to the unusually high temperatures observed during the months of September, October and November 2022. Hence a possible phytoremediation strategy to be applied for the following years of the field campaign could be based only on the seeding of *Sorghum sp.*

The following graphs are depicting the average daily temperature (ºC) and precipitation (mm) detected by the meteorological station installed at the pilot site, during the months of May and November 2022. In Table 4.2, the monthly average temperature and precipitation recorded at the Spanish pilot site between the months of June-November 2022, are reported.

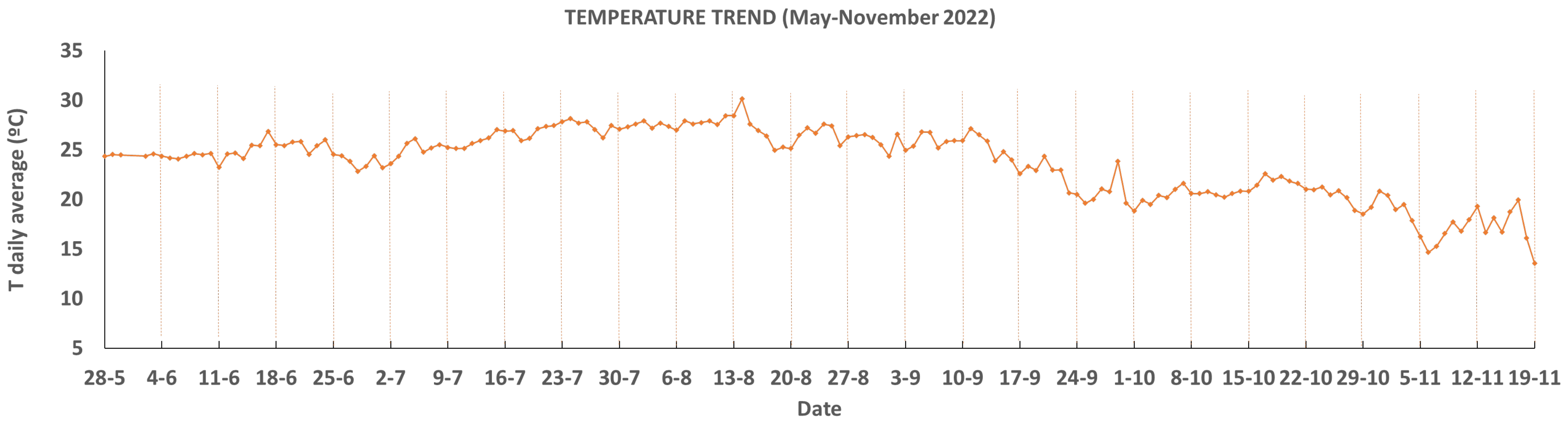


Figure 4-8. Daily average temperature (ºC) detected between May and November 2022

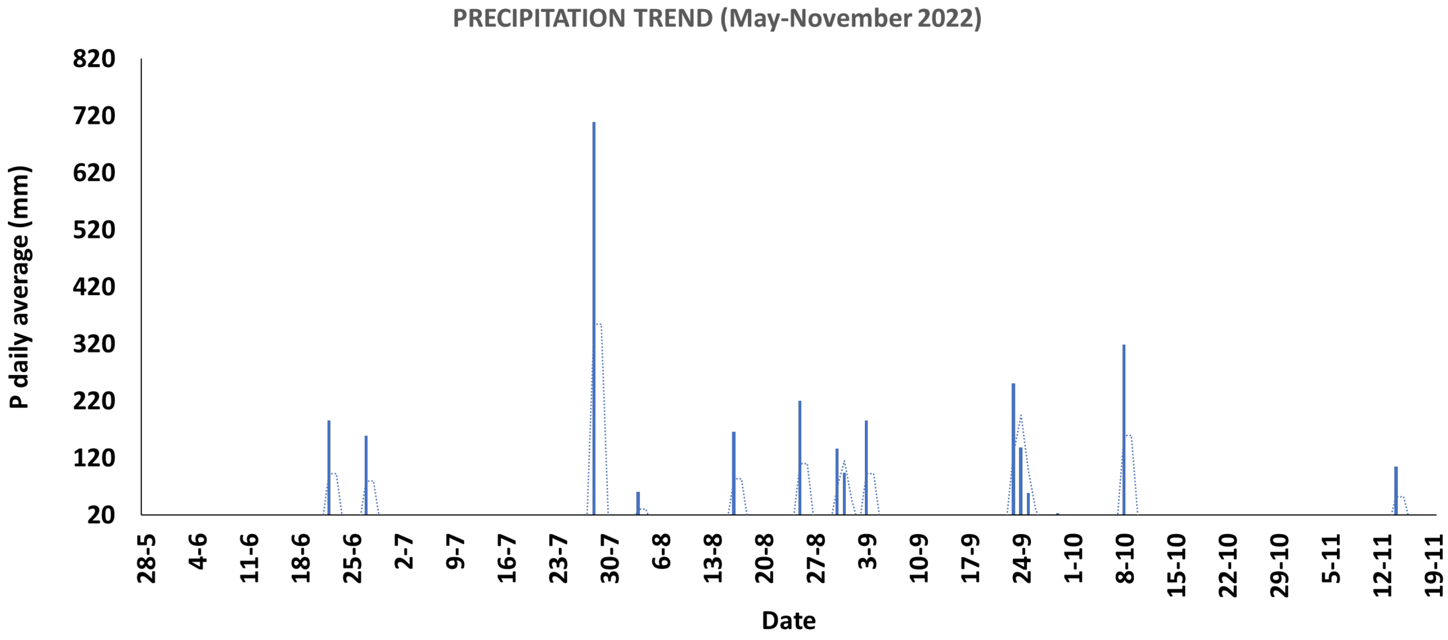


Figure 4-9. Daily average precipitation (mm) detected between May and November 2022

Table 4.2. Monthly average temperature and precipitation recorded at the Spanish pilot site between the months of June-November 2022

	T monthly average (ºC)	P monthly average (mm)
JUNE 2022	24.68	11.87
JULY 2022	26.23	22.89
AUGUST 2022	27.05	21.86
SEPTEMBER 2022	23.83	21.95
OCTOBER 2022	20.65	10.29
NOVEMBER 2022	17.24	5.27

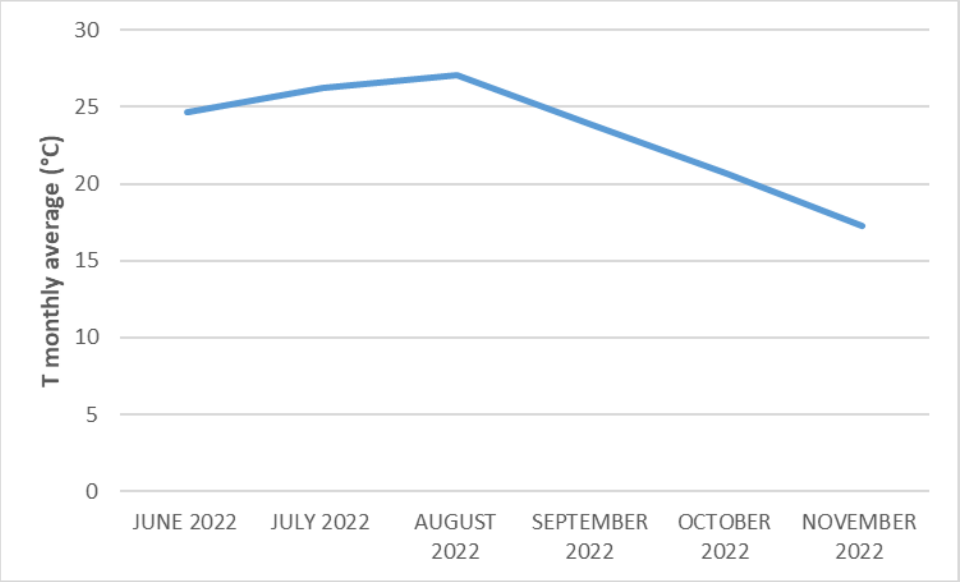


Figure 4-9. Average Temperature values (June 2022-November 2022)

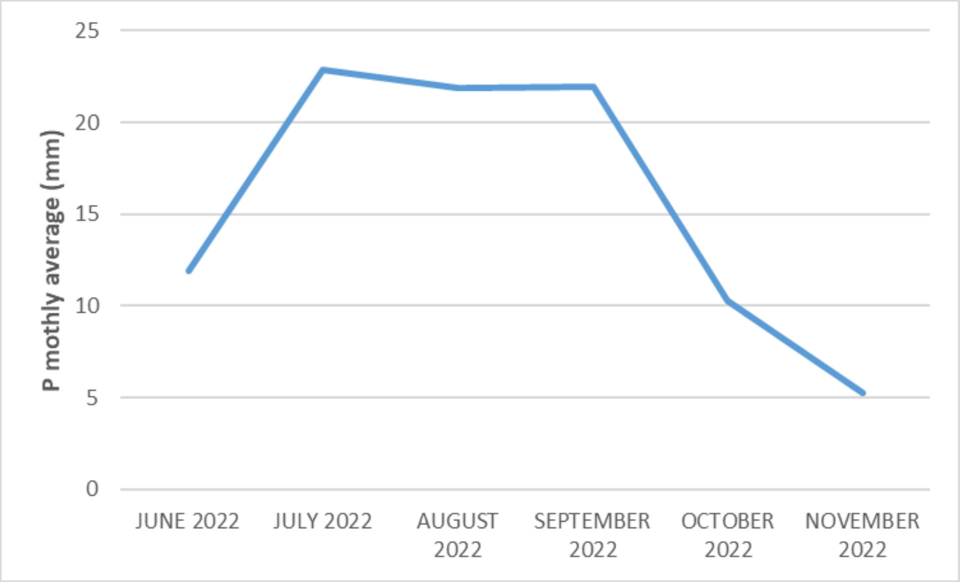


Figure 4-10. Average Precipitation values (June 2022-November 2022)

4.6 Harvest and pelletizing

The *Sorghum sp.* harvesting campaign was carried out between August 31st and September 2nd, 2022. Harvesting consisted of the collection of the whole plant, including all plant materials (roots, leaves, stems and seeds). The harvested fresh biomass was weighted for each of the subparcels and was left to dry in a warehouse, until its shipping to the pelletization facilities.

Prior to pelletizing, the biomass will be completely dried and shredded to size 4-5 mm. An initial characterization will be performed, in which water content; ash; elemental carbon, hydrogen and nitrogen; sulphur and chlorine; and oxygen content will be analysed. Several trials will also be performed to ensure the pellet quality requested by WP3. In particular, the investigated variable will be the compression ratio of the matrix, with three compression ratios: 1:4, 1:6 and 1:10. For each of those, mechanical durability, apparent density, total humidity and size of the pellets will be determined. The best compression ratio will later be used to pelletize the total amount of dry biomass, which will be later shipped to WP3.

Sorghum sp. biomass has been collected all at once (stems+ leaves + roots + seeds).

On the contrary, when *Brassica napus* will have to be harvested, seeds will be collected manually first, then the other part of the aboveground and belowground biomass (leaves, stems, and roots) will be collected.

Harvesting will always be performed manually, with the help of conventional farming tools such as sickles or scythes and no harvesting pre-treatment will be implemented only drying.

The aboveground (stems+seeds) and belowground (roots) biomass of *Sorghum sp.* has been analysed to prove that no translocation of TPH/PAH has occurred. If the translocation factor will result in a value <1, the detection of TPH/PAH in the harvested biomass won't be performed for the following harvesting campaigns and only biomass production will be estimated.

The physico-chemical characterization of the collected soil samples, before the seeding of *Sorghum sp.* and after its harvesting, has been performed at LEITAT facilities. For each parcel 3 composite samples of soils (obtained by collecting 5 samples per each subparcel constituting the parcel) have been analysed.

4.7 Phytoremediation performance

As above mentioned, for each parcel in which the pilot site has been divided, 3 composite samples of soils (obtained by collecting 5 samples per each subparcel constituting the parcel) have been analysed before and after the phytoremediation campaign based on the use of *Sorghum sp.* mixed with compost+biochar+PGPR, to carry out the physico-chemical characterisation of the site and determine the phytoremediation efficiency.

The performance of the phytoremediation strategy has been established by determining the TPH percentage removal efficiency as

$$TPH\ \%\ removal = \frac{TPH\ F - TPH\ 0}{TPH0}\ x\ 100$$

where TPH 0 and TPH F are an average value (3 replicates) of TPH concentrations at the beginning and at the end of the phytoremediation campaign, respectively.

However, phytoremediation actions conducted in WP2 have the further objective of improving soil quality to get to an arable quality. Therefore, it is essential to monitor, not only the decontamination capacity of the remediation strategy but also its effect on enhancing soil quality. To this purpose and, as mentioned in the introduction section, the soil quality index (SQI) proposed by Klimkowicz-Pawlas et al. in 2019 was selected to be estimated during the 3.5 years field campaign. As previously commented, the selection of the parameters to be followed to assess the SQI depends on the level of anthropopressure of the site, and the Spanish Pilot Site has been classified as of high anthropopressure. Hence in this case the parameters to be followed during the field campaign to determine the SQI will be the Humins, Zn, Basal microbial respiration, pH, and Silt (see Table 3.2). Analysis of these parameters are on-going to determine the SQI for the first year of field campaign and will be presented in the next deliverable.

4.7.1 Soil parameters

Results of the physico-chemical characterisation of soil before the seeding of *Sorghum sp.* (pre-sowing) have been collected in Table 4.3, Table 4.5 and Table 4.7 and the results obtained after the harvesting of *Sorghum sp* (post-harvesting) have been collected in Table 4.4, Table 4.6 and Table 4.8, so to facilitate their comparison.

Concerning physical parameters, the moisture content of soil, also referred to as water content, shows mean values below 4.2% in pre-sowing analysis while it decreases to 0.9% (the lowest value obtained) in post-harvesting analysis.

Concerning chemical parameters, the results show that pH average values remain almost constant to basic conditions (in accordance with high values of Mg and Ca) in both pre-sowing and post-harvesting analysis.

The values of pH at the beginning of the first year of *Sorghum sp.* campaign (pre-sowing), range between 7.2 and 7.7 for the contaminated parcels (E1, E2, E3 and E4) and between 7.6 and 7.7 for the non-contaminated parcels (C1 and C2), while the average values of pH at the end of the first year of *Sorghum sp.* campaign (post-harvesting) range between 8.4 and 8.6 for the contaminated parcels (E1, E2, E3 and E4) and between 8.3 and 8.6 for the non-contaminated parcels (C1 and C2). In conclusion, no differences in pH values are observed between contaminated parcels and non-contaminated parcels, but there is an increasing trend of pH observed at the end of the first year of *Sorghum sp.* campaign, probably due to the application of biochar.

Concerning the electrical conductivity (EC), the results shows that EC average values at the end of *Sorghum sp.* campaign increases to 418 µS/cm in contaminated parcels and 578 µS/cm in non-contaminated parcels. However, the EC average values in both cases are below 4dS/m, indicating that the soils are not saline.

Concerning the organic matter (OM), the results shows that OM average values at the end of *Sorghum sp.* campaign decreases to 3.84% (the lowest average value). Total N content has shown very low values in both pre-sowing and post-harvesting analysis, with a high prevalence of C (3.48 to 4.84 mg/kg dry matter at the end of *Sorghum sp.* campaign).

Concerning the available P concentrations, results show that P concentrations values at the end of *Sorghum sp.* campaign range between 91-97 mg/kg dry matter for the contaminated parcels (E1, E2, E3 and E4) and between 98-122 mg/kg dry matter for the non-contaminated parcels (C1 and C2) while the available K concentrations range between 2185-2428 mg/kg dry matter for the contaminated parcels and between 1989-2395 mg/kg dry matter for the non-contaminated parcels (also referred as controls). In conclusion, P concentrations have increased with the addition of amendments (compost) reaching slightly higher values, while the K concentrations are adequate, although they have significantly reduced due to consumption by *Sorghum sp.* plants.

By comparing the values obtained at the end of the first year of *Sorghum sp.* campaign presented in Table 4.4, Table 4.6 and Table 4.8 with the initial values presented in Table 4.3, Table 4.5 and Table 4.7, the following conclusions regarding metal, metalloids, and other elements of interest can be made:

- Mean values of Mo, S, Cd and B remain below LQ;
- Cr, Pb and Mn do not significantly change their mean values;
- Mean values of Mg, Ca, Cu, Fe and Na slightly increases.

Currently the study area is for industrial use, despite this and according to the objectives of the project, the values established for metal/metalloids concentrations for other uses (more restrictive in general), will also be taken into account. In relation to the Generic Reference Levels established in Cataluña region (DL 1/2009), the studied soils do not exceed the limits established for the protection of ecosystems or health for the concentrations of Cu vary between 21 and 37 mg/kg dry matter on average, nor of As with concentrations of approximately 10 mg/kg.

In the case of Zn, in the control parcels the limits established for the protection of ecosystems (110 mg/kg), and for other uses (170 mg/kg) at the level of protection of human health are exceeded, not for industrial use (1000 mg/kg). Pb exceeds the limit concentrations for health protection for urban and other uses (60 mg/kg), not for industrial use (550 mg/kg) and for the protection of ecosystems (60 mg/kg), with the exception of parcel E1. Mo and Cd concentrations are below the detection limit.

Table 4.3. Pre-sowing physico-chemical analysis to determine the effectiveness of the investigated phytoremediation strategy

Parcel	Texture			Texture class	pH$_{(water)}$	EC	Water content	Mg	Ca	Cu	Fe
	Clay	Silt	Sand								
	%	%	%	-	-	*µS/cm*	%	*mg/kg DM*	*mg/kg DM*	*mg/kg DM*	*mg/kg DM*
E1	14	26	60	Sandy-loam	7.2±0.03	301±4	3.8±0.03	22796±795	183177±5724	19.3±0.6	12415±537
E2	14	23	63	Sandy-loam	7.4±0.06	266.3±2	3.5±0.02	24119±1317	187511±2708	18±0.0	13019±239
E3	14	20	66	Sandy-loam	7.5±0.02	308±4	4.2±0.10	22804±418	188068±4546	19±1.0	13385±476
E4	14	26	60	Sandy-loam	7.7±0.06	287.3±4	4.2±0.21	22859±896	186451±2002	17.3±0.6	13116±1,022
C1	10	14	76	Sandy-loam	7.7±0.04	317.3±4	1.4±0.12	20975±674	147428±5669	21.7±1.5	14210±346
C2	10	12	78	Sandy-loam	7.6±0.05	623±15	1.8±0.03	19117±1666	158280±7673	38±21.7	15193±22

Table 4.4. Post-harvesting physico-chemical analysis to determine the effectiveness of the investigated phytoremediation strategies

Parcel	Texture			Texture class	pH$_{(water)}$	EC	Water content	Mg	Ca	Cu	Fe
	Clay	Silt	Sand								
	%	%	%	-	-	*µS/cm*	%	*mg/kg DM*	*mg/kg DM*	*mg/kg DM*	*mg/kg DM*
E1	15	25	60	Sandy-loam	8.6 ± 0.01	390±8	2.1±0.25	31676±1338	218755±2337	21±1	17321±740
E2	13	23	64	Sandy-loam	8.4 ± 0.02	357±13	1.6±0.28	31996±545	223327±1506	21±0.2	18080±379
E3	13	22	65	Sandy-loam	8.4 ± 0.1	418±20	0.9±0.01	30385±747	218701±5928	25±3	16858±1050
E4	13	27	60	Sandy-loam	8.5 ± 0.02	380±12	1.4±0.44	31426±267	220521±6377	37±26	16730±504
C1	10	15	75	Sandy-loam	8.6 ± 0.1	383±27	0.9±0.43	28605±1706	177404±6928	30±1	16815±279
C2	10	13	77	Sandy-loam	8.3 ± 0.03	578±16	1.2±0.54	29260±2793	205391±1121	33±1	15517±280

Table 4.5. Pre-sowing physico-chemical analysis to determine the effectiveness of the investigated phytoremediation strategy

Parcel	Organic matter	Mn	Mo	Zn	Total C	Total N	Cd	Cr	Pb
-	***%***	***mg/kg DM***	***mg/kg DM***	***mg/kg DM***	***mg/kg DM***	***mg/kg DM***	***mg/kg DM***	***mg/kg DM***	***mg/kg DM***
E1	5.56±0.32	305±9	<LQ	55±5	5.90±0.10	0.030±0.004	2.3±0.6	12±1	57±2
E2	*	318±7	<LQ	49±1	6.13±0.40	0.033±0.003	<LQ	10±1	70±9
E3	8.08±2.74	326±19	<LQ	50±2	5.87±0.21	0.038±0.004	<LQ	11±1	76±3
E4	7.06±0.37	327±15	<LQ	53±4	6.06±0.70	0.033±0.003	<LQ	11±1	76±3
C1	3.83±0.13	264±8	<LQ	204±9	5.66±1.20	0.034±0.001	<LQ	12±0	65±6
C2	4.24±0.26	260±7	<LQ	254±13	6.28±1.18	0.031±0.008	<LQ	16±4	73±4

** Inconclusive value. It will be included in the next deliverable.*

Table 4.6. Post-harvesting physico-chemical analysis to determine the effectiveness of the investigated phytoremediation strategies

Parcel	Organic matter	Mn	Mo	Zn	Total C	Total N	Cd	Cr	Pb
-	***%***	***mg/kg DM***	***mg/kg DM***	***mg/kg DM***	***mg/kg DM***	***mg/kg DM***	***mg/kg DM***	***mg/kg DM***	***mg/kg DM***
E1	3.84±0.06	372±12	<LQ	62±3	4.55±0.64	0.023±0.004	<LQ	12±0	55±6
E2	4.14±0.24	398±3	<LQ	68±4	4.69±0.42	0.026±0.002	<LQ	14±1	61±2
E3	4.27±0.14	384±8	<LQ	76±4	4.72±0.34	0.028±0.002	<LQ	14±1	69±3
E4	4.43±0.11	386±11	<LQ	66±13	4.84±0.29	0.031±0.003	<LQ	13±0	69±3
C1	4.01±0.03	331±10	<LQ	441±60	3.48±0.91	0.028±0.003	<LQ	17±1	76±8
C2	4.23±0.20	353±10	<LQ	264±14	4.02±0.97	0.030±0.008	<LQ	16±0	84±2

Table 4.7. Pre-sowing physico-chemical analysis to determine the effectiveness of the investigated phytoremediation strategy

Parcel	P available	K available	S	B	As	Na	Microbial biomass
-	*mg/kg DM*	*mg/kg DM*	*mg/kg DM*	*mg/kg DM*	*mg/kg DM*	*mg/kg DM*	*CFU/ml*
E1	<LQ	2185±153	<LQ	<LQ	7±1	158±9	7.56x10^{6}
E2	<LQ	2316±100	<LQ	<LQ	6±0	120±10	7.63x10^{6}
E3	<LQ	2428±164	<LQ	<LQ	6±1	122±8	8.53x10^{6}
E4	<LQ	2278±446	<LQ	<LQ	6±1	105±14	1.15x10^{7}
C1	<LQ	2395±55	<LQ	<LQ	5±1	117±17	1.43x10^{6}
C2	<LQ	1989±367	<LQ	<LQ	5±1	101±28	1.76x10^{6}

Table 4.8. Post-harvesting physico-chemical analysis to determine the effectiveness of the investigated phytoremediation strategies

Parcel	P available	K available	S	B	As	Na	Microbial biomass
-	*mg/kg DM*	*mg/kg DM*	*mg/kg DM*	*mg/kg DM*	*mg/kg DM*	*mg/kg DM*	*CFU/ml*
E1	94±2	156±21	<LQ	<LQ	9±0	311±8	1.47x10^{6}
E2	97±6	147±10	<LQ	<LQ	9±0	330±24	1.83x10^{6}
E3	91±7	159±11	<LQ	<LQ	9±0	310±8	1.30x10^{6}
E4	93±4	119±15	<LQ	<LQ	9±0	320±7	1.37x10^{6}
C1	98±7	181±18	<LQ	<LQ	9±0	337±13	1.70x10^{6}
C2	122±7	267±7	<LQ	<LQ	10±0	318±22	1.83x10^{6}

Finally, in the following table the results in terms of BTEX, EPH C10-C40, PAH and TPH, including the % of TPH removal, are reported for each of the experimental and control parcels in which the Spanish pilot site has been divided, before (T0) and once finalised (TF) the first year of the phytoremediation campaign based on the use of *Sorghum sp.* mixed with compost+biochar+PGPR.

Particularly, % of TPH removal, used to determine the performance of the phytoremediation strategy, has been calculated as follows:

$$TPH\ \%\ removal = \frac{TPH\ F - TPH\ 0}{TPH0}\ x\ 100$$

where TPH 0 and TPH F are an average value (3 replicates) of TPH concentrations at the beginning and at the end of the phytoremediation campaign, respectively.

Table 4.9. Concentrations of petroleum hydrocarbons determined for each of the experimental (E1, E2, E3, E4) and control (C1, C2) plots in which the site has been divided, before (T0) and once finalised (TF) the phytoremediation campaign based on the use of *Sorghum sp*. mixed with compost+biochar+PGPR (May 4th-September 2nd, 2022)

E1 PLOT				
Sampling time	T0	T41	T90	T120 (TF)
Sampling date	04/05/2022	14/06/2022	02/08/2022	01/09/2022
BTEX	7.9	10	4.93	0.25
EPH C10-C40	177.5	390	192.5	94.5
HAP 16 EPA	0.605	0.89	1.34	0.26
HAP VROM	0.522	0.77	1.26	0.2
TPH C5-C35	260	553	255	112
TPH removal (%)	**57**			
E2 PLOT				
Sampling time	T0	T41	T90	T120 (TF)
Sampling date	04/05/2022	14/06/2022	02/08/2022	01/09/2022
BTEX	131.75	0.72	14.2	0.7
EPH C10-C40	375	170	300	111
HAP 16 EPA	1.505	0.16	1.21	0.28
HAP VROM	1.422	0.1	1.15	0.24
TPH C5-C35	907	174	397	119
TPH removal (%)	87			
E3 PLOT				
Sampling time	T0	T41	T90	T120 (TF)
Sampling date	04/05/2022	14/06/2022	02/08/2022	01/09/2022
BTEX	0.58	1.2	0.25	<0.25
EPH C10-C40	94.5	180	50	50
HAP 16 EPA	0.29	0.89	1.34	0.21
HAP VROM	0.21	0.77	1.262	0.16
TPH C5-C35	98	236	66	62
TPH removal (%)	37			
E4 PLOT				
Sampling time	T0	T41	T90	T120 (TF)
Sampling date	04/05/2022	14/06/2022	02/08/2022	01/09/2022
BTEX	34.25	0.25	0.25	0.35
EPH C10-C40	472.5	120	230	96
HAP 16 EPA	3.03	0.25	0.16	0.37
HAP VROM	2.80	0.21	0.1	0.29
TPH C5-C35	681	143	248	103
TPH removal (%)	85			

C1 PLOT				
Sampling time	T0	T41	T90	T120 (TF)
Sampling date	04/05/2022	14/06/2022	02/08/2022	01/09/2022
BTEX	0.65	-	-	<0.25
EPH C10-C40	74	-	-	83
HAP 16 EPA	3.60	-	-	3.7
HAP VROM	2.63	-	-	2.7
TPH C5-C35	64	-	-	67
TPH removal (%)	-5			
C2 PLOT				
Sampling time	T0	T41	T90	T120 (TF)
Sampling date	04/05/2022	14/06/2022	02/08/2022	01/09/2022
BTEX	0.65	-	-	<0.25
EPH C10-C40	74	-	-	110
HAP 16 EPA	3.60	-	-	1.8
HAP VROM	2.63	-	-	1.3
TPH C5-C35	64	-	-	94
TPH removal (%)	-48			

In the analysis of the subsamples, some anomalous values have been identified but have been classified as normal consequence of the plot's heterogeneity and method variability.

As shown in the table above, the total TPH concentration in the experimental plots has been considerably reduced by the establishment of the phytoremediation technique. The average values of the control plots, show a slight increase in concentrations, although some subsamples have shown a decrease, these results contrast since the removal rate obtained for TPH has been in any case considerably less than for the rest of the plots.

Polycyclic aromatic hydrocarbons have also shown a decrease in their concentration. In general, polycyclic aromatic hydrocarbons show concentration levels below the reference level established as more restrictive (other uses).

In some cases, as is the case of Naphthalene (E2, E4), at the beginning of the tests the plots presented concentration values slightly higher than the limit established by Spanish legislation (R.D 9/2005).

Currently, concentrations have been significantly reduced in the approximately three months of the trial, with concentrations of 0.01 mg/kg naphthalene.

In the case of benzo(a)pyrene, has shown a different trend in part of the subplots with values above the established most restrictive legal limit (0.02 mg/kg) dry matter), as it is the case of E3. Some exceptional cases have also been observed, according to replicated samples, so special attention will be paid to its evolution.

As can be observed (Figure 4.9), most polluted plots have shown a decrease in the total concentration of TPHs. The most contaminated plots have achieved TPH removal values of up to 87% efficiency. The control plots are the ones that presented less removal capacity for some C1 subsamples, highlighting the particular case of the average values obtained for C2, a consequence of the heterogeneity of the soil, since as in the rest of the results most TPHs have been decrease, C2 presented higher dispersion which makes the average values are considerably increased. The results obtained will be considered in future actions.

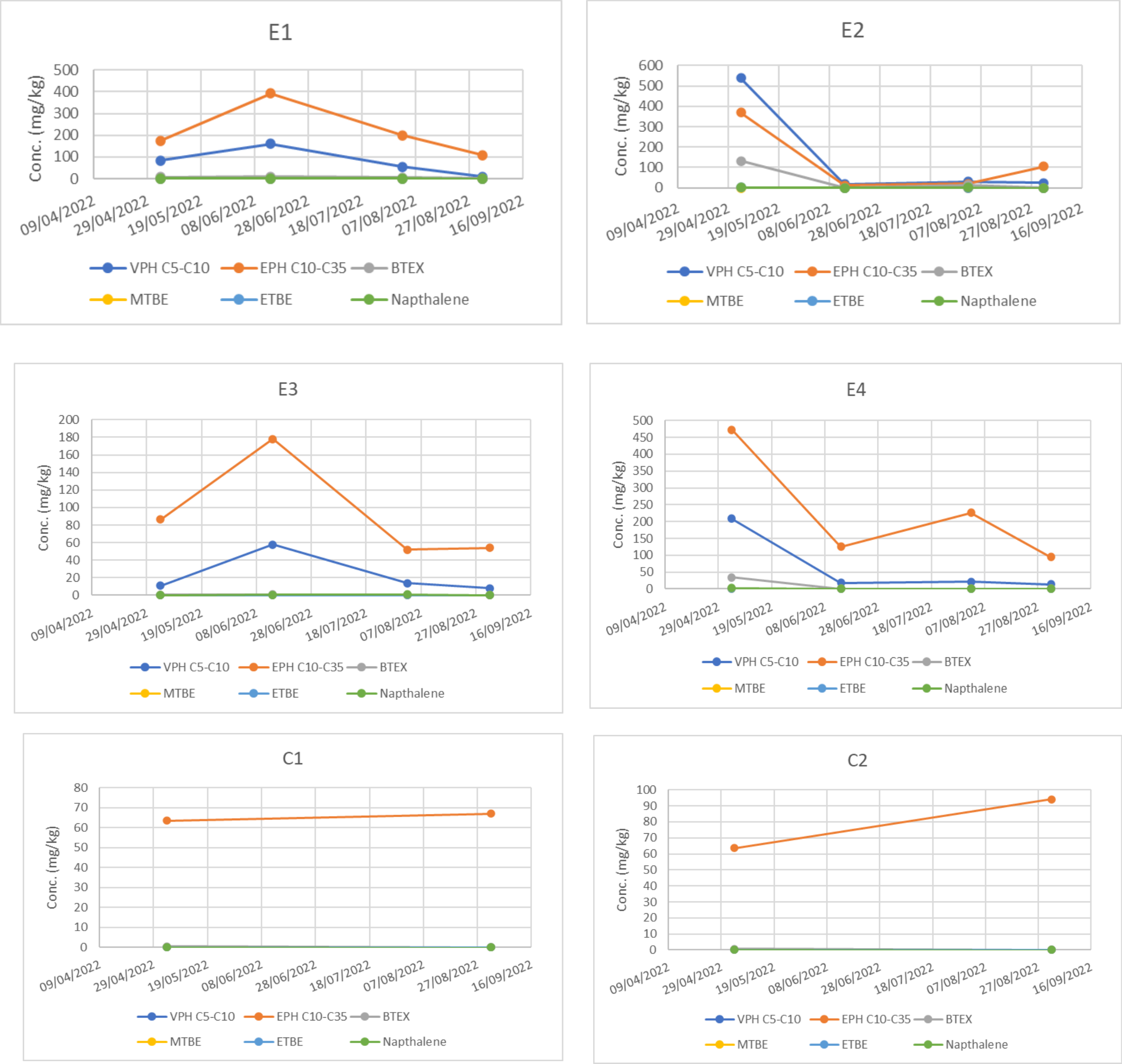


Figure 4-10. Pollutants concentration evolution, during the technique execution (from may 2022 to september 2022)

4.7.2 Biomass output

Aboveground biomass (leaves/stems/seeds) was measured gravimetrically at the end of the trials, using both wet and dry weights. To determine dry weight, the aboveground biomass was dried by leaving it in a warehouse. Results of wet weight of stems and leaves (collected as a bulk sample) are presented in the following Table, dry weight will be obtained on November 25th, 2022.

Table 4.9. Biomass production determined for each of the experimental (E1, E2, E3, E4) and control (C1, C2) parcels in which the site has been divided, once finalised the phytoremediation campaign based on the use of Sorghum sp. mixed with compost+biochar+PGPR (May 4th-September 2nd, 2022)

Parcel	Surface (m^2)	Wet weight (kg)	Dry weight (kg)	Biomass production (t/ha)
E1	128	513	271	19
E2	216	301	154	7
E3	121	612	195	34
E4	158	213	61	7
C	117	799	376	26

Comparing the performance of the trials in the different established parcels is observed that biomass production in the most contaminated parcels (E2 and E4), is estimated to be approximately 72% lower than in the parcels established as control (C); 24% less for parcels with medium contamination (E1); and similar production (even higher) in parcels with low concentration of pollutants (E3).

Figure 4-10. Harvested biomass of *Sorghum sp.* dried in a warehouse (field campaign that started on May 4th and ended on September 2nd, 2022)

4.8 Encountered problems and amendments

A modification of Spanish site activities compared to the initially proposed was found to be necessary. Particularly, it was decided to perform the following activities:

- Contaminated soil excavation+ movement of soil+ geotextile fabric (PEAD) installation

Instead of the initially proposed ones such as:

- Contaminated soil excavation, refilling and platform construction, platform dismantling

Furthermore, as above mentioned, the climatic changes observed during the month of September 2022 and above all the abundant rainfall, have affected the germination of *Brassica* *napus*, already limited by the high concentration of TPH (as above mentioned the seeding of *Brassica napus* was only performed at the most contaminated subparcels). Replanting strategy was not applied because when rainfall ended the optimal time for sowing the colza was already passed. On the contrary, some plants of *Sorghum sp.* that were not harvested to test possible re-germination, actually started to re-germinate, probably due to the unusual high temperatures observed during the months of September, October and November 2022. Hence a possible phytoremediation strategy to be applied for the following years of the field campaign could be based exclusively on the seeding of *Sorghum sp.* (no rotation with *Brassica napus*).

4.9 Other information

No additional information.

4.10 Overall summary of phytoremediation performance in M12-M24

The parcels established for 3 months in a paved area of 800 m^2 of the facilities of Exolum, have shown good development. *Sorghum sp*. It has been established and developed in all the parcels, reaching in the parcels established as control heights of up to 169 cm luxuriant specimens and with the presence of panicles.

Similar results have been obtained in the parcels with intermediate contamination, and although the growth parameters are lower in the most of the contaminated parcels after three months, they reached average heights of more than 80 cm.

The objectives of biomass production during the time of execution of the test have been met, obtaining in some parcel's quantities similar to the controls.

Total TPHs concentration of the experimental parcels has been reduced by the establishment of the phytoremediation technique. In general, Polycyclic aromatic hydrocarbons have also shown a decrease in their concentration. Achieving TPH removal values of up to 86% efficiency. Regarding the concentrations of metal(loid)s, special attention will be paid to the concentrations of Pb and Zn for future actions.

5. FIELD TRIALS ON THE SERBIAN PILOT SITE

5.1 Landscape preparation

The Serbian pilot site is situated along Begej canal near Serbian-Romanian border where app. 5900 m^3 of sediments from Begej canal is placed in a confined area. The pilot site has a total area of app. 3800 m^2. For the Phy2Climate project the site is divided in two sections – Landfill 1 and Landfill 2 – each of approximately 1200 m^2 (Figure 5-1).

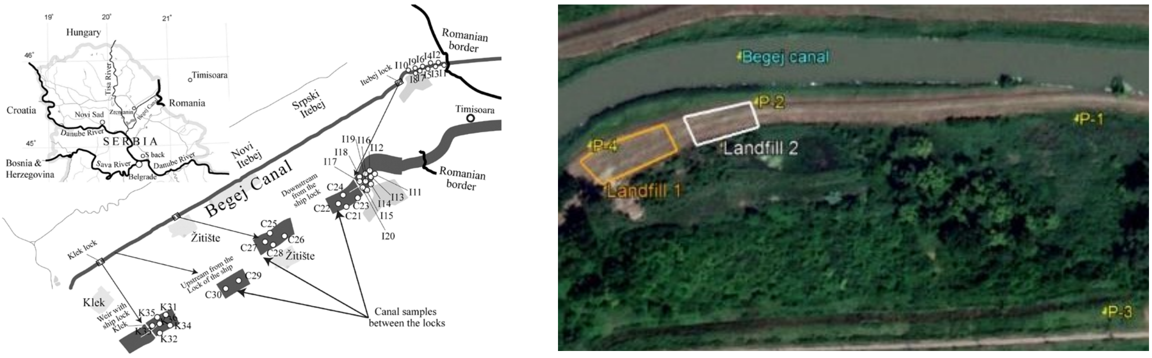


Figure 5-1. Position of landfills and piezometers

Dredging and disposal of sediments at the landfill in 2017 are shown in Figure 5-2.

Figure 5-2. Sediment dredging and aerial view of disposal site (2017)

Four piezometers were installed in 2017 to monitor the impact of leachate from experimental landfill on groundwater (Figure 5-3). Dredging and disposal of sediments in April 2021 are shown in Figure 5-4.

Debris removal has not been necessary.

Figure 5-3. Construction of piezometers for groundwater monitoring (2017)

Landfill 2 was prepared to accommodate fresh canal sediments by PWMCVV. Dredging of fresh sediments from canal was finalised by the end of 2021 (Figure 5-4), sowing of the Landfill 2 are done in September 2022.

Figure 5-4. Dredging and deposition of sediments at Landfill 2

5.2 Soil preparation and seeding campaign

Ploughing and tilling. Sediment that was dredged during 2017 and was moved to Landfill 1 by PWMCVV. By 2020 the area was completely overgrown with vegetation. In February 2021 reeds and other vegetation were first removed from the site. Dredged material was transferred to Landfill 1 and then soil was slowed and levelled by the PWMCVV (Figure 5-5). The pilot site was treated with herbicide (glyphosate) at the beginning of June 2021 to prevent common reed from growing. After that, pilot site was treated with triclopyr to prevent broadleaf weeds from growing (pre-sowing treatment).

November 2020

February 2021

April 2021

September 2021

Figure 5-5. Pilot site preparation activities

Sub-plot division - For the purpose of initial monitoring Landfill 1 will be divided into 10 experimental (1-10) and 2 control (11,12) sections (Figure 5-6).

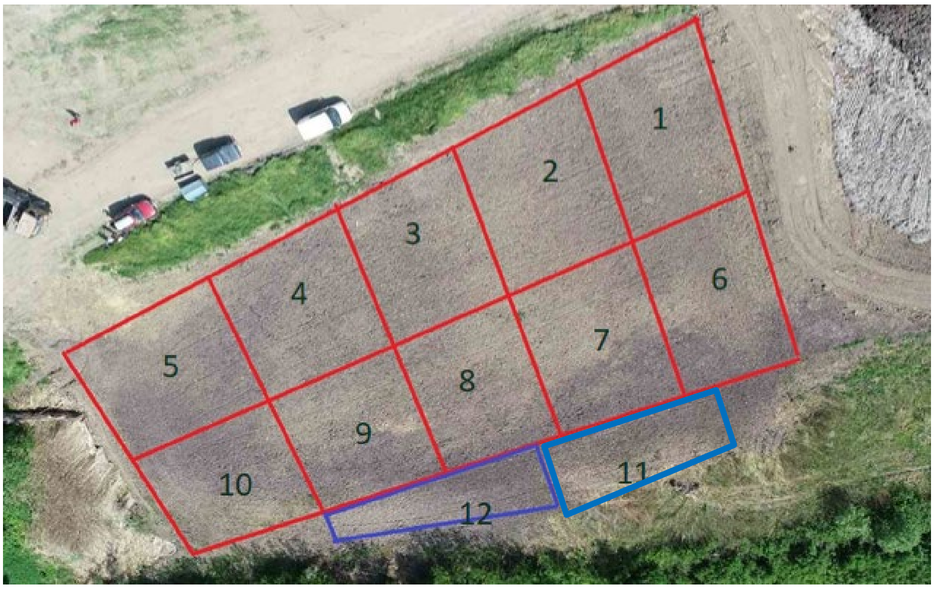


Figure 5-6. Division of Landfill 1 into control (11,12) and experimental (1-10) sections

Based on the results of the initial characterization and on the results of preliminary pot tests a specific phytoremediation strategy was established to be investigated in field. The selection of the most suitable plants for growing on the pilot site was done on the basis of the pot test results. Five different crops were tested for their suitability to be used for growing on pilot site: rapeseed (*Brassica napus*), white mustard (*Brassica alba*), sunflower (*Helianthus annuus*), hemp (*Cannabis sativa*) and sorghum (*Sorghum bicolor*). Beside crops, three commercial products containing PGPR were also tested in the pot tests for its suitability to promote metal uptake by plants. Based on the obtained results, rapeseed (*Brassica napus*) winter variety Zlatana owned by Institute of Field and Vegetable Crops was selected for seeding at the pilot site at Landfill 1 for the first growing season. Since PGPR amendment didn't increase uptake of metals significantly, this amendment was not applied at the pilot site in the first growing season.

Sowing of rapeseed was performed in September 2021. Seeding rate was approximately 60-80 seeds per m^2.

5.3 Monitoring program

Fences and surveillance systems were not applied at the pilot site.

Weather conditions was monitored through AgroSense digital platform that provides support to farmers and agricultural companies in monitoring the weather conditions (https://agrosens.rs) and through portal of the Republic Hydrometeorological Service of Serbia (RHMZ) (Table 5.1.). Both digital platforms enable daily data collection. Daily minimum, maximum and average values for weather condition parameters was collected.

Table 5.1. Weather conditions

WEATHER CONDITIONS	Units	Meteo station and distance from the pilot site
Precipitation	precipitation, mm	Krajisnik (13.44 km) Torak (13.69 km) Banatski brestovac (14.46 km) Zrenjanin - Biosens meteo station (34.5 km) Zrenjanin - RHMZ meteo station (35.8 km)
Air temperature	°C	
Wind speed and direction	m/s	
Humidity	%	
Light regime	lux	

Irrigation of the pilot site was not done. The risk of hot weather is already mitigated by selection of winter variety of rapeseed as test plant for phytoextraction. Rapeseed is normally harvested in the second half of June and hot weather would only induce earlier ripening of seeds and little bit earlier harvest. Possible yield reduction is negligible compared to costs of setting irrigation system.

For initial monitoring Landfill 1 was divided into 10 experimental and 2 control sections (5/6). Sampling for the initial characterisation was done at 4 depths from each section (0-20 cm; 20-40 cm; 40-60 cm; 60-100 cm). At each section composite samples were obtained by collecting three samples from each section at 4 defined depths and composite samples per section were created for each depth. In this way the 48 soil samples from the pilot site were collected (10+2 sections x 4 depths). Physical and chemical characterisation was done in accordance with the defined common framework program (including parameters defined for Soil Quality Index (for the sampling depth 0-20 cm), Table 5.2.

Soil monitoring after the harvesting is based on the results of initial characterisation, minimum 50% of initial sample sites were sampled for monitoring. Criteria for selection was the type and the level of contaminants determined in the initial characterisation. At each defined section three samples were collected at 4 defined depths (0-20 cm; 20-40 cm; 40-60 cm; 60-100 cm) and composite sample per segment was created for each depth. In this way the 20 soil samples from the pilot site were collected (min. 5 segments x 4 depths) for the purpose of monitoring of phytoremediation activity. Additional samples were taken from the unmanaged control parcel, applying the same methodology – three samples were collected at 4 defined depths and composite sample was created for each depth (4 samples in total).

Table 5.2. Parameters for the soil characterization

Physical parameters - common	Chemical and microbiological parameters	
	Common	Specific
• Water content (%) • Texture (granulometric composition %)	• pH, • Organic matter (mg/kg) • Total organic carbon (mg/kg) • Microbial biomass (CPU/ml) • Total nitrogen (mg/kg) • Total phosphorous (mg/kg) • Sulphate (mg/kg) • Boron (mg/kg) • Molybdenum (mg/kg) • Available K (mg/kg) • Metals (Mg, Ca, Fe, K, Mn, Cu, Cd, Cr, Pb, Zn) (mg/kg) • Arsenic (mg/kg) • Hydrocarbons (TPH) (mg/kg) • Polyaromatic Hydrocarbons (PAH) (mg/kg) • Parameters for soil quality index (CO_2 respiration, nitrification potential and dehydrogenase activity)	• Nickel (mg/kg) • Organohalogen pesticides (OCP) (mg/kg) • Polychlorinated biphenyles (PCB) (mg/kg) • Heavy metals distribution in soil fractions (sequential extraction BCR method) - Cr, Cu, Pb, Cd, Zn (mg/kg) • Bioavailable fraction of organics (PAH, OCP and PCB) (mg/kg)

The ground water samples from the 4 installed piezometers on the pilot site was collected before sowing and after harvesting. Site location of the piezometers, upstream and downstream from the pilot site, enables the assessment of the effect of the pilot site activities on the groundwaters.

The ground water samples will be analysed for: pH, nitrogen compounds (mg/L), total organic compound, phosphorous (mg/L), sulphate (mg/L), boron (mg/L), metals and metalloids (Ca, Mg, K, Na, Cu, Cd, Cr, Pb, Zn, Ni, As, Mo) (mg/L), TPH (µg/L), PAH (µg/L), OCP (µg/L).

5.4 Plant development

Progress of the in-situ phytoremediation is presented in Figure 5-7. Germination and growth of rape seed before winter hibernation phase was satisfactory, with high rate of germinated seeds (approximately 90% based on visual inspection). However, in spring 2022 small part of the pilot site was covered in water due to inadequate water drainage which caused inhibition of plant growth. Overall plant growth at the whole pilot site was satisfactory.

Visual inspection of the energy crops on the site was provided at the regular intervals (IFVCNS). During the vegetation phase, rapeseed was monitored carefully for pest occurrence, especially rape beetle, hairy beetle, cabbage stem weevil, brassica pod midge, rape winter stem weevil, turnip sawfly and pollen beetle. The treatment of crops against pests was done using alpha-cypemethrin, boscalid and dimoxystrobin. Plant growth in most of the field is satisfactory (Figure 5-7).

For the purpose of BAF and TF calculation, plants on pilot site were sampled three times during growth season. Plants were sampled at the 50% of initial soil sampling sites (the same sites as defined for the soil monitoring). Five plants per section were collected. Plant sampling included stems and leaves; Flowers/Seeds -aboveground (composite); and roots – belowground (composite). Composite samples from 5 collected plants (separated by plant parts) were obtained for each sampling section. Sampling was performed after plant emergence (February 2022), during the flowering phase (April 2022), and just before the harvest (June 2022). Additionally, two random samplings of one section were done between these two-sampling periods. The energy crops were characterised for metals content, and its bioaccumulation and translocation factor were calculated (UNSPMF).

January 2022 March 2022

April 2022 June 2022

Figure 5-7. In-situ phytoremediation progress

5.5 Environmental conditions

Weather conditions

Precipitation. According to the data on the amount of precipitation (Figure 5-8), December 2021 is the month with the largest amount of rain (56.2 mm), while the least rain fell during March 2022 (only three rainy days - 3.2 mm). In comparison with the average amounts of precipitation, it can be concluded that except for December 2021, in all other months there was significantly less precipitation, and we can consider that it was a dry period

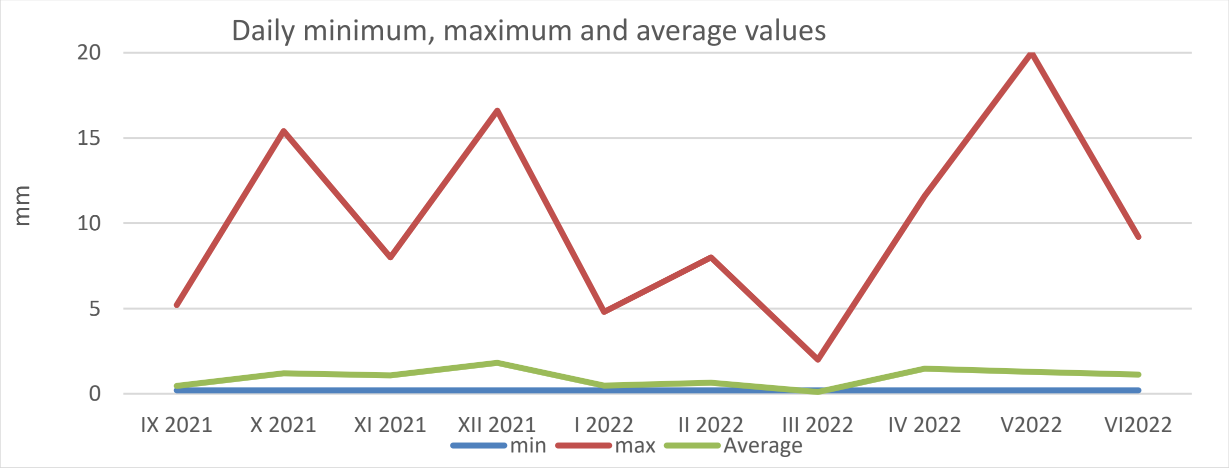


Figure 5-8. Precipitation daily minimum, maximum and average values (September 2021 – June 2022)

Temperature. The warmest month was June 2022 with the highest maximum temperature of 34.5°C, while the minimum temperature of -10.6°C was measured in January 2022.

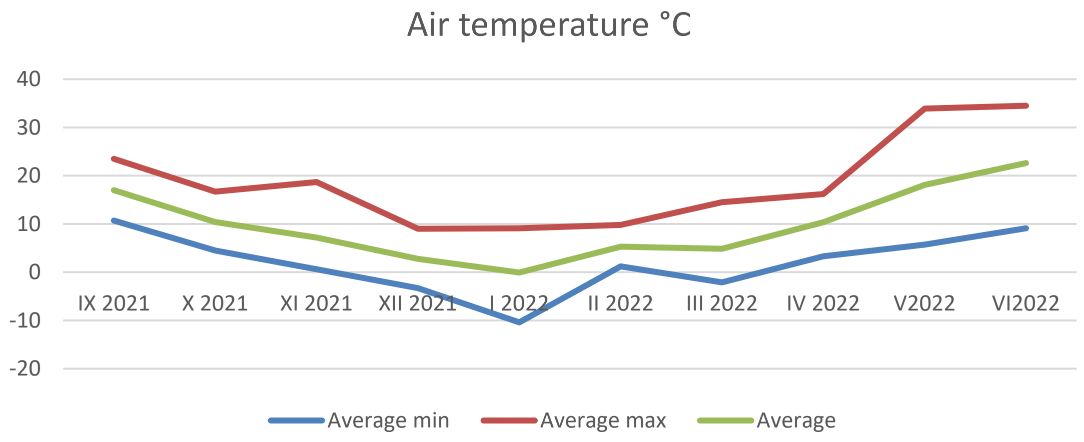


Figure 5-9. Minimum, maximum, and average air temperature values (September 2021 – June 2022)

Wind speed. The most common winds in this part of the country are due northerly. Košava comes to the Banat area from the southeast, and to the northern Banat area sometimes from the south. It is most common in winter, and it is more common in autumn than in spring. Northerly winds blow throughout the year, although it is more frequent in summer. Average wind speeds are from 2 m/s to 3 m/s.

Humidity. Air humidity depends on several indicators, especially on the amount of evaporation, air temperature, degree of continentality. It is higher in winter than in summer. Cloudiness and precipitation are directly proportional, and inversely proportional to insolation and visibility on the horizon. The average air humidity in the test period was lower than the average values, except in December and January, which is in compliance with the results of precipitation.

Light regime. The lowest average value for light regime was recorded in the month of December and increased from month to month, so that the highest values were recorded in the months of May and June 2022 (34.72 and 37.67 lux respectively).

Pests and nutritional deficiency*.* Soil fertilization was done with ammonium sulphate 40 kg/ha nitrogen in February 2022. During the vegetation phase rapeseed was monitored carefully for pest occurrence. In March 2022 occurrence of cabbage moth, *sclerotinia* and *alternaria* was detected so rapeseed was treated with pesticides containing alpha-cypemethrin, boscalid and dimoxystrobin.

5.6 Harvest and pelletizing

First rapeseed growing season was successfully completed in June 2022 (Figure 5-10).

Figure 5-10. First season harvesting and palletization

Harvest was performed by IFVCNS on June 22nd, 2022. 530 kg of seeds were collected, and it is estimated that over 2500 kg of fresh harvest residues were produced (based on number of planted seeds per m^2 and its average mass at the moment of harvest). 5 kg of seeds, needed as mitigation measure for CUJ[2], were collected manually. Approximately 100 kg of fresh harvest

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[2] Former Indian project partner

residues were collected at the Landfill 1 and transported to Novi Sad for further processing. Fresh biomass was spread in a thin layer and dried for next 5 days in open air, using solar energy. After that, the dried material was collected in bags and sent to palletization facility. Palletization was completed in August 2022 and pellets were shipped to Fraunhofer at the end of September 2022.

5.7 Phytoremediation performance

5.7.1 Soil parameters

General parameters and SQI monitoring. The general chemical and physical parameters of samples characterization are presented in the Tables 5.3. General parameters are expressed as average value of all sample measurements. Based on the TOC content contaminated sample can be considered as rich in organic carbon. According to the CEC value samples before sowing (August 2021) and after harvesting (August 2022) can be classified as loams and silty clays.

Table 5.3. General chemical and physical parameters

Parameter	Unit	Field samples	
		Before sowing	After harvesting
pH		7.44±0.21	7.21±0.31
Eh	μS/cm	441.5±37.4	421.6±70.0
TOC	%	3.25±0.95	3.69±1.24
CEC	C_{molc}/kg DW	34.6±5.54	30.2±2.43
OM	%	8.58±1.68	8.45±0.96
Total N	mg/kg	2209.6±124.3	2350±94.3
Total P	mg/kg	1593.3±164.2	1269±241.2
Available P	P_2O_5/100g	89.6±14.2	83.7±4.74
S	mg/kg	46.9±7.22	42.1±5.13
Na	g/kg	716.5±190.4	412.8±48.1
K	g/kg	7986±320.9	6789±698.4
Available K	K_2O/100g	15.9±1.54	26.2±2.41
Mg	g/kg	16898±607.1	7364.6±93.7
Ca	g/kg	25951±340.8	410.9±50.8
Texture	% 0.05-2	60.4±6.22	60.4±5.99
	%0.002-0.05	8.28±2.88	10.9±2.41
	0.002%	31.3±6.26	28.7±3.07
FA	g/kg	0.812±0.60	0.991±0.13
HA	g/kg	2.070±0.56	1.552±0.42
HU	g/kg	29.8±1.95	39.1±1.43
NIT	μgNO_2^-/g dw h	0.74±0.11	0.40±0.09
BR	μgCO_2/g dw h	8.12±1.14	9.75±1.07
C_{mic}	μg/g dw	275.9±27.6	459.7±21.7

FA – fulvic acids, HA – humic acids, HU – humins, NIT – potential of nitrification, BR – basal respiration, Cmic – microbial biomass;

Both field samples, at the start and after one year, can be considered as slightly alkaline. Also, electro conductivity, soil texture and organic matter content didn't change in time. At the beginning, soils are rich in organic matter and nutrients (N, P, K). After one year we can observe slightly lower values in samples for all nutrients. Values of Mg, Na and Ca were lower at the end compared to the start of the experiment. No significant changes in the soil quality index parameters were observed after one year, except for the C_{mic} were obtained value at the end was almost two times higher than in samples at the start.

Metals and metalloids monitoring. The content of the Cr, Cu, Zn, Pb, Cd and Ni in the soil before sowing and after harvesting is presented in Figure 5-11.

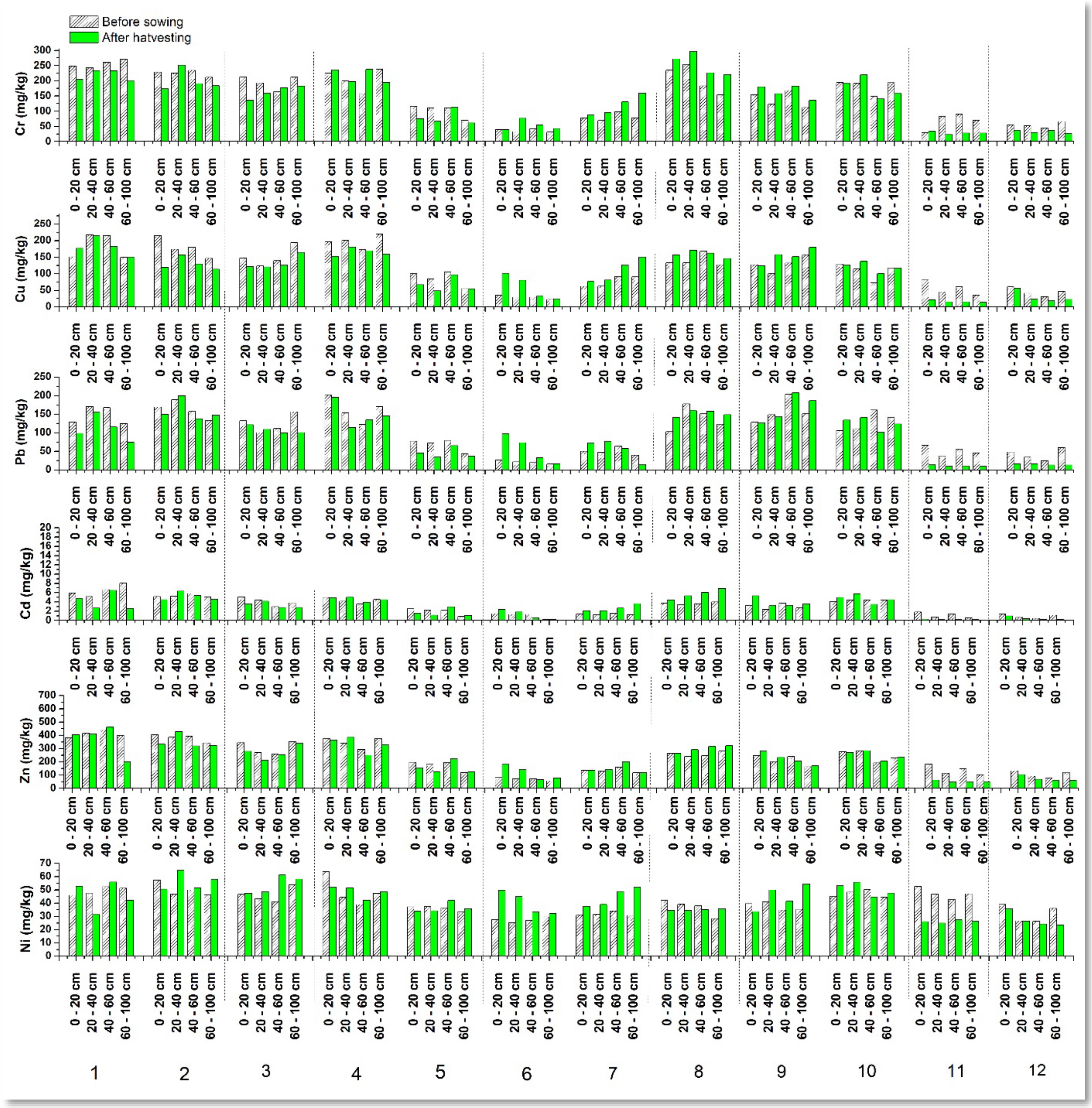

Figure 5-11. Cr, Cu, Cd, Pb, Zn and Ni concentration in the soil before sowing and after harvesting. No 1 to 12 sampling locations given in the fig. 5-6

The concentration of those selected metals is presented separately for each sampling point and each sampling depth because they are the most toxic and they are identified in the high concentration. The obtained results indicate high heterogeneity of the heavy metals concentration on the pilot site. The Cr, Cu, Pb and in some sampling point Cd are detected above remediation value[3].

As expected, there is no significant change in the total concentration of observed heavy metals. The presented changes are mostly due the heterogeneity of the pilot site. Maximum amount of heavy metals which can be bioaccumulated by the plants are about 0.01% for Cd; 0.1% for Cr, Cu, Pb and Ni; 1% for Mn and Zn[4]. Based on this for a given experimental conditions the expected changes of the total heavy metal's concentration in soil during the experiment are less than 1%. This is less than the measurement uncertainty of the analytical methods used for analysing these analytes.

Concentration of Fe, Mn, As, Mo, B and P are given as average value of 12 sampling points for each sampling depth (Figure 5-12). Those analytes are represented as averages, given the fact, that they are not considered toxic (Fe, Mn, B, P) or they are detected in low concentration (As, B). No significant changes of the given analytes have been observed during the field experiment. Except in the case of Mo in the surface layer where significant decreasing of concentration has been detected. The Mo is one of micronutrients and could be accumulated by rapeseed. But also, it is considered as a mobile element and some of the Mo could be lost by leaching in the deeper layers. A slight increase of Mo in deeper layers indicate that this happened.

Heavy metal toxicity is not only related to the total concentration of heavy metals, but also to the distribution of its speciation. Different forms exert different environmental effects, which directly affects the toxicity of heavy metals, their migration, and natural cycling. For the integration of these various classifications and methods, European Community Bureau of Reference proposed the BCR method, divided the heavy metals into four types of genera, namely: exchangeable, reducible fraction, oxidizable fraction and residual. The more mobilizable metals correspond to the two first fractions, which can be released simply by increasing the ionic strength and by slight pH changes. The fractionation methods provide relevant information about the possible metal content that could be bioaccumulated by the plants.

The results of the BCR extraction are presented as average value of 12 surface soil samples, from 12 sampling points (see Figure 5-13). Based on the obtained results showed on the Figure 5-13 most of the present metal(oid)s are in non-available fractions at the start of the experiment. The fractions from this first step of sequential extraction was bound to acid-soluble fractions included water soluble, ion exchange and carbonate binding states, which were absorbed in clay and soil humus and were vulnerable to environmental changes and easier to be transformed and migrated under acidic conditions. The high bioavailability, mobility and potential toxicity of acid-soluble metals in aquatic organisms are of great concern and changes in salinity and increase in pH value have been reported to increase metal mobility in aquatic ecosystems. Although, the percentage in these samples ranged for this first phase up to 20%, the potential ecological risks still exist and cannot be ignored since environmental conditions

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[3] Regulation on limit values of pollutants in surface waters, groundwater and sediment and timelines for reaching of the values ("Official Gazette RS" no. 50/12)

[4] Baker AJM, Brooks RR (1989) Terrestrial higher plants which hyperaccumulate metal elements—a review of their distribution, ecology and phytochemistry. Biorecovery 1:81–126

could modify this scenario[5]. After the end of this one-year experiment significant changes in the metal distribution in different fraction has been observed for almost all investigated metal(oid)s.

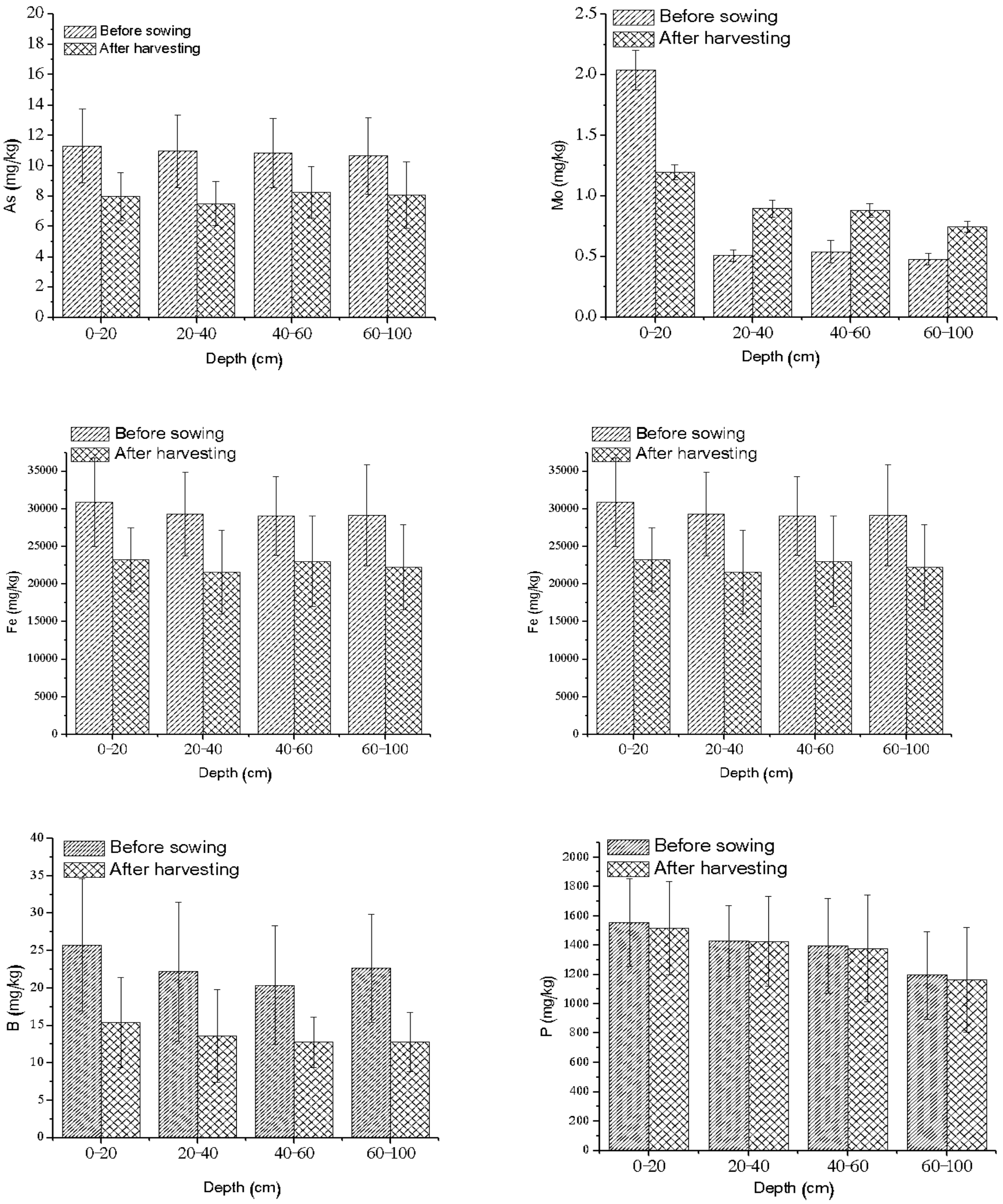

Figure 5-12. As, Mo, Fe, Mn, B and P concentration in the soil before sowing and after harvesting.

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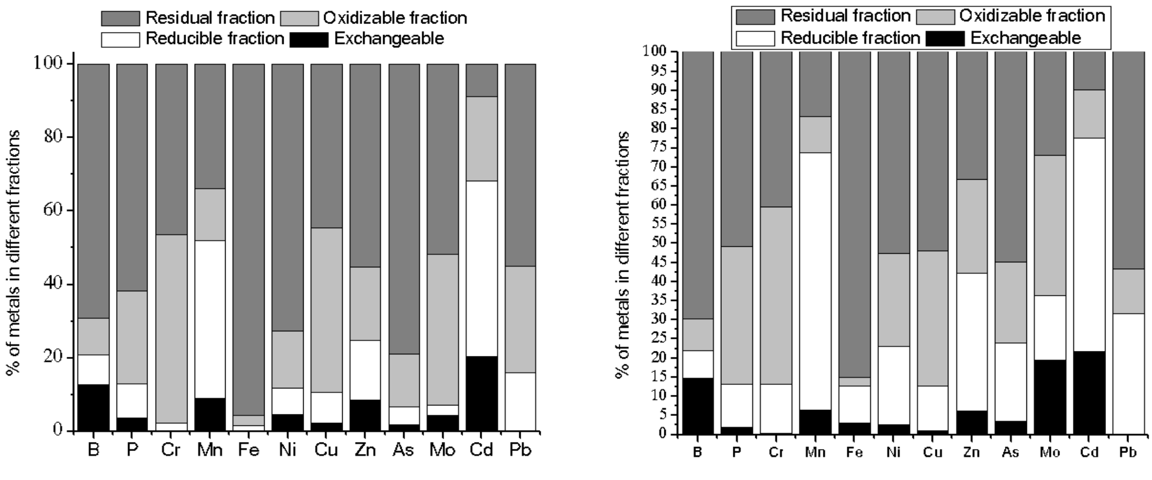
[5] Concentration and pollution assessment of heavy metals within surface sediments of the Raohe Basin, China | Scientific Reports (nature.com)



a) b)

Figure 5-13. Results of the sequential metal(oid)s extraction – BCR (a) before sowing and (b) after harvesting

Organic pollutants monitoring

The quantitative results of the pesticides (organochlorine, atrazine, simazine, alachlor, chlorpyrifos, trifluralin and other impurities such as pentachlorbenzene and hexachlorobenzene), PCBs, TPH and PAHs in soils are presented in Figures 5-14 – 5.17 and expressed as the initial and final concentration before harvesting and after harvesting. Additionally, the bioavailable fraction of the organic pollutants was also measured by Tenax extraction. The average bioavailable fraction was investigated and generally were in range TPHs 11%; PAHs 12.5%; PCBs 13.6; Pesticide 27%. The PAHs concentration was in the range of 22 to 6624 µg/kg before sowing. The highest PAHs concentration up to 12200 µg/kg was observed for the experimental section 4 of Landfill 1. However, during the experiment, the concentration of all PAHs decreased and was in the range of 34 to 285 µg/kg. Therefore, PAHs removal at the end of the field experiment was about 95%. It is important to note that experimental sections no. 5, 6, 7, 9, and 11 were not sampled at the end of the experiment due to previously detected low concentrations of PAHs, pesticides, PCBs and TPH.

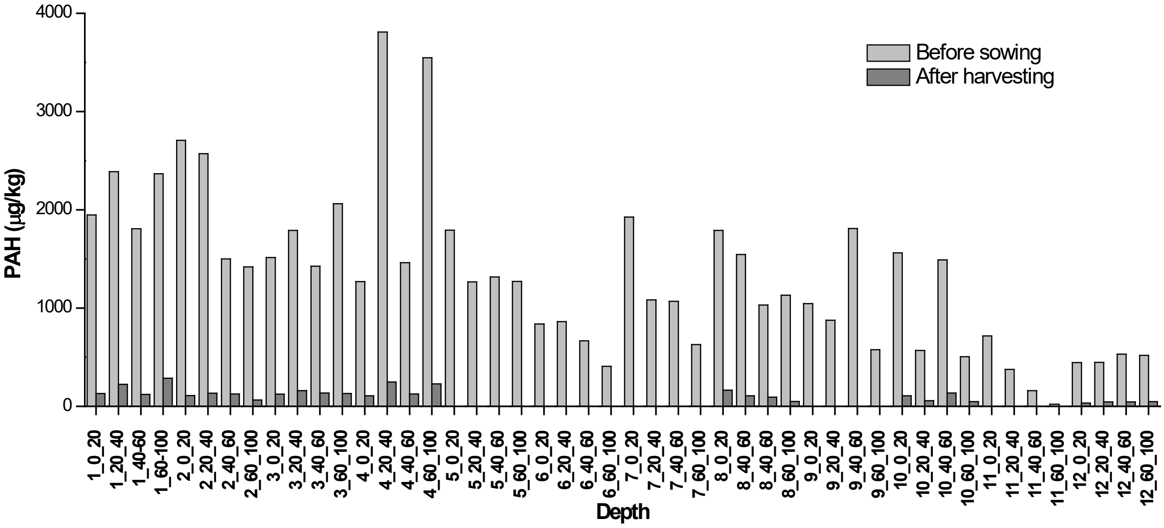


Figure 5-14. PAHs at the start and after one year of the field experiment

TPHs were between 150 and 4545 mg/kg for start of experiment and decreased to 111 to 3978 mg/kg at the end (Figure 5-15).

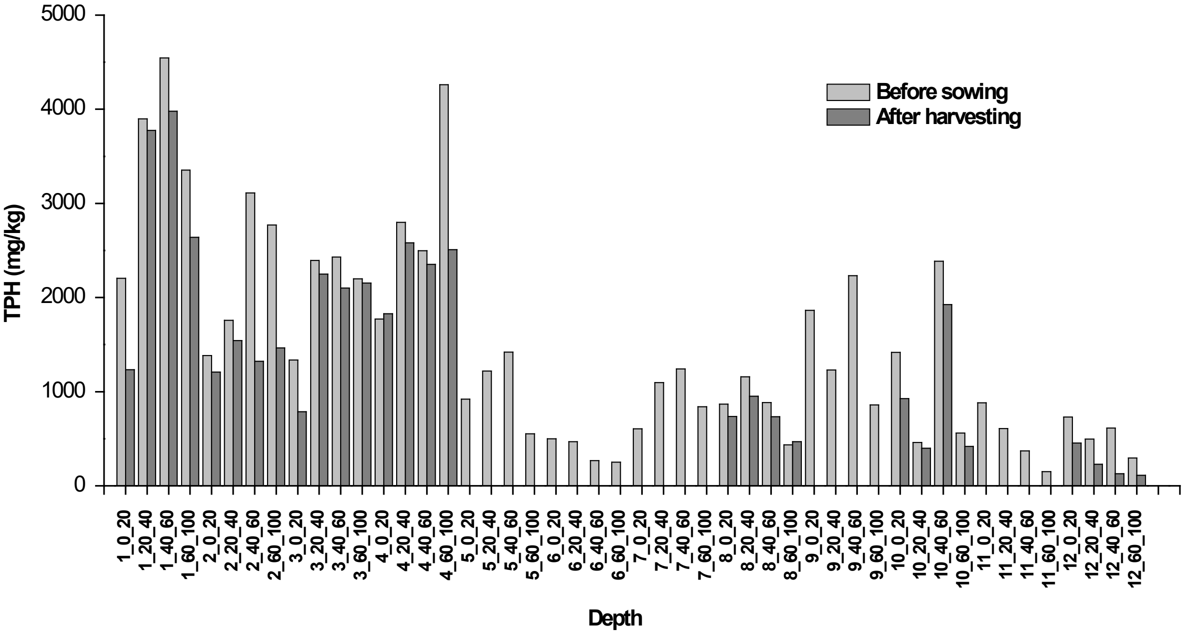


Figure 5-15. TPH at the start point and after one year of field experiment

The percentage of TPH removal was in the range of 15-38% for the experimental section (1-10) and was lower compared to removal obtained for other organic pollutants. At the end of the field experiment, removal of TPHs generally increased in the following experimental section order 3 < 8 < 4 < 1 < 10 < 2. The highest removal was observed for experimental section 2 (32%) of Landfill 1. This could be a consequence of the intensive sorption of TPHs on the root parts of the investigated plants. Additionally, the leaching of all investigated organics in the subsurface and exposure to different weather conditions during the experiment could be a reason for their lower detection.

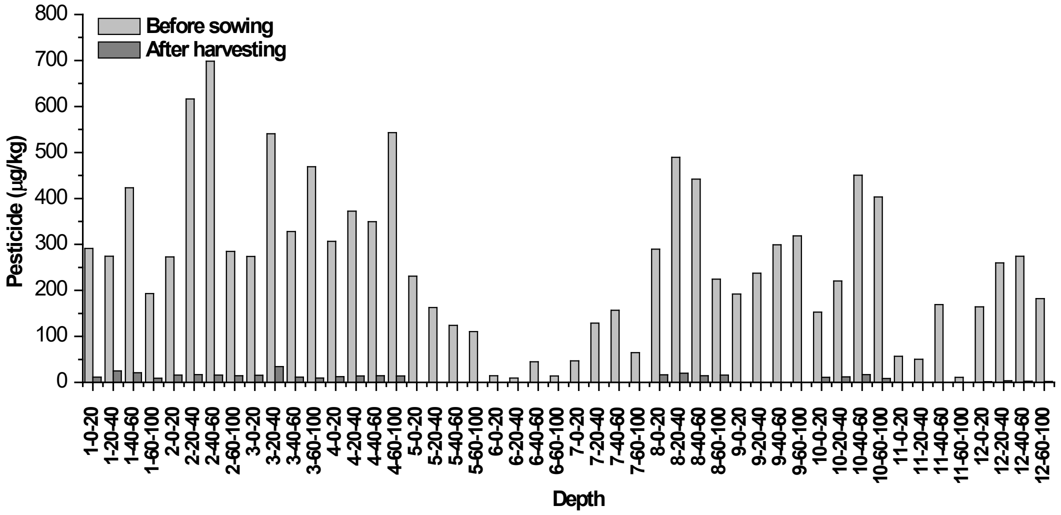


Figure 5-16. Detected concentration for pesticides

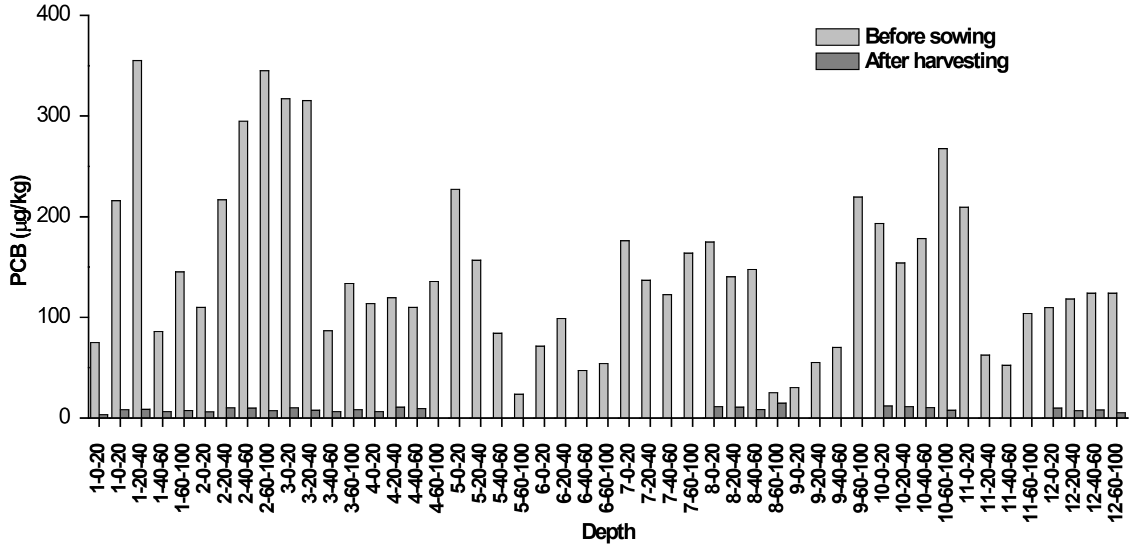


Figure 5-17. Detected concentration for PCB

For all analysed samples, pesticides and PCB concentration were between 9 and 699 µg/kg and 24 and 670 µg/kg at the start point of the field experiment, respectively (Figure 5-17). The range between 2 - 34 µg/kg for pesticides and 3 - 15 µg/kg for PCB obtained at the end of the experiment were lower than the values at the start, indicating a decrease of detected concentration over one year. The percentage of removal for both during the experiment was about 98%. The highest pesticides and PCB removal for all investigated depths were obtained for experimental sections no. 1 and 3 and control no. 12 of Landfill 1.

Basic microbiological properties of soil

Soil microorganisms are a crucial element of soil ecosystems and play a necessary function in terrestrial ecosystem processes, especially the regulation of carbon and nutrient cycles. They rely on carbon sources provided by means of plant litter and root exudates and they can be influenced through modifications in plant-derived organic matter. Basic microbiological properties of soil are given in the table 5.4. as average value of 12 sampling points for each sampling depth. Those microorganisms play an essential function in the weather, ecosystems, and plant health. They may activate the germination capacity of seeds, improve crop performance, inhibit plant illnesses, and stimulate stress tolerance and general health[6].

Table 5.4. Basic microbiological properties of soil

Layers (cm)	***Azotobacter* sp. ×10^1**	*** Ammonifiers ×10^6**	**The total number× 10^6**	***Oligonitrophiles ×10^5**	***Fungus ×10^3**	***Actinomycetes ×10^3**	**Dehydrogenase activity (DHA)**
	Number of microorganisms(CFU g^{-1} absolutely dry soil)						mU g^{-1} dry soil
0-20	180.5	197.2	278.6	326.2	51.5	51.0	11.3
20-40	153.5	135.5	219.9	260.7	44.0	31.4	8.84
40-60	140.7	82.0	155.7	190.3	25.8	20.0	7.36
60-100	109.9	49.7	83.8	101.6	17.5	7.21	5.91

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[6] https://www.sciencedirect.com/science/article/pii/B9780128216569000031

Energy crop characterization

The energy crop was sampled in December, April, and June from the sampling location 1, 2, 3, 4, 8, 10 and 12 (control) (Fig. 5-6). Additional sampling from the sampling point 10 was done in March and beginning of the September. From each sampling location 5 plants were taken and composite samples were made for aboveground, and belowground biomass. The heavy metals concentration is presented in Table 5.5, bioaccumulation factor (BAF) is presented in the Figure 5-18 and translocation factor (TF) on Figure 5-19. BAF and TF are presented as average value of the 1, 2, 3, 4, 8 and 10 sampling location for contaminated soil, and single value for 12 sampling location of the control parcel for the period between April and June.

Table 5.5. Heavy metals concentration in the energy crops

Time		Cr	Ni	Cu	Zn	As	Cd	Pb	B
Below ground biomass (mg/kg)									
10 weeks	Control	2.18	5.13	4.04	27.31	0.48	0.16	0.57	15.82
	Contaminated	3.08	1.49	7.29	38.01	0.50	0.79	1.11	15.16
32 weeks	Control	5.91	3.26	9.29	18.63	0.61	0.26	1.63	25.53
	Contaminated	6.39	3.12	7.69	39.32	0.55	0.78	1.29	33.30
38 weeks	Control	12.03	5.48	2.85	10.00	0.29	0.16	1.30	12.99
	Contaminated	10.45	4.54	7.07	35.85	0.31	1.40	1.50	21.17
Above ground biomass (mg/kg)									
10 weeks	Control	3.75	1.77	4.60	24.99	0.18	0.29	1.37	23.13
	Contaminated	2.64	3.31	6.03	40.50	0.18	1.17	0.72	22.66
32 weeks	Control	2.63	1.61	4.20	19.32	0.30	0.16	0.33	22.09
	Contaminated	2.72	1.85	6.82	35.36	0.22	0.65	0.91	31.23
38 weeks	Control	1.28	0.99	2.95	10.36	0.33	0.10	0.47	28.55
	Contaminated	1.47	1.08	5.21	31.05	0.12	0.76	0.84	20.90

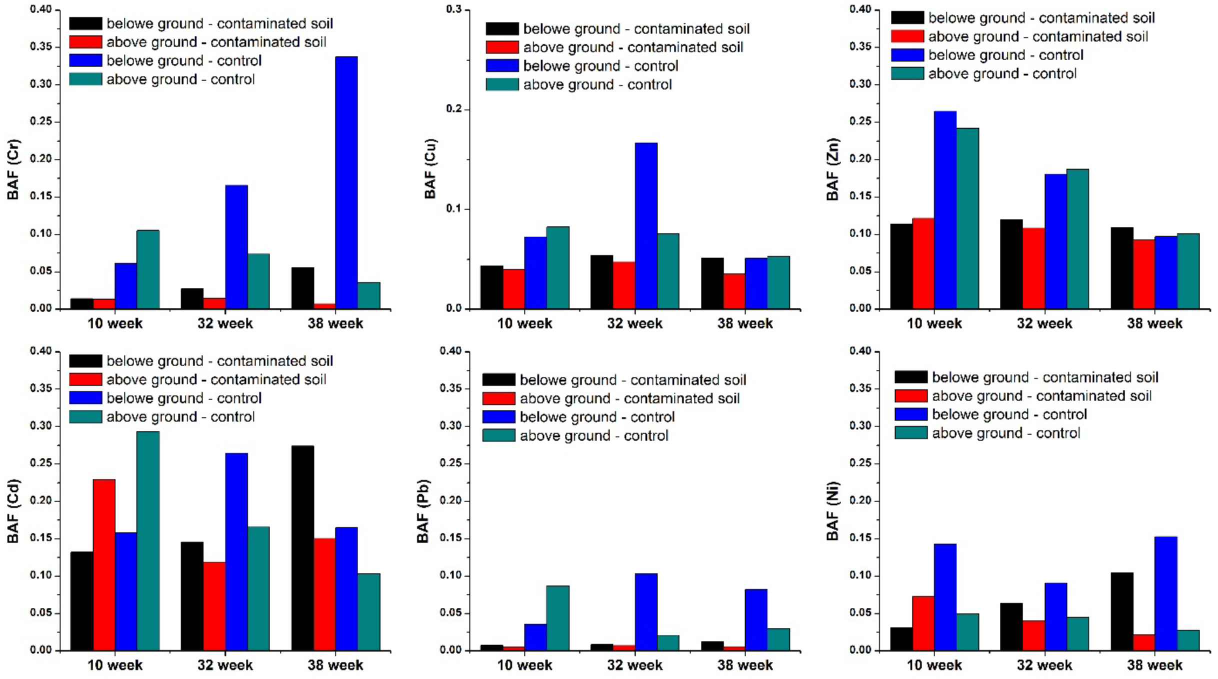

Figure 5-18. Heavy metals bioaccumulation factor

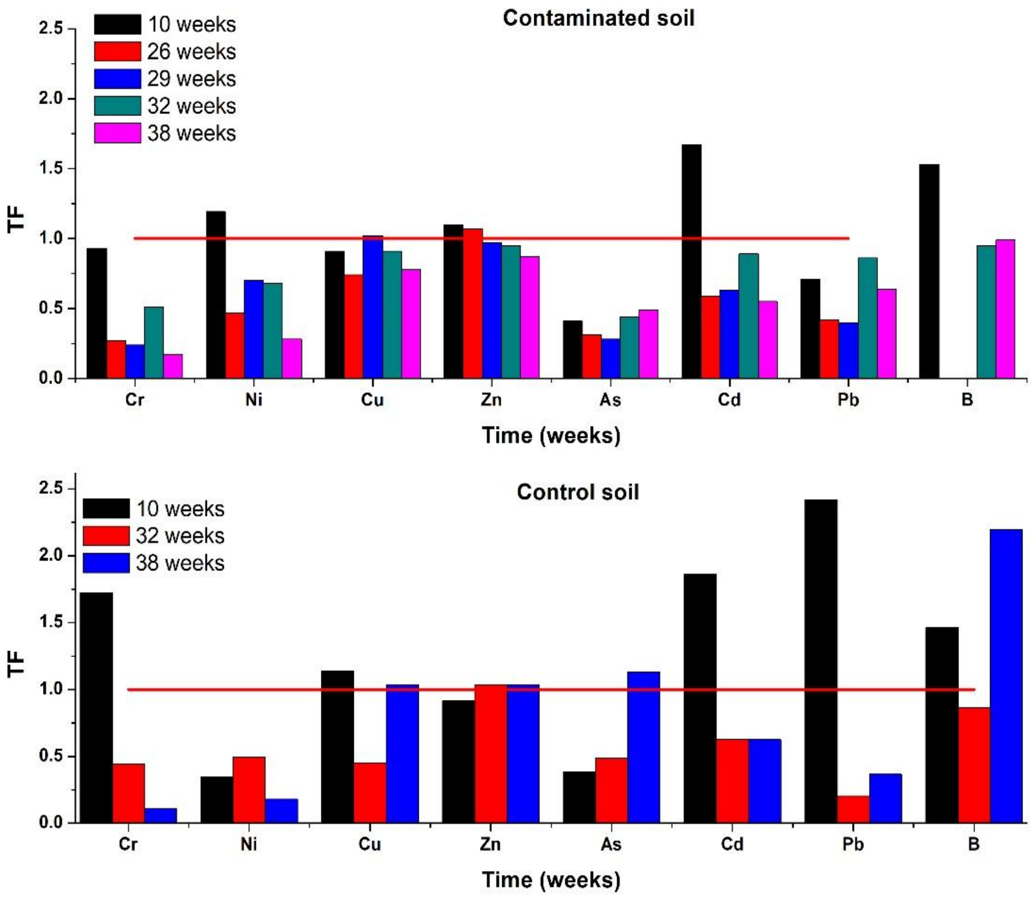

Figure 5-19. Translocation factor

The concentration of the metal(oids) in the energy crop is generally higher in the contaminated soil, compared to the control parcel. This was expected beforehand because of the higher available metal(oids) for accumulation. Generally, BAF for Cr of the belowground biomass has significantly higher, and correspondingly TF was <1 which indicate that the main mechanism of the Cr removal is phytostabilisation and not phytoextraction[7]. For Cu, Zn, Pb and Cd BAF is approximately at the same level in above- and belowground biomass which is in line with TF obtained. TF decreased in the 38 weeks of sampling; however, this is mostly due the removal of seeds and fallen off leaves in the last phase of rapeseed growth (both represent the aboveground biomass). The concentration of the metals in the seeds is presented in Table 5.6. Therefore, the 32 weeks are more representative for TF assessment. Namely, for Cu and Zn TF > 1 is reached. For Pb and Cd TF were close to 1. Generally, a much higher TF factor has been observed compared to the results obtained in the pot test (Deliverable 2.2), this can be attributed to the longer contact time of energy crop with the contaminated soil. The BAF and TF in control soil are generally higher compared to the contaminated, because of lower concentration of the metal(oids) in soil (BAF is calculated as C_{plant}/C_{soil}, and in case of TF enough time to translocate small amounts available for accumulation in plant in low contaminated soil).

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[7] Baker, A. J. M., (1981). Accumulators and excluders-strategies in the response of plants to heavy metals. Journal of Plant Nutrition, 3: 643-654.

Table 5.6. Metal(oid) concentration in the seeds

Cr	Ni	Cu	Zn	As	Cd	Pb	B
mg/kg							
0.40	0.87	4.60	36.29	0.07	0.12	0.21	14.01

Groundwater monitoring

To assess the impact of the phytoremediation activity, and check for possible leaching of contaminants to the groundwater at the pilot site, four piezometers for groundwater monitoring were previously installed – two piezometers upstream, and two downstream of the pilot site. To determinate the background level of contamination, before phytoremediation activities, groundwater samples were taken from four piezometers (April 10, 2021). Monitoring of groundwater was done after harvesting in the August as well. Results are presented in Table 5.7. According to the results given in Table 5.7, the values obtained for the upstream and downstream sampling location are very similar, indicating that there is no current impact of the sediment landfill to the groundwater. Heavy metals have been detected at the low levels, only the arsenic contamination in the one downstream sample has exceeded the remediation threshold. However, this can be attributed to the natural geochemistry at this part of Serbia[8]. Regarding the organic contaminants, a few polycyclic aromatic hydrocarbons and trifluralin have been detected, but at low concentrations which were below the remediation threshold level.

Table 5.7. Groundwater characterisation before sowing (BS) and after harvesting (AH)

Parameters	Units	Upstream - GPS sample location				Downstream – GPS sample location			
		N 45° 34' 51"		N 45° 34' 46"		N 45° 34' 51"		N 45° 34' 50"	
		E 20° 45' 45" P1		E 20° 45' 43" P3		E 20° 45' 32" P2		E 20° 45' 27" P4	
Field parameters		BS	AH	BS	AH	BS	AH	BS	AH
Depth of sampling	m	4.12	3.2	2.7	1.98	4.38	3.4	4.52	3.84
Air temperature	°C	17.6	27	17.5	25	19.5	27	20.1	26
Water temperature	°C	18.5	24.8	14.5	21.5	18.2	21.2	16.6	24
pH	/	7.71	7.35	7.87	7.86	7.97	7.4	7.89	7.2
Conductivity	µS/cm	521	540	610	800	460	430	665	810
Dissolved oxygen	mgO$_2$/L	2.2	1.8	2.31	1.18	1.25	2.1	1.86	1.2
General parameters									
Total solids	mg/L	376	499	374	450	307	358	391	507
Chemical oxygen demand	mgO$_2$/L	46	60.3	<32	80.9	34	50.3	<32	80.4
Biochemical oxygen demand	mgO$_2$/L	19.2	31.2	6	81	8	30.7	11	93
Ammonium ion	mg N/L	6.14	0.71	5.23	0.62	6.35	0.24	4.25	6.3
Nitrate	mg N/L	0.0216	0.13	0.0455	0.57	0.0388	0.23	0.616	<0.02
Nitrite	mg N/L	0.0012	<0.005	0.00472	0.017	0.0012	<0.005	0.016	<0.005
Chloride	mg Cl/L	52.1	21	54.6	19.27	51	19.3	54.9	24.5
Sulphate	mg SO4/L	1.32	25.7	1.23	49.4	0.374	19.9	2.48	67.5

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[8] Watson M.A., Tubić A., Agbaba J., Nikić J., Maletić S., Molnar Jazić J., Dalmacija B. (2016) Response surface methodology investigation into the interactions between arsenic and humic acid in water during the coagulation process, Journal of Hazardous Materials, 312,150-158.

Phosphate	mg P/L	1.01	0.52	/	0.065	/	0.061	/	0.13
Fluoride	mg F/L	0.844	0.756	1.21	0.234	0.546	0.134	0.267	0.325
Metals									
Fe	mg/L	21.1	6.28	9	0.66	20.3	9.63	15.7	6.71
Mn	mg/L	1.3	0.32	0.622	0.89	1.22	1.3	0.312	0.52
Ni	µg/L	<2.2	3.2	<2.2	2.93	<2.2	4.05	<2.2	2.76
Zn	mg/L	0.66	0.25	0.182	0.17	0.219	0.21	0.686	0.23
Cd	µg/L	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15
Cr	µg/L	6.55	5.04	<0.90	<0.90	<0.90	1.72	8.47	2.19
Cu	µg/L	9.75	14.7	7.03	22.7	10.1	29.6	10.7	16.4
Pb	µg/L	18.5	33.2	20.3	6.62	<5.9	<5.9	59.2	8.58
As	µg/L	56.5	156.7	7.8	2.05	14.8	17.7	75.5	22.12
Hg	µg/L	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50
Na	mg/L	35.2	40.1	45.8	42.3	33.8	33.9	41	31.5
K	mg/L	3.76	4.45	3.96	4.55	3.74	4.01	4.35	3.67
Ca	mg/L	92.5	100.2	132	123.5	147	111.1	95.4	92.4
Mg	mg/L	16.4	20.5	26.9	21.3	13.7	15.6	21.4	18.7
VOC									
Chloroform	µg/L	<1.60	<1.60	<1.60	<1.60	<1.60	<1.60	<1.60	<1.60
1.1.1-trichloretane (1.1.1-TCE)	µg/L	<0.260	<0.260	<0.260	<0.260	<0.260	<0.260	<0.260	<0.260
1.2-dichloretane (1.2-DCE)	µg/L	<0.245	<0.245	<0.245	<0.245	<0.245	<0.245	<0.245	<0.245
Benzene	µg/L	<0.365	<0.365	<0.365	<0.365	<0.365	<0.365	<0.365	<0.365
Trichlorethylene	µg/L	<0.605	<0.605	<0.605	<0.605	<0.605	<0.605	<0.605	<0.605
BDHM	µg/L	<0.480	<0.480	<0.480	<0.480	<0.480	<0.480	<0.480	<0.480
Toluol	µg/L	<1.06	<1.06	<1.06	<1.06	<1.06	<1.06	<1.06	<1.06
DBHM	µg/L	<0.480	<0.480	<0.480	<0.480	<0.480	<0.480	<0.480	<0.480
Tetrachlorethylene	µg/L	<0.510	<0.510	<0.510	<0.510	<0.510	<0.510	<0.510	<0.510
Chlorbenzene	µg/L	<0.620	<0.620	<0.620	<0.620	<0.620	<0.620	<0.620	<0.620
Ethylbenzene	µg/L	<0.650	<0.650	<0.650	<0.650	<0.650	<0.650	<0.650	<0.650
m+p-Xylene	µg/L	<0.780	<0.780	<0.780	<0.780	<0.780	<0.780	<0.780	<0.780
o-Xylene	µg/L	<1.03	<1.03	<1.03	<1.03	<1.03	<1.03	<1.03	<1.03
Bromoform	µg/L	<0.720	<0.720	<0.720	<0.720	<0.720	<0.720	<0.720	<0.720
1,2-dichlorobenzene	µg/L	<1.15	<1.15	<1.15	<1.15	<1.15	<1.15	<1.15	<1.15
1,4-dichlorobenzene	µg/L	<1.32	<1.32	<1.32	<1.32	<1.32	<1.32	<1.32	<1.32
Vinylchloride	µg/L	<0.500	<0.500	<0.500	<0.500	<0.500	<0.500	<0.500	<0.500
Poly aromatic hydrocarbons									
Naphthalene	ng/L	<10.0	<10.0	<10.0	<10.0	<10.0	<10.0	94.1	<10.0
Acenaphthylene	ng/L	12.2	<10.0	12	<10.0	12.5	<10.0	13.5	<10.0
Acenaphthene	ng/L	<10.3	<10.3	<10.3	<10.3	<10.3	<10.3	<10.3	<10.3
Fluorene	ng/L	7.7	<6.15	<6.15	<6.15	<6.15	<6.15	<6.15	<6.15
Phenanthrene	ng/L	<6.90	<6.90	<6.90	<6.90	<6.90	<6.90	<6.90	<6.90
Anthracene	ng/L	19.2	<10.3	19.9	<10.3	18.7	<10.3	16.5	<10.3
Fluoranthene	ng/L	16.2	<10.3	17.6	<10.3	17.5	<10.3	19.6	<10.3
Pyrene	ng/L	21.2	<20.5	22.5	<20.5	21.4	<20.5	21.1	<20.5
Benzo(a)Anthracene	ng/L	54.6	<20.5	50.6	<20.5	53.5	<20.5	52.7	<20.5

Chrysene	ng/L	<20.5	<20.5	<20.5	<20.5	<20.5	<20.5	<20.5	<20.5
Benzo(a) Fluoranthene + Benzo(k) Fluoranthene	ng/L	47.5	<30.0	45.9	<30.0	49.2	<30.0	50.8	<30.0
Benzo(a)Pyrene	ng/L	<30.0	<30.0	<30.0	<30.0	<30.0	<30.0	<30.0	<30.0
Benzo (g, h, i) perylene	ng/L	<30.0	<30.0	<30.0	<30.0	<30.0	<30.0	<30.0	<30.0
Dibenzo (a, h) anthracene+	ng/L	<30.0	<30.0	<30.0	<30.0	<30.0	<30.0	<30.0	<30.0
Indene (1,2,3-cd) pyrene									
Organochlorine pesticides									
4,4’-DDT	ng/L	<0.500	<0.500	<0.500	<0.500	<0.500	<0.500	<0.500	<0.500
4,4’-DDD	ng/L	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00
4,4’-DDE	ng/L	<4.75	<4.75	<4.75	<4.75	<4.75	<4.75	<4.75	<4.75
Aldrin	ng/L	<2.50	<2.50	<2.50	<2.50	<2.50	<2.50	<2.50	<2.50
Dieldrin	ng/L	<6.25	<6.25	<6.25	<6.25	<6.25	<6.25	<6.25	<6.25
Endrin	ng/L	<6.25	<6.25	<6.25	<6.25	<6.25	<6.25	<6.25	<6.25
Alpha – HCH	ng/L	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00
Beta – HCH	ng/L	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00
Gama – HCH	ng/L	<3.25	<3.25	<3.25	<3.25	<3.25	<3.25	<3.25	<3.25
Delta – HCH	ng/L	<2.50	<2.50	<2.50	<2.50	<2.50	<2.50	<2.50	<2.50
Alpha Endosulfan	ng/L	<2.50	<2.50	<2.50	<2.50	<2.50	<2.50	<2.50	<2.50
Endosulfan sulfat	ng/L	<2.50	<2.50	<2.50	<2.50	<2.50	<2.50	<2.50	<2.50
Heptachlor	ng/L	<2.50	<2.50	<2.50	<2.50	<2.50	<2.50	<2.50	<2.50
Heptachlor-epoxide	ng/L	<2.50	<2.50	<2.50	<2.50	<2.50	<2.50	<2.50	<2.50
Priority pesticides									
Alachlor	ng/L	<20.0	<20.0	<20.0	<20.0	<20.0	<20.0	<20.0	<20.0
Atrazine	ng/L	<40.0	<40.0	<40.0	<40.0	<40.0	<40.0	<40.0	<40.0
Simazine	ng/L	<20.0	<20.0	<20.0	<20.0	<20.0	<20.0	<20.0	<20.0
Chlorpyrifos	ng/L	<20.0	<20.0	<20.0	<20.0	<20.0	<20.0	<20.0	<20.0
Trifluralin	ng/L	17.3	10.5	11.5	7.3	16.3	<5.45	9.26	<5.45
Pentachlorobenzene	ng/L	<5.45	<5.45	<5.45	<5.45	<5.45	<5.45	<5.45	<5.45
Hexachlorobenzene	ng/L	<9.67	<9.67	<9.67	<9.67	<9.67	<9.67	<9.67	<9.67
Phenols									
4-nonilfenol	ng/L	<40.0	<40.0	<40.0	<40.0	<40.0	<40.0	<40.0	<40.0
4-oktilfenol	ng/L	<20.0	<20.0	<20.0	<20.0	<20.0	<20.0	<20.0	<20.0

5.7.2 Biomass output

530 kg of seeds were collected. It is estimated that over 2500 kg of fresh harvest residues were produced (based on number of planted seeds per m^2 and its average mass at the moment of harvest). Therefore, the aim to produce >40 kg (dry basis) of energy crops per growing season was achieved and exceeded. The yield of rapeseed was 2.6 to 2.9 t per ha, yield on the uncontaminated agricultural soil was 2.5 to 3.5 t per ha. Therefore, the yield of the energy crops was >85% in comparison with the crop yield in clean soil conditions. Few plants of Ricinus have been sown on the field also.

Figure 5-20. Biomass output of rapeseed and Ricinus

5.8 Encountered problems and amendments

Progress of the in-situ phytoremediation is presented in Figure 5-5. Germination and growth of rape seed before winter hibernation phase was satisfactory, with a high rate of germinated seeds (approximately 90% based on visual inspection). However, in spring 2022 small part of the pilot site was covered in water due to inadequate water drainage which caused an inhibition of plant growth. Overall plant growth at the whole pilot site was satisfactory.

Since the PGPR amendment didn't increase uptake of metals significantly during the pot tests, this amendment was not applied at the pilot site in the first growing season. Nutrient deficiency was observed in February. Therefore, soil fertilization was done with ammonium sulphate 40kg/ha nitrogen in February 2022.

5.9 Other information

Set II of pot tests

Based on the set I of pot tests (Deliverable 2.2), set II of the pot tests focused on the increasing the bioavailability of metals in the soil by adding acidifying fertilisers and low molecular weight organic acids. Soil/sediment used in pot tests was collected at the Serbian pilot site in April 2022. Approximately 300 kg of polluted soil/sediment were collected from the site, then transported to IFVCNS facilities, and manually mixed and homogenized before placed in pots. 5 kg of sediment were used for the pot experiments. Experiments were performed in open air under natural weather conditions. Spring variety of rapeseed "Jovana" was used for the tests. All treatments were performed in 3 replicates in polluted sediment. Seeding density for rapeseed was 10 seeds per pot. After plant emergence, pots were trimmed to 4 plants per pot. The soil in the pots was treated with different low molecular weight organic acids (LMWOA), acidifying fertilisers and elemental Sulphur at different concentration. The first soil treatment was performed four weeks after the second treatment and five weeks after seeding. Experiment treatment set up was as follow: citric acid, tartaric acid and glutamine acid have been added at a concentration of 10 mmol/kg, 20 mmol/kg and 40 mmol/kg; successive addition of the citric acid, tartaric acid, glutamine acid has been performed (10+10 mmol/kg and 10+10+20 mmol/kg), oxalic and malonic acids were applied in concentrations of 20 mmol/kg each. Acidifying fertilisers used in experiments were ammonium nitrate, ammonium sulphate and urea. All fertilisers were applied in concentrations of 150 mg of N/kg of soil and 300 mg of N/kg of soil. Treatments with elemental sulphur were conducted at doses of 300 and 500 mg/kg of soil. In total 96 pots were set up (Figure 5-21). Plants were harvested in May.

Figure 5-21. Second season of pot test

Additionally, pot test with the Ricinus as a mitigation measure for CUJ partner have been setup (Figure 5-22). 5 kg of sediment was used for the pot experiments. Experiments were performed in open air under natural weather conditions, without amendments and in triplicate. Plants were harvested in September. Characterisation of the soil and Ricinus biomass from pot setup II is in progress.

Figure 5-22. Pot test with Ricinus

Metals and metalloids monitoring – selected results

Regarding the pseudo-total metal(oid) concentration in the sediment, there was not significant difference between the start and end of the experiments for all treatment.

BCR analysis was conducted as well. The obtained results are shown in Figure 5-23. It has shown that some treatments can increase the availability of heavy metals in the soil samples in the exchangeable and reducible phase. The most significantly, addition of the citric, glutamic, and tartaric acid lead to the increase of the Cr exchangeable fraction. This is important because

Cr is one of the less mobile metals in soil, since it is almost always present in the form of its oxide (non-mobile form). Regarding the treatment with acid fertilisers, in case of Cr, Pb and Cu increasing of reducible phase was observed, especially for the combination of the acid fertiliser with oxalic acid. No significant changes were observed for Cd for all phases.

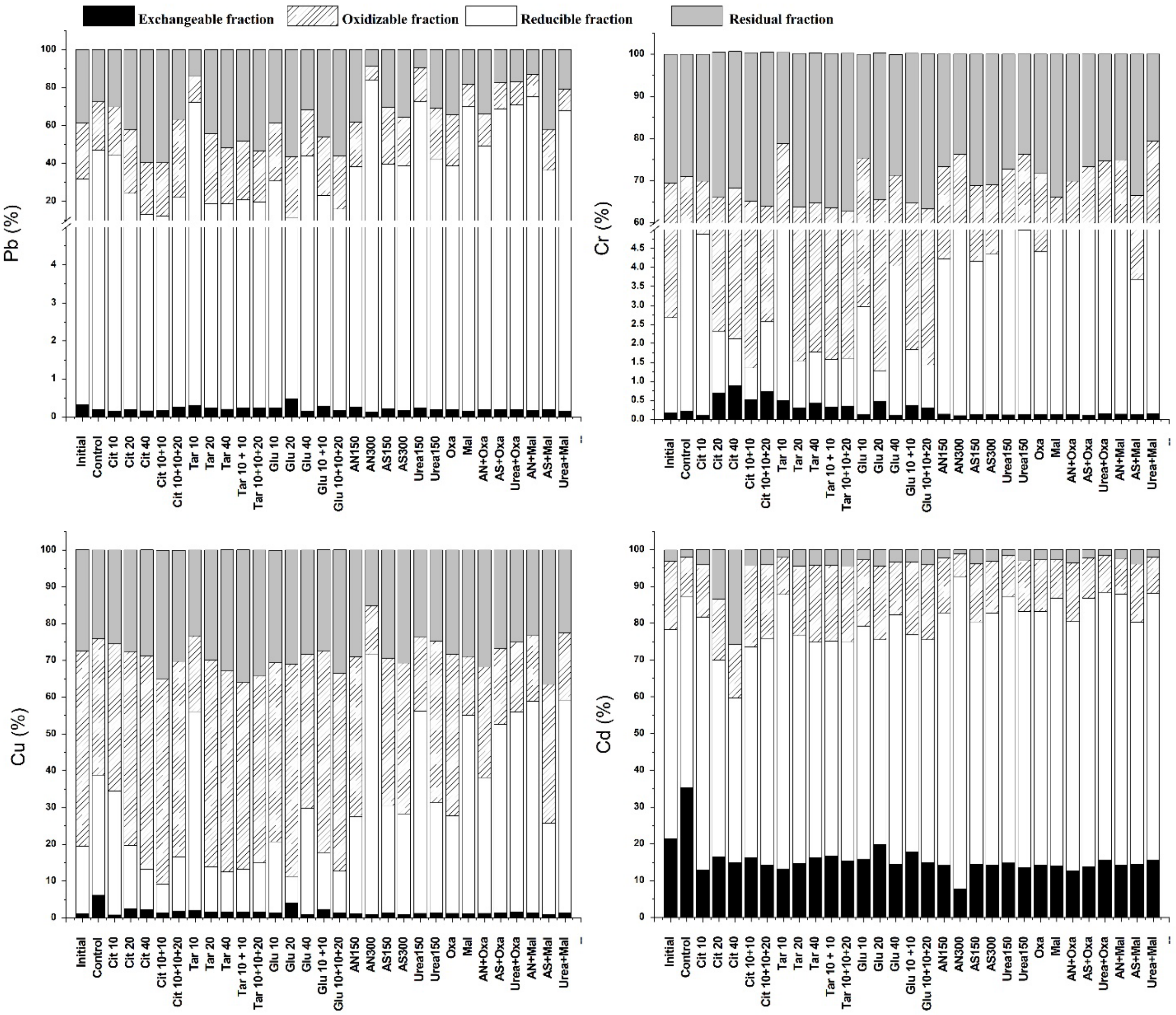


Figure 5-23. Results of the sequential heavy metals extraction – BCR

The translocation factor after first and second harvesting is presented on the Figure 5-24. The main findings can be summarized as follows:

- Cd – TF above 1 reached in all cases for all treatment and in the first and second harvesting. However, addition of citric, tartaric, and glutamic acid and combination of Urea and malic acid additionally increased TF.
- Cu – TF above 1 reached in all cases except in the treatment with ammonium sulphate malic and combination AN+oxa, and AS +Oxa. Addition of citric, tartaric, and glutamic acid and combination of Urea and malic acid additionally increased TF.

- Cr – TF above 1 reached in treatment with addition of citric, tartaric, and glutamic acid and combination of Urea and malic acid and Ammonium nitrate and malic acid. But, only in case of treatment Ammonium nitrate and malic acid TF above 1 was observed after the second harvesting.
- Pb – TF reached in treatment with addition of citric, tartaric, and glutamic acid and treatment with Ammonium nitrate. In the second harvesting TF below 1 was observed in all treatments but in case of Ammonium nitrate TF was at its highest level.
- Generally, the TF for all metals was reduced in the second harvesting (loosing leaves and seeds in the aboveground biomass).

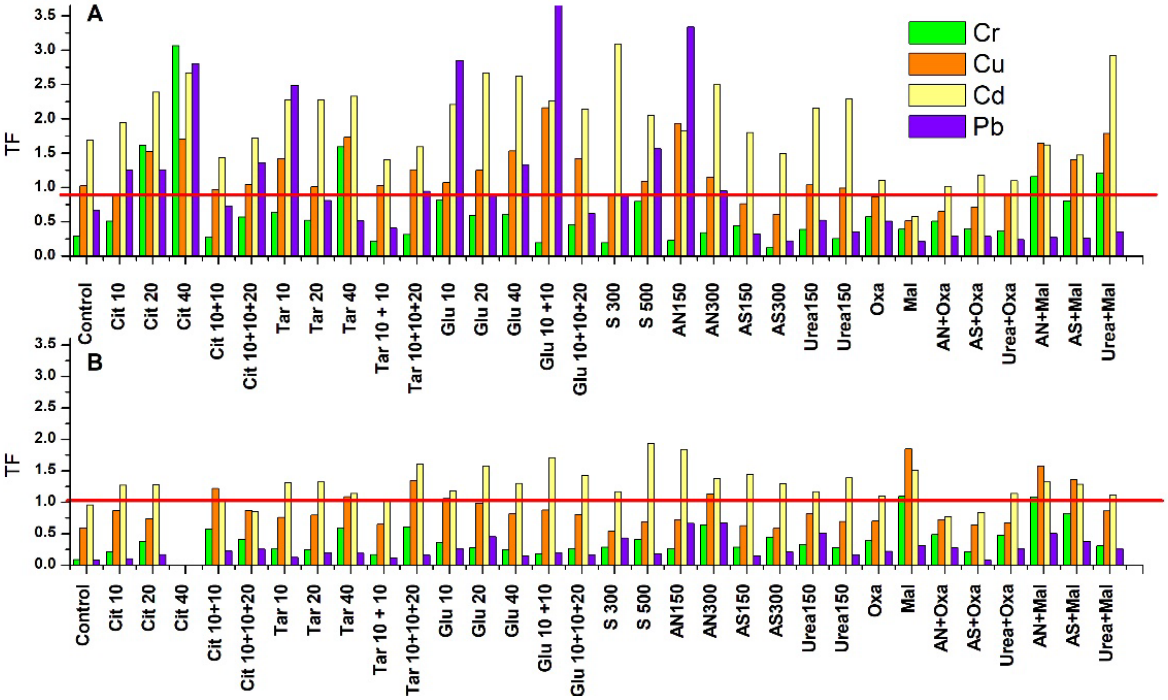


Figure 5-24. Translocation factor of the selected heavy metals

Organic pollutants in POT experiment

The Σ16 PAHs were about 2000 µg/kg and 1200 µg/kg for initial and control concentrations. The total PAHs for all treated samples ranged from 527 to 1800 µg/kg (Figure 5-25). It could be observed that there was no significant difference between the control and treated samples as well as among differently treated samples.

The bioavailable fraction of Σ16PAHs decreased more than twice compared to the total concentration. Additionally, Σ16PAHs of the bioavailable fraction for treated samples was in the range between 102 and 351 µg/kg, indicating there was no significant difference.

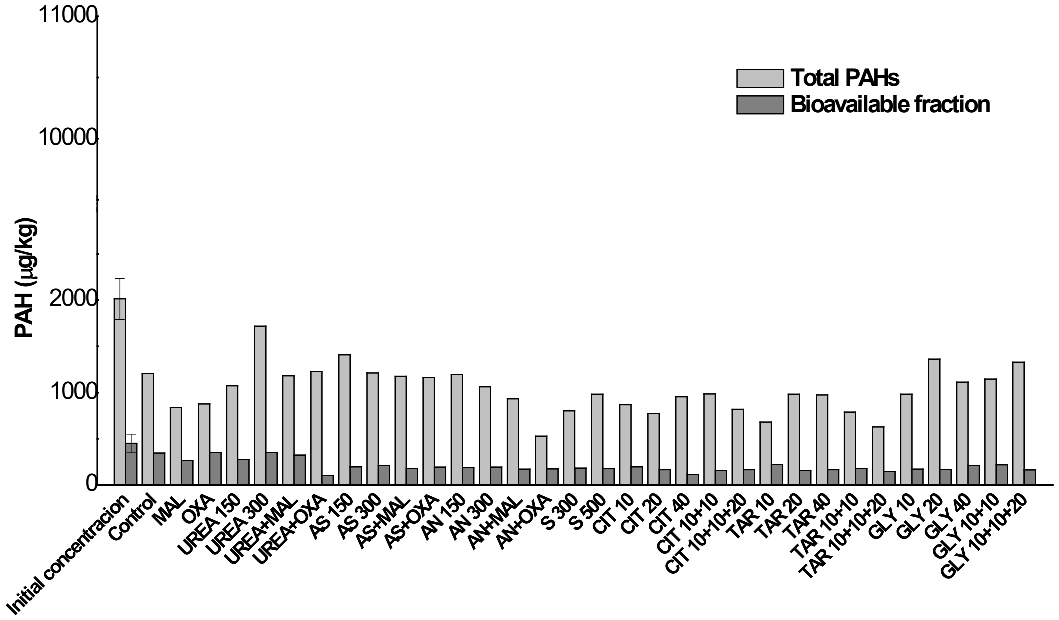


Figure 5-25. Total and bioavailable fraction of PAHs during different treatments

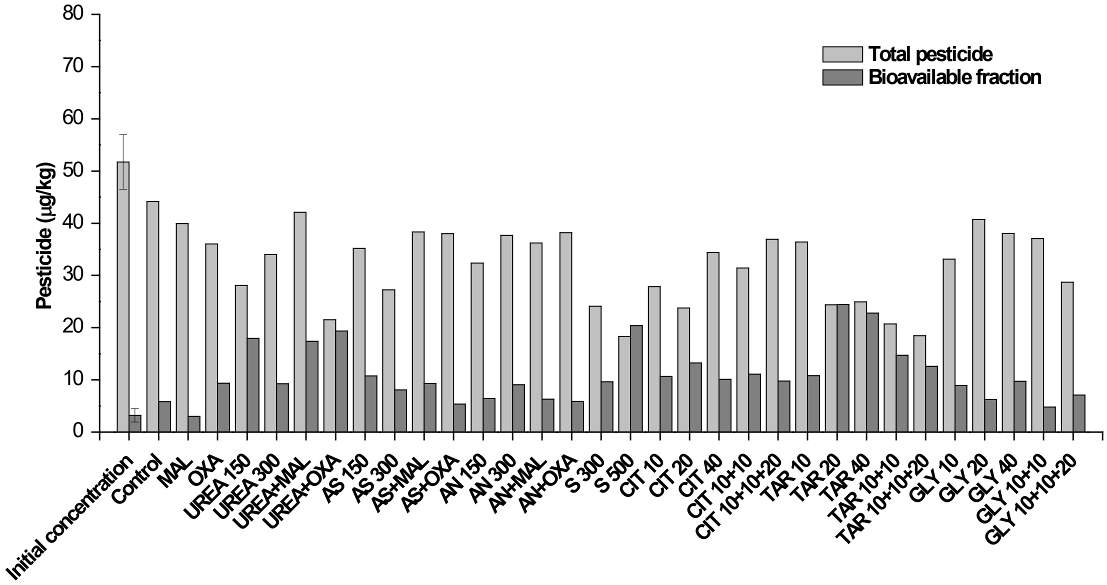


Figure 5-26. Total and bioavailable fraction of pesticides during different treatments

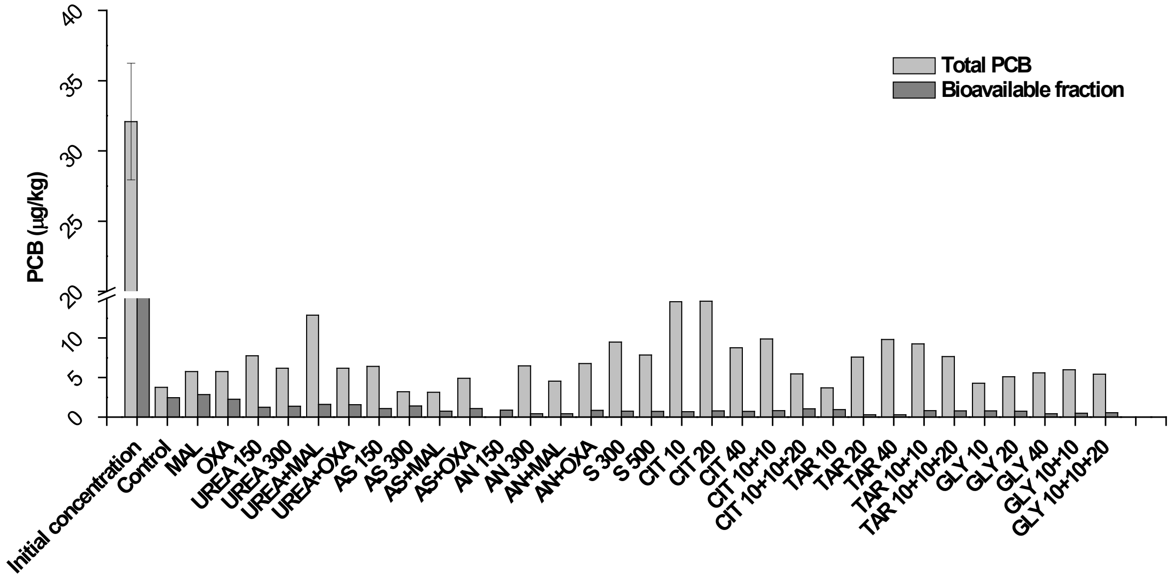


Figure 5-27. Total and bioavailable fraction of PCBs during different treatments

For both cases, the bioavailable fraction was lower compared to the total amount. The figures were in the range between 2.83 and 24 µg/kg for pesticides and up to 3 µg/kg for PCBs. Of the PCB congeners measured, the higher molecular weight (HMW) congeners was abundant and dominated by hexa-, penta-PCB.

For all treatments, total TPHs were between 1674 and 4777 mg/kg (Figure 5-28). The initial concentration was about 4000 mg/kg, while for control samples were about 3000 mg/kg. For all other treated samples, there was no clear trend between samples. Generally, most of the treated samples have the same concentration as the control one.

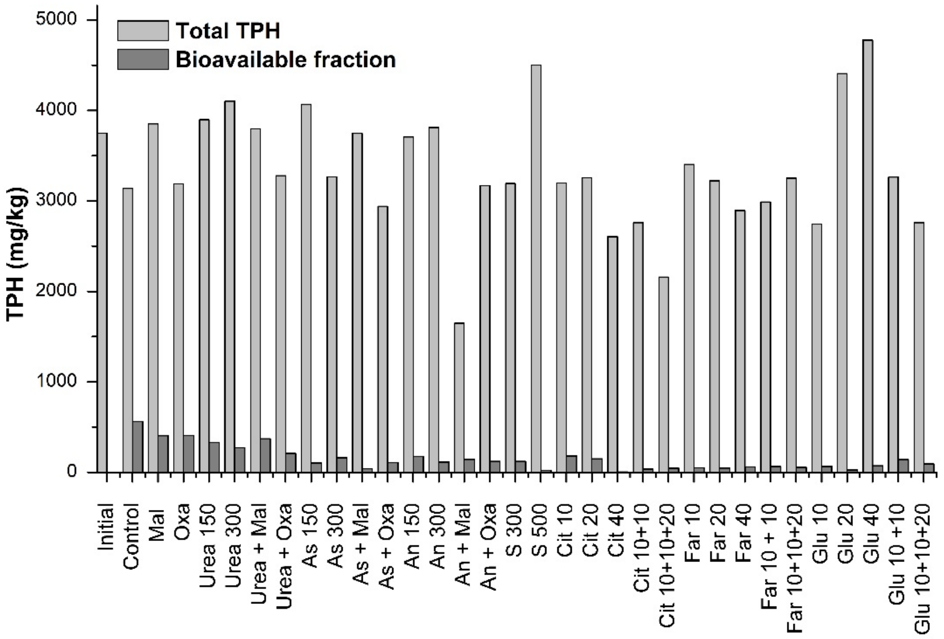


Figure 5-30. Total and bioavailable fraction of TPH at the end of POT experiments

The content of TPHs in the bioavailable fraction was investigated and generally ranged from 4 to 407 mg/kg. The bioavailable fraction decreased for the experiment with citric (average 83.3 mg/kg), glutamic (79.4 mg/kg) and tartaric acid (55.1 mg/kg) compared to other treatments. Generally, no clear trend was observed for all organic pollutants and applied treatments.

Plant yield

Common parameters for energy crop characterisation included yield of production of biomass. The biomass obtained for the tested treatments is presented in the Table 5.8. Generally, the highest yield was obtained in the case of treatment with the glutamic acid, increasing Glu concentration led to the further biomass increase. After the first harvest in almost all treatments there was no reduction of biomass. On the contrary, an increase of biomass was observed when treated with Tar, Glu, AN, AS and Urea. After the second harvest, treatment Tar, AN and AS has similar biomass yield as control sample. Glu had significantly higher yield compared to the control unit, and all other treatments exhibited the yield reduction.

Table 5.8. Total biomass of the rapeseed (Brassica napus) after the first and the second harvest

	Total biomass (g)	
Treatment	**First harvest**	**Second harvest**
Control	3.87 ± 0.82	10.92 ± 1.16
Cit 10	3.08 ± 0.92	5.89 ± 0.40
Cit 20	2.95 ± 0.55	2.15 ± 0.69
Cit 40	2.63 ± 0.29	/
Cit 10+10	5.01 ± 0.97	4.43 ±1.81
Cit 10+10+20	3.30 ± 0.70	2.11 ± 0.52
Tar 10	4.75 ±1.51	7.01 ± 1.19
Tar 20	3.31 ±0.49	11.35 ±1.11
Tar 40	5.42 ± 0.91	11.69 ± 3.70
Tar 10 + 10	2.18 ± 0.37	3.81 ± 1.76
Tar 10+10+20	9.39 ± 3.80	6.37 ± 0.62
Glu 10	3.16 ± 0.88	14.87 ± 2.96
Glu 20	5.06 ± 0.93	11.36 ± 1.73
Glu 40	8.37 ± 1.43	16.12 ± 5.40
Glu 10 +10	4.30 ± 0.74	9.79 ± 1.48
Glu 10 +10+20	8.40 ± 3.40	11.39 ±1.44
S1	3.76 ± 0.88	4.12 ± 2.61
S2	3.39 ± 0.55	7.31 ± 0.28
AN1	6.19 ± 2.23	9.79 ± 1.02
AN2	4.92 ± 0.54	7.35 ± 4.10
AS1	5.30 ± 1.97	7.70 ± 4.12
AS2	4.61 ± 0.99	8.82 ± 0.67
Urea 150	5.43 ± 2.30	8.35 ± 3.16
Urea 300	3.73 ± 1.76	9.50 ± 1.63
Oxa	2.49 ± 1.49	2.40 ± 0.82
Mal	1.87 ± 0.72	2.29 ± 0.47
AN+Oxa	2.14 ± 1.23	3.63 ± 0.29
AS+Oxa	3.12 ± 0.56	3.75 ± 1.27
Urea+Oxa	2.88 ±0.74	6.80 ± 0.74
AN+Mal	3.72 ± 0.97	5.73 ± 1.17
AS+Mal	3.19 ± 1.13	2.80 ± 1.08
Urea+Mal	3.53 ± 0.71	3.95 ± 1.25

The selection of the best performing amendment will strongly depend on the final aim of the treatment. Whether the aim is to obtain maximum yield, or to extract the maximum of selected heavy metals or to degrade organic pollutants. It can be stated however that Glu-treatment leads to the best performance regarding biomass yield. Cit, Tar, and AN (+mal) provide the best results regarding the extraction of metals and degradation of organic pollutants.

5.10 Overall summary of phytoremediation performance in M12-M24

The first growing season has been completed successfully. The soil (sediment) on the Serbian pilot site is highly contaminated with heavy metals, such as Cu, Cr, Pb and Zn, and to some extent Cd. Higher levels of PAHs were also detected. Additionally, organochlorine compounds (OCP) such as DDT, DDE, DDD and PCB congeners were detected in the sediment samples. Based on the first set of pot test (D2.2) the Rapeseed (*Brassica Napus*) was chosen as the most adequate plant species to be tested on the pilot site. During the first growing season plant growth at the whole pilot site was satisfactory, no visual plant stress was observed. The KPI to produce more than 40 kg of biomass and yield more than 85% compared to the uncontaminated soil was achieved.

Metal(oids) bioaccumulation have been also satisfactory, given the fact that most metals were present in the non-available fraction. The translocation factor was also satisfactory. Namely, for Cu and Zn TF > 1 was reached. For Pb and Cd TF was close to 1. Regarding the organic pollutants polycyclic aromatic hydrocarbons have been reduced significantly. While the total petroleum hydrocarbons have been reduced to a smaller extent. Nevertheless, no leaching of heavy metals or organic pollutants to the groundwater during phytoextraction process has been observed.

Therefore, we consider that there is a good potential to decontaminate the soil to the level which fits for its intended usage (fit for purpose) – the cultivation of energy crop in the quality range suitable for energy production, not for human and animal consumption. The remediation strategy for the soil is essentially based on removal of mobile of residue and reducing risks for toxicity effect manifestation. Up to now we can confirm that efficiently growing energy crops on highly polluted soil is possible.

6. FIELD TRIALS ON THE LITHUANIAN PILOT SITE

6.1 Landscape preparation

The contaminated site is in the northern part of Lithuania, in Šiauliai city. The soil on the site is contaminated with petroleum hydrocarbons. The contamination is of historical origin as the site was exploited as oil base in the Soviet time. Last oil tanks were demounted and removed from the site in 2009. Since then, the site was left without any maintenance. Due to this, the site was found overgrown with bushes and trees at the start of Phy2Climate project. There were piles of debris on the site as well because it was accessible for the passing public for years.

Trees and bushes were removed in March 2021. In the following months, cement blocks and other debris were removed from the site, while the biggest holes in the surface were covered using an excavator. Deep tillage was performed in March 2022 before the start of the field trials to level out the soil surface and to shred larger roots that were still present in the soil (Figure 6-1).

Figure 6-1. Deep tillage of the soil in March 2022

6.2 Soil preparation and seeding campaign

Harrowing was carried out to supply plants and microbial species with oxygen. It was done after deep tillage in April 2022. It also helped to loosen the soil after pressing it with deep tiller.

The site was then subdivided into 3 different size experimental parcels (squares). The colour of the parcel frame (Figure 6.2.1) indicates which plant species were sown/planted: green parcel - herbaceous plants mix (overall green biomass); red parcel – amaranth (*Amaranthus caudatus,* blooms in red); yellow parcel – Jerusalem artichoke (*Helianthus tuberosus*, blooms in yellow). The subdivision was based on initial characterization of soil carried out in 2021. The green parcel contained the highest and the deepest contamination, thus it was designated for herbaceous plants that have dense and deep root system. The red parcel exhibited moderate contamination levels yet still deep, therefore it was designated for amaranth. While, J. tuberosus has a shallow root system, so it was designated to grow it in the yellow parcel where contamination was the lowest and located in the top layers. Parcel sizes were as follow: herbaceous mix – 1,234 m^2, J. artichoke - 870 m^2, amaranth - 310 m^2. Figure 6-2 also presents real-scale measurements of each parcel.

Figure 6-2. Subdivision and real-scale measurements of each parcel. Green parcels – herbaceous plants, red parcel – amaranth, yellow parcel – J. artichoke

The soil on the site was amended with organic compost and mineral fertilizers before seeding using the following amounts:

Herbaceous plants:

- Compost – 600 kg wet weight (ww)/parcel (4.8 t ww/ha);
- Mineral fertilizer – NPK(S) 12-11-18 - 8S (%), 30 kg/parcel (0.2 t/ha), and KCl (60%): 8 kg/parcel (0.064 t/ha).

Amaranth:

- Compost – 420 kg ww/parcel (13.5 t ww/ha);
- Mineral fertilizer – NPK(S) 12-11-18 - 8S (%), 25 kg/parcel (0.8 t/ha).

Jerusalem artichoke:

- Compost – 780 kg ww/parcel (8.9 t ww/ha);
- Mineral fertilizer – NPK(S) 12-11-18 - 8S (%), 50 kg/parcel (0.6 t/ha).

Herbaceous plant mix was seeded in the green parcel. Seeding was done by hand and the soil surface was slightly raked after spreading the seeds. The mix comprised of species selected as the best-performed ones during the pot experiment:

- 37.5% tall fescue (var. *Medainis*) - 0.6 kg/parcel (68 kg/ha),
- 25% perennial ryegrass (var. *Elena DS*) - 0.4 kg (4.5 kg/ha),
- 25% alfalfa (var. *Malvina*) - 0.4 kg (4.5 kg/ha),

- 6.25% festuca perennis (var. *Ugne)* - 0.1 kg (1.1 kg/ha),
- 6.25% bird's-foot trefoil (var. *Gelsvis)* - 0.1 kg (1.1 kg/ha).

Amaranth was also seeded by hand and the surface was slightly raked after spreading the seeds. For amaranth, variety *Raudonukai* was selected, and about 580 g were seeded in the red parcel (18 kg/ha).

Variety *Sauliai* of J. artichoke was planted in the yellow parcel. About 100 kg ww of planting material were used in the parcel (1.1 t ww/ha). Tubers were planted using shovel at the depth of 0.10-0.15 m. Distance among tubers in a row was ~0.4 m, distance between the rows was 0,7 m. One tuber was planted if large, while 2-4 tubers were planted if smaller.

Control parcels were installed next to the contaminated site on fresh and non-contaminated sandy-loam. Sandy-loam was chosen because it's granulometric composition is similar to the granulometry of the contaminated soil. The clean soil was poured into a raised bed (about 0.5 m). One square meter was designated for every plant species. Each parcel received about 7 kg ww of compost. About 20 g of herbaceous mix seeds, 30 g of amaranth seeds and 12 tubers of J. artichoke were seeded/planted into the designated parcel. The control parcels did not receive any fertilizers.

Bacterial additive was spread out in mid-June 2022. About 100 kg (dw) of the additive, consisting of various *Bacillus spp* and *Pseudomonas spp* strains, was added to a tank (12 m^3) with luke-warm water. Additionally, meat and bone meal (MBM) was added to help activate the bacteria. The mixture was aerated and then poured onto the soil. Only the contaminated site received bacterial additive.

6.3 Monitoring program

The monitoring program consisted of three main parts:

Monitoring of the plants. This was carried out every 10-14 days. The following parameters were evaluated: germination rate, soil cover with plants, plant density, luxuriant (lushness of the plants), and morphological parameters, such as stem high and root length.

Fences and surveillance. It was planned to install fence and surveillance cameras in April 2022. However, later it was decided to repair the existing fence instead of installing a new one. Surveillance cameras were installed in July 2022.

Weather monitoring. This was carried out through the Lithuanian Hydrometeorological Service Station. The station provided hourly data sets every 10 days on air temperature, air humidity, amount of precipitation, sunny hours, average wind speed and wind direction.

6.4 Plant development

Plant development was monitored for 24 weeks throughout all vegetation periods. The trends are presented in Figure 6-5 where the green line shows the development of the plants in the control parcels with the clean soil, while the red line shows the development of plants from the contaminated parcels. The main observations are as follows:

Germination:

- Herbaceous mix plants in the contaminated soil germinated weaker than in the clean soil. It never reached 100%, but stayed slightly below 75%.
- J. artichoke in the contaminated soil germinated (sprouted) slower than in the clean soil, nonetheless it reached 100%.
- Amaranth germinated very poorly in the contaminated soil. There was a major delay between plant germination in the clean and the contaminated soil. The weak germination in both cases was partly caused by dry conditions right after the sowing. While, the most important reason for the weak germination, was poor quality seeding material. However, the reason appeared much later in the year.

Soil cover:

- Herbaceous mix. Soil cover was higher in the control soil, where it remained about 75%, and was very homogeneous. While the cover in the contaminated soil was only about 50%, and there were patches that had sparse coverage or very dense coverage. This occurred due to the uneven soil contamination with TPH.
- J. artichoke. Soil cover in the clean soil reached 100% and remained at this level. Soil cover in the contaminated reached 50% at its maximum. Such difference was due to the planting density. There were 12 plants per square meter in the clean soil versus 4.6 plants per square meter in the contaminated soil.
- Amaranth. Soil cover in amaranth parcels was very low in both cases. In the clean soil it reached 30% at max. While, in was about 48% maximum in case of the contaminated soil. However, it is worth mentioning that there were many weeds in the contaminated soil, that were not excluded when evaluating soil cover. Thus, the actual soil cover by amaranth was much lower.

Plant density:

- Herbaceous mix plants in the control soil were denser than the ones grown in the contaminated soil. The density of plants in the control soil was always evaluated with the maximum score (9), while plants in the contaminated soil for plant density was scoring around 6. Very likely such differences were caused by different seeding density and different seeding technique (wide-spread hand gesture for the larger contaminated parcel, thus less dense, and almost pouring seeds from above for the small control parcel).
- J. artichoke exhibited maximum plant density in both cases. The only difference was that plants in clean soil reached maximum density sooner than the ones grown in the contaminated soil. This could be caused by higher plant density in the control parcel.
- Amaranth exhibited very low plant density. This coincides with other parameters, like low soil coverage and low score in luxuriant.

Luxuriant:

- Herbaceous mix grown in the clean soil were lush. The luxuriant of these plants reached its maximum at the same time as the maximum plant density. Plants grown on the contaminated soil were less luxuriant, it’s overall score was around 6 – the same as for plant density. It is important to note, that some patches in the contaminated parcel had more contamination and herbaceous plants were barely growing there, thus it decreased the overall score both for the luxuriant and for the plant density.

- J. artichoke grown on the clean soil was more luxuriant than the ones growing on the contaminated soil, 9 and 7-8 point, respectively. In addition, J. artichoke grown on the contaminated soil reached maturity faster and began to dry out earlier than the ones on the clean soil.
- Amaranth was very poor in both soils, and the luxuriant was lingering around 3. This was caused by poor quality seed.

Plant height:

- Herbaceous mix plants were of similar height throughout all growing season. No major differences were observed.
- J. artichoke in both cases was growing similarly and the height was similar. The plants grown in the contaminated soil began to dry out in early September, thus the height started to drop. While, plants in the clean soil were still growing. The development of the plant height of J. artichoke coincides with the luxuriant scores.
- Amaranth did not reach its maximum height not in the clean soil, nor in the contaminated soil. Development of the height was similar in both cases.

Overall, plant development in the contaminated soil was slightly worse than in the clean soil. Additionally, it did not fully meet expectations after the great results achieved in pot tests, especially in the case of amaranth.

Figure 6-3. Jerusalem artichoke grown on soil contaminated with TPH blooming in August 2022

Figure 6-4. LEFT: amaranth grown on soil contaminated with TPH beginning to bloom (red florets) in mid-August 2022. RIGHT: first harvest of herbaceous plants grown on the contaminated soil in mid-August 2022. The field is already cut and the hay is raked into swaths and ready for transportation

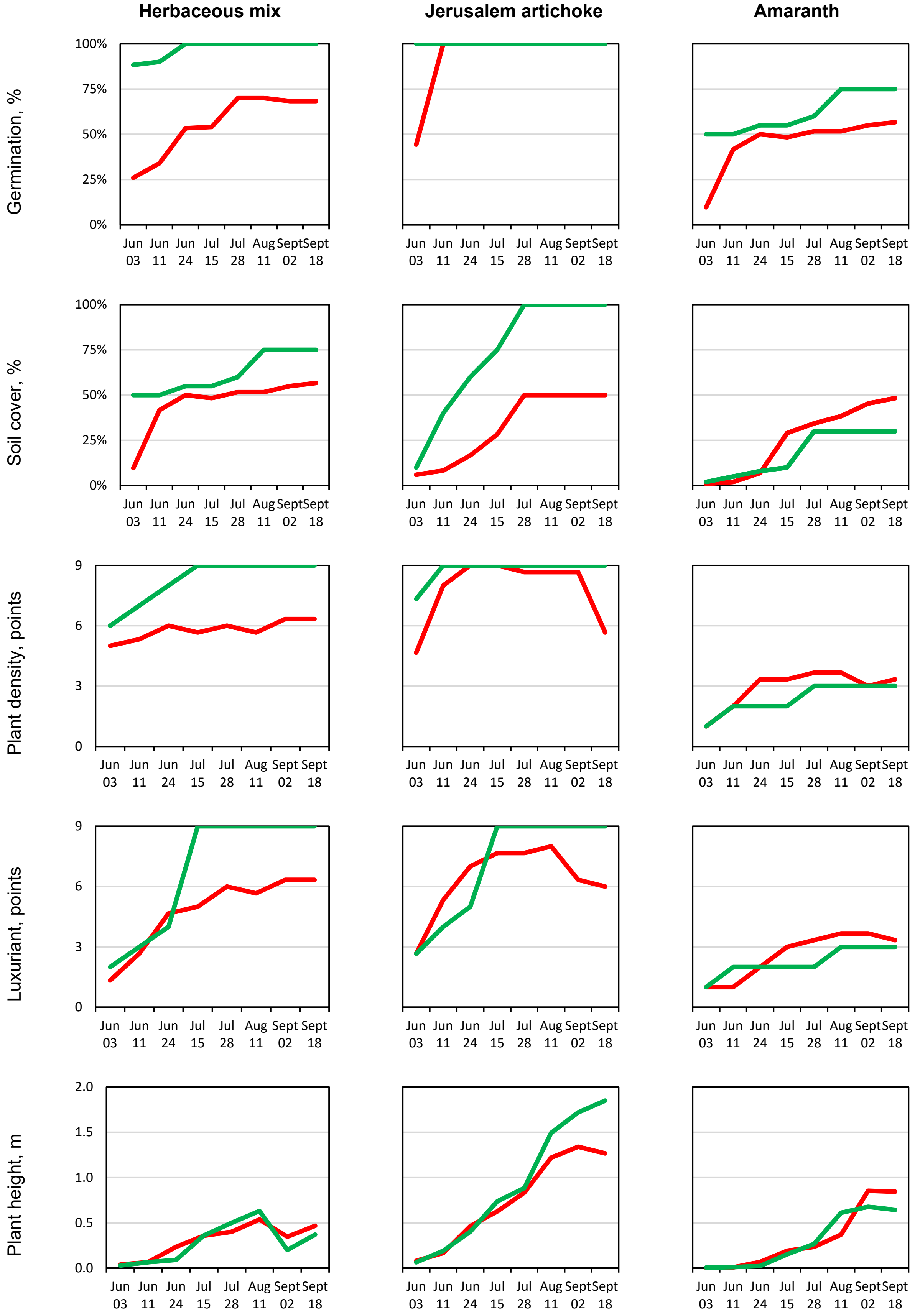


Figure 6-5. Plant development. Green line – plants grown on control (clean) soil; red line – plants grown on contaminated soil

6.5 Environmental conditions

Weather. Growing season of 2022 in Šiauliai region where the pilot site is located can be divided into 3 periods: 1) cold and dry, 2) hot and wet, and 3) hot and dry. Graphs representing average air temperature and precipitation are given in Figure 6-6. The cold and dry period was especially critical to amaranth as the seeds, that are typically sown into very shallow depth (3-5 mm), did not have enough moisture to germinate. Thus, the germination was delayed. The hot and wet period was favoured by herbaceous plants when the plants spurred into the aboveground biomass development. The hot and dry period in August caused that plants stopped developing aboveground biomass quite early and in case of J. artichoke even started to wilt. However, as irrigation was not foreseen in the initial planning, no watering was provided.

Fortunately, there were no strong storms, heavy rainfalls or hailstorms which could have destroyed the plants. Overall, it was a typical summer without strong weather anomalies.

In addition, no signs of pests or disease were observed during the field trial.

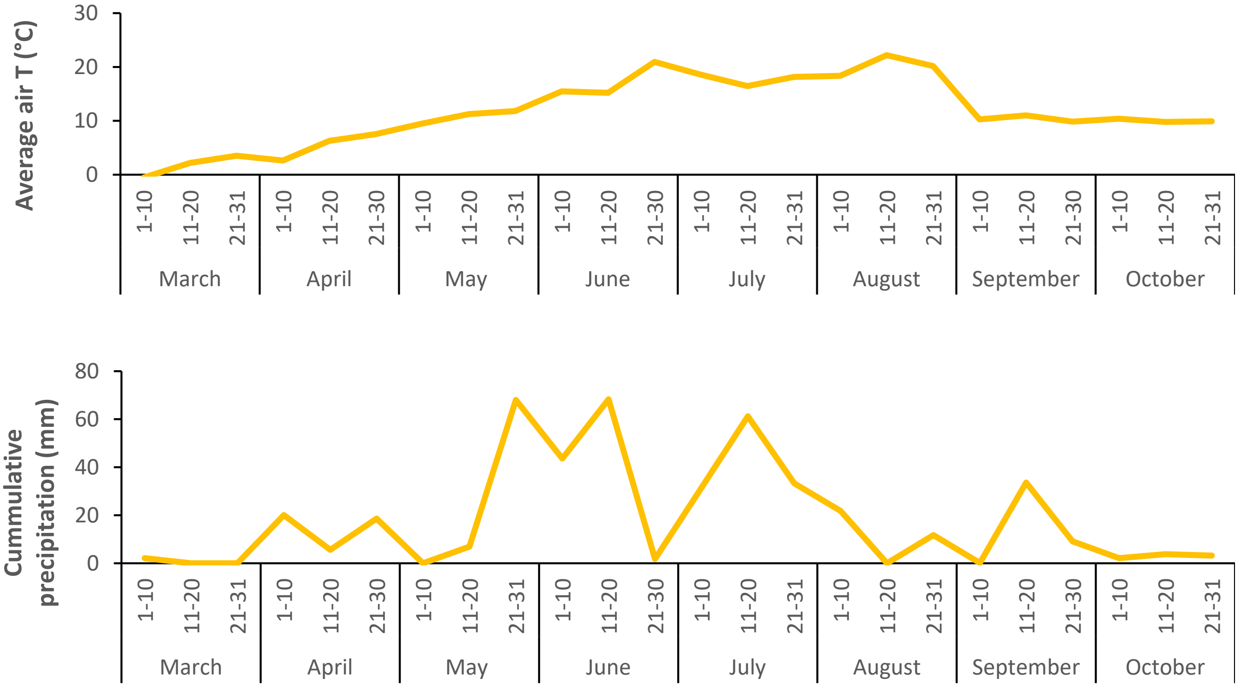


Figure 6-6. Weather monitoring data of March-September, 2022. Temperature is presented as decade average; precipitation is presented as a cumulative value for each decade. The data was provided by the Lithuanian Hydrometeorological Service Station. The meteorological station to the pilot site was less than 3 km.

6.6 Harvest and pelletizing

One of the main and most critical tasks in the "Phy2Climate" project is the supply of processed biomass from the pilot sites to the TCR reactor for biomass conversion. To ensure successful biomass conversion, the harvested biomass need to be pelletized. During this step, biomass processing starting from the harvest to the drying, biomass milling and biomass pelletization will be discussed.

Three different plant species were grown in Lithuanian pilot site, that leads to three different harvesting dates. Lithuanian pilot site harvest campaign was performed in the following order:

- Herbaceous plants mix harvest – August 2022;
- Jerusalem artichoke harvest, amaranth harvest – October 2022.

Harvesting herbaceous plants. Based on the pot test in 2021, it was estimated that one harvest will be sufficient to provide required biomass for the further testing at TCR facility. Harvesting of the herbaceous plants was performed after the plants fully developed in early August, just before the blooming phase, in order to obtain higher biomass output, at phenological stage BBCH 59. Harvesting was done using disc trimmers. Then the wet biomass (approximately about 995 kg) was laid into swaths for drying for 7 days. After drying on field, air-dried biomass was collected and transported to the drying facility on 2022 08 11 (Figure 6-4 Right).

Harvesting Jerusalem artichoke and amaranth. Due to similar vegetation length, Jerusalem artichoke aboveground and belowground, and amaranth were harvested at the same time in mid-October 2022. Jerusalem artichoke and amaranth plants were at the end of blooming phase, phenological stage BBCH 69, when stems of the plants were starting to lose its first leaves.

Jerusalem artichoke aboveground biomass and amaranth biomass were harvested using disc trimmers, biomass was cut into swaths, collected and transported to the drying facilities after 2 days on field pre-drying. For J. artichoke about 300 kg of wet biomass was obtained from the parcel (870 m^2). While for amaranth, it was about 360 kg of wet biomass from the parcel (310 m^2).

Jerusalem artichoke belowground biomass (tubers) was harvested using manual tools and picking by hands. Only about 218 m^2 from the entire J. artichoke area of 870 m^2 was harvested for tubers. It resulted in 250 kg of wet tubers being collected. The remaining tubers were left in the field to evaluate plant's ability to regrowth in contaminated site as a perennial plant. In addition, 250 kg of biomass was sufficient amount for further biomass processing.

Drying of the biomass. The aboveground biomass of herbaceous plants was dried in the hay-shed type facility and dried with atmospheric air. It was left there for about one month (from mid-August till mid-September) until further processing. The aboveground biomass of J. artichoke and amaranth and Amaranthus biomass was dried in the same hay-shed type facility, also for one month, from mid-October till mid-November. Tubers of J. artichoke were drying together with the aboveground biomass. However, there was no weight loss, so it was decided to intensify the drying by putting the tubers into heated (up to 50 °C) drying chamber. The tubers were dried in the drying chamber for 3 days.

Processing of the biomass. Biomass of the herbaceous plants was first shredded into smaller particles and then milled with a regular feed-type grain mill, 6 kW of power through 6 mm sieve, and with the 50 kg/hour output. Biomass of J. artichoke and amaranth as well as tubers of J. artichoke were successfully milled with the same type of mill, without prior shredding.

Pelletization of the biomass. Milled biomass material was pelletized using pellet mill CPM-2000 series (California pellet mill) (Figure 6-7). Pelletizer chamber compression ring die ratio was 1:5, and holes had 8 mm in diameter.

All biomass obtained in the Lithuanian pilot site was prepared in accordance with the specification for pellets suitable for biomass conversion in TCR feed, i.e., 8 mm in pellet diameter and <50 mm in pellet length. Photos of the pellets are presented in Figure 6-8. It can be noted, that despite different plant sources, visually there is no difference between the pellets obtained from the aboveground plant material. Whereas pellets from the tubers of J. artichoke have rough surface and are longer (Figure 6-9). Although, it seems brittle, after drying it out, it holds the pellet structure nevertheless.

Figure 6-7. CPM-2000 pelletizer and its compression ring die

Herbaceous plants | *Amaranth* | *J. artichoke (aboveground)*

Figure 6-8. Pellets of the biomass obtained at Lithuania pilot site field trials in 2022

Figure 6-9. Pellets of the J. artichoke tubers obtained at Lithuanian pilot site field trials in 2022

6.7 Phytoremediation performance

Phytoremediation performance was evaluated in two aspects: i) changes in the soil parameters, including general soil parameters and contaminants, and ii) biomass output, which is of critical importance not only within the Phy2Climate framework, but also in order to make phytoremediation commercially available.

6.7.1 Soil parameters

General soil parameters. Tables 6.1 and 6.2 present soil parameters before and after the field trials. Initial samples were collected in April 2021 before any soil movement with agricultural machinery. While, another set of samples were collected in October 2022, right after harvest of the last plants in the pilot site, meaning, that the soil was tilled, fertilized and vegetated. A joint-soil sample comprised of minimum 3 sub-samples was collected for every depth. Although, control subplots were installed on the site as well, control soil was not analysed for the soil parameters.

General parameters of the contaminated soil did not have significant differences within different soil (plant) parcels, thus are described together. Analysis showed that after the first year of the field trials, several very important soil parameters have improved due to complex of the applied remediation means: bacterial additive, fertilizers, compost and vegetation:

- organic matter, improved by 1 % on average,
- electrical conductivity, improved by 3.6 mS/m on average,
- microbial biomass, improved by 16 times,
- total C, improved by 1% on average,
- total N, improved by 360 times.

Soil parameters that decreased and need to be attenuated are as follows:

- pH, increased by 0.3 on average. Acidifying means need to be applied to avoid further alkalisation,
- total P (decreased by 5.4 times on average), total K (decreased by 8.0 times on average), and Mg (decreased by 28.8 times on average). To avoid soil depletion of macronutrients, mineral fertilizers will be applied before during the next vegetation season.

Table 6.1. Soil parameters in the contaminated soil determined during the initial characterization in April 2021

BEFORE (iniatial chracterisation)													
Plants	**Sampling depth**	**Total solids**	**Organic matter**	**pH$_{KCl}$**	**Electrical conductivity**	**Microbial biomass**	**Peroleum hydrocabons, C6-C10**	**Peroleum hydrocabons, C10-C40**	**Total C**	**Total N**	**Total P**	**Total K**	**Mg**
	cm	*%*	*%*		*mS/m*	*CPU/ml*	*mg/kg*	*mg/kg*	*%*	*mg/kg*	*mg/kg*	*mg/kg*	*mg/kg*
Herbaceous plants	0-20	99.5	2.18	8.2	8.25	370000	<0.25	685	3.05	2.3	322	999	11440
	20-40	99.6	1.79	8.5	8.14	21000	0.71	3753	3.61	2.35	235	1041	8793
	40-60	99.3	3.8	8.4	12.4	4900	0.84	7132	4.49	1.7	229	1020	9363
	60-100	99.3	3.6	8.8	16.7	8700	0.73	2492	3.81	3.28	355	1124	10313
Amaranth	2 sq. 0-20	99.5	2.04	8.2	9.01	230000	<0.25	245	2.3	0.61	321	1334	8743
	2 sq. 20-40	99.5	2.41	8.3	8.57	110000	<0.25	790	2.88	1.63	309	1332	12450
	2 sq. 40-60	99.7	2.03	8.4	8.48	520000	<0.25	1029	2.92	1.13	286	1332	10800
	2 sq. 60-100	99.4	3.06	8.00	13.8	770000	<0.25	557	2.76	9.1	427	2081	8680
Jerusalem artichoke	3 sq. 0-20	99.6	4.39	8.3	10.4	1 000000	<0.25	698	2.96	1.62	321	1041	9580
	3 sq. 20-40	99.4	3.16	7.7	9.65	41000	<0.25	431	2.25	2.72	386	1415	6843
	3 sq. 40-60	99.1	3.13	7.5	13.7	44000	<0.25	<100	1.92	3.85	553	2019	4277
	3 sq. 60-100	99.2	2.46	7.8	11.8	51000	<0.25	<100	2.29	1.78	504	3184	4820

Table 6.2. Soil parameters in the contaminated soil determined after 1st year field trial

AFTER field-trial (1st cycle)													
Plants	**Sampling depth**	**Total solids**	**Organic matter**	**pH_{KCl}**	**Electrical conductivity**	**Microbial biomass**	**Peroleum hydrocabons, C6-C10**	**Peroleum hydrocabons, C10-C40**	**Total C**	**Total N**	**Total P**	**Total K**	**Mg**
	cm	*%*	*%*		*mS/m*	*CPU/ml*	*mg/kg*	*mg/kg*	*%*	*mg/kg*	*mg/kg*	*mg/kg*	*mg/kg*
Herbaceous plants	0-20	93.0	3.58	8.7	11.2	1800000	<25.0	1261	4.64	867	18.5	155	379
	20-40	92.3	3.67	8.7	13.2	200000	<50.0	1667	4.08	947	22.3	137	380
	40-60	91.8	3.44	9.3	16.6	2 300000	<50.0	1931	4.48	729	18.7	95.8	1020
	60-100	89.2	5.58	8.1	15.6	9 300000	<50.0	1939	4.54	1010	44.1	91.4	174
Amaranth	0-20	94.7	2.71	8.3	12.2	170000	<25.0	462	3.28	636	22.1	119	168
	20-40	92.7	3.7	8.7	13.2	5 000000	<25.0	500	3.7	1033	38.0	158	292
	40-60	93.6	2.6	8.7	11,00	3 000000	<25.0	494	4,00	816	19.1	105	123
	60-100	86.8	4.85	7.8	21.8	7 300000	<50.0	1532	3.26	1840	56.4	154	188
Jerusalem artichoke	0-20	93.0	3.68	8.1	----	5 000000	<25.0	511	4.24	1390	369	367	432
	20-40	93.2	3.88	8.6	13.3	200000	<25.0	651	3.42	1120	53.8	179	204
	40-60	92.8	3.73	8.7	14.8	5 000000	<5.0	<100	3.41	430	54.9	306	142
	60-100	88.9	4.75	8.2	17.2	12 000000	<5.0	<100	3.48	717	58.8	358	176

Contamination. Figure 6-9 shows concentration of heavy (C10-C40) petroleum hydrocarbon fraction in the contaminated soil in Šiauliai site before and after field trials. Due to the fact that the site was used as oil base in the past, diesel and oil fractions were prevailing among other contaminants. The light fraction petroleum hydrocarbons (C6-C10) was almost undetectable already during the initial characterisation; therefore, it is not included in the graph (Figure 6-9), although data on it can be found in the Tables 6.1 and 6.2. Heavy metals, PAH and PCB were not detected during the initial characterisation; therefore, these analytes were not analysed after the field-trials.

According to Lithuanian legislation, the limit value for petroleum hydrocarbons in the soil in areas with low sensitivity is 200 mg/kg. The initial characterisation showed that the soil in Šiauliai site contains values above the limit value, and that the contamination is uneven on this site. The highest contamination was in the parcel which was vegetated with herbaceous plants (Figure 6-2, the lowest green parcel). Furthermore, deeper layers exhibited more contamination than the topsoil. Whereas yellow and red parcels (Figure 6-2) exhibited less contamination and it was distributed in within the soil layers more evenly.

The phytoremediation potential was evaluated as a *ratio between contaminant's concentration in the soil before and after the experiment*, and the results are presented in Table 6.3. Only values above unity show that phytoremediation (degradation) process has come about. The higher the value, the more intensive the process is. However, there are values below unity, and in this case, it shows that contaminants concentration in the soil after the field trial was higher than before. There are several reasons for this phenomenon: i) soil was deep tilled after taking samples for initial characterisation. This was done to shred roots and to homogenise the soil. It is likely that more contaminated soil was upturned and brought to the surface; ii) TPH analysis before and after the field trials were performed in different laboratories, so there could be instrumental error, especially for lower values, and finally iii) soil contamination in the Šiauliai site is uneven. It is likely that samples were not take at exactly same spot, so the sampling error is also possible.

Despite all these reasons, the most intensive remediation effect occurred in the most contaminated areas, where the contamination dropped almost 4 times. Herbaceous plants were sown in this parcel. As expected, one cycle was insufficient to reach the limit values. Furthermore, there were patches where the contamination was visibly higher (poor soil structure, diesel-specific odour, oily stones) and herbaceous plants were significantly weaker. In the most contaminated part of the sown area, the mixture of perennial grasses has germinated very unevenly, and there are completely bare areas where there are no plants at all. These areas account for about 30 percent. *Poaceae* species predominate in the sown mixture. Legume plants germinated significantly less and among them there are more sedges than *alfa alfa*. In some areas, there are also pink clovers that have not been sown. In contrast to the less polluted areas, there is almost no white-sweet clover. The issue of uneven contamination needs to be taken into account and additional tools needs to be planned for the next growing season to unify vegetation within the parcel.

Regarding the parcels where amaranth and J. artichoke was growing, it is difficult to evaluate the real phytoremediation potential due to the above-named reasons and due to the reasons explained in Chapter 6.4 (amaranth – seed quality, J. artichoke – planting density), but it is expected that higher phytoremediation potential will be reached next year.

Table 6.3. Phytoremediation potential for every sampling depth

Sampling depth, cm	Herbaceous plants	Amaranth	J. Artichoke
0-20	0.54	0.53	1.36
20-40	2.25	1.58	0.66
40-60	3.69	2.08	1.00
60-100	1.28	0.36	1.00

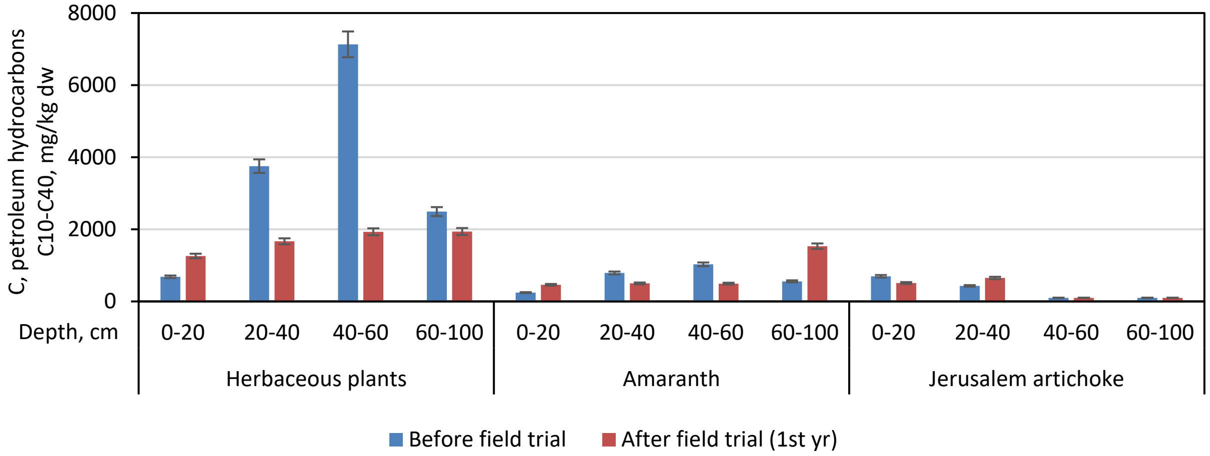


Figure 6-9. Concentration of heavy petroleum hydrocarbon fraction in the contaminated soil in Šiauliai site before and after field trials

6.7.2 Biomass output

Biomass output during the field trials was very important and closely monitored because it is inseparable to MS3 - *to deliver first batch of biomass for biofuel production.*

Table 6.4 presents biomass output obtained in the Šiauliai site during the first year of field trials and a recalculated output for one hectare. Based on the results from our pot-experiments (2021) it was estimated that herbaceous plants could produce about 1287 kg/ha, J. artichoke – about 23497 kg/ha, and amaranth – about 27169 kg/ha of dry biomass. However, field-trials exhibited different results: biomass output from herbaceous mix field was 1296 kg/ha, from J. artichoke – 3878 kg/ha, and from amaranth – 1382 kg/ha of dry biomass. The expectations were met only in the case of herbaceous plants, but the J. artichoke and amaranth harvests were drastically smaller. For J. artichoke plant density played a major part, as it dropped more than 20 times in the field as compared to the pot experiments (23 plants/m^2 versus 5 plants/m^2). Density was high in the pot tests and it did not affect plant development, but it needed to be adjusted (dropped) in the field to fit agricultural machinery. In the case of amaranth, prolonged dry conditions slowed down the germination of seeds, which allowed spread and overshadowing by weeds that are typically more resistant to unfavourable weather conditions. Furthermore, unlike the monocultures, herbaceous mix was comprised of five different species and it is likely that it allowed better adaptability to harsh conditions, thus it was possible to achieve the yield as estimated.

Table 6.4. Biomass output in Šiauliai site after the first year of field trials (ww…wet weight; dw … dry weight)

Biomass origin	Parcel area, ha	Total biomass yield, kg ww	Total biomass yield, kg dw	Biomass yield, kg/ha dw
Herbaceous plants mix	0.1234	995	160	1296
J. artichoke aboveground	0.0870	300	110	1264
J. artichoke tubers	0.0218	250	57	2614
Amaranth	0.0311	360	43	1382

6.8 Encountered problems and amendments

One of the main problems encountered can be considered the purchase of poor-quality amaranth seed. The seedlings had weaker germination rate than those used in the greenhouse experiment. In addition, due cold and dry spring it took time for the amaranth to start germinate. Later, when the plants began to sprout, their density was low, so the weeds quickly began to grow, and later overshadowed the amaranth. No additional action was taken this year, as it was certain that the required amount of biomass would be obtained anyway. However, next year, glyphosate will be used in the site to reduce weeds. Also, before sowing, a germination test will be performed in order to make sure of the quality of the seed.

Secondly, the field trial showed that the biomass output for J. artichoke calculated based on the results from the pot test can't be reached due to different planting density. Planting density in the pot experiments was several times higher than in the field trials. However, lower planting density is in line to the technical parameters of the currently used agricultural machinery. Higher plant density is expected next year, as tubers were left in the larger part of the J. artichoke parcel. It is expected that the tubers will sprout denser in comparison to manual seeding.

Thirdly, calculation of phytoremediation potential was incomplete due to possible sampling issues and due to applied agrotechnical tools that could have upturned the contaminant so that the initial characterization doesn't exactly correspond to it. Since no heavy machinery work is planned for the next growing season, the soil will remain undisturbed, thus it is expected that sampling will be more precise.

6.9 Other information

Pot experiments. Greenhouse pot experiments were continued in 2022 but only with herbaceous plant mixes as the selected species are perennial. After the final harvest in September 2021, pots with the plants were left in the greenhouse. The greenhouse remained heated throughout the winter, but the temperature was maintained only at 5-7 °C. The vegetation restarted in February. The plants were fertilized with urea (N-46.2 %) in March; 19.5 g of solid urea fertilizer were spread to the surface of each box. Control plants did not receive fertilization.

Herbaceous plants were cut on three occasions, in March, in July and in August. Table 6-5 shows the total dry biomass output recalculated for 1 ha based on results from the pot experiment.

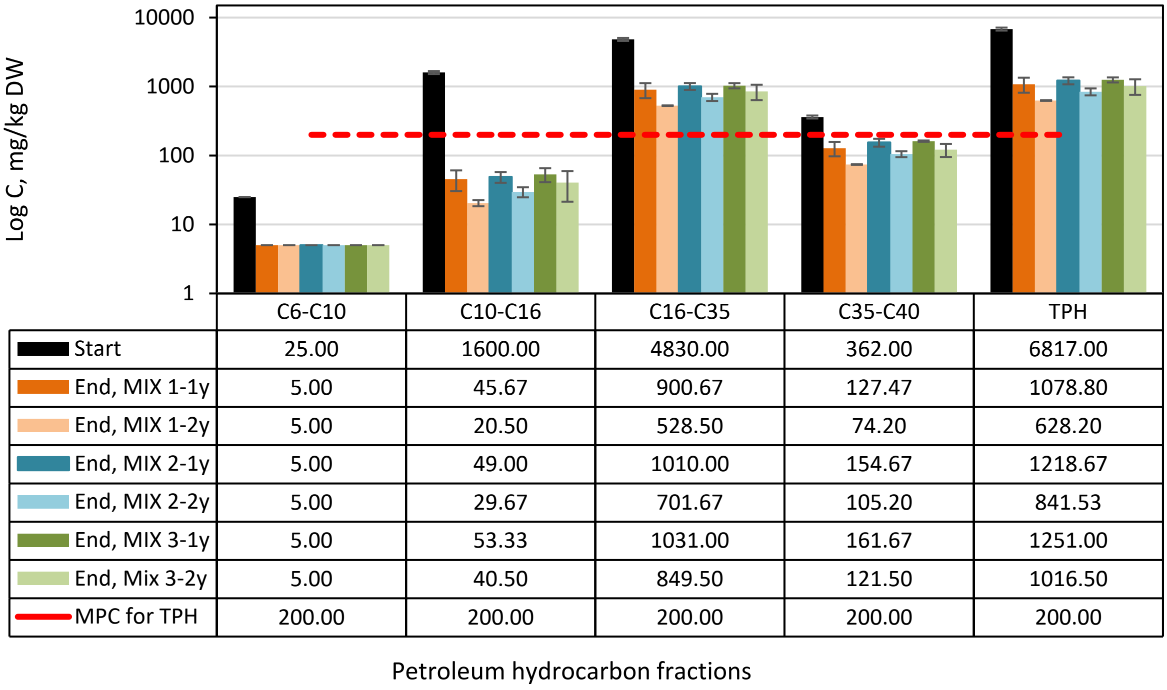
Table 6.5. Total biomass output, kg of dry weight/ha (n=3)

Mix I		Mix II		Mix III	
Contaminated	Control	Contaminated	Control	Contaminated	Control
1748.2	729.6	900.8	1,009.5	2,291.1	757.5

The results are that herbaceous plants in Mix I and Mix III grew better on the contaminated soil than on the clean soil. Whereas biomass output was very similar for the contaminated and the clean soil for Mix II. The very likely reason why biomass output was higher in the contaminated soil is that contaminated soil received mineral fertilizers. It was fertilized with NPK+S fertilizers during the first-year experiments, and with urea fertilizer during the second-year experiments. While, control soil did not receive fertilization and soil became depleted of nutrients.

It was observed that some species did not survive the winter and died out or became sparse. Meanwhile, some other species, like *Festulolium* and *Tall fescue* became dominant and contributed the most biomass. All these observations were considered when preparing herbaceous plant mix for the field trials.

Phytoremediation potential as a ratio between TPH concentration in the soil before and after the experiment was calculated after second year. The trends remained the same as after the first year – the highest TPH removal efficiency was determined in the MIX I, the lowest in the Mix III. Figure 6-5 shows changes of different petroleum hydrocarbon fractions in the contaminated soil after two consecutive years in phytoremediation process. The light fractions were easily degraded during the first year, and no changes occurred after the second year (it remained below detection limit of the instrument). Despite that the reduction of heavier fractions was less significant after the first year, it was continuous in the second year and concentration of C6-C40 decreased even more. However, two years were insufficient to reach maximum permissible concentrations in soil forced by Lithuanian law.



	C6-C10	C10-C16	C16-C35	C35-C40	TPH
Start	25.00	1600.00	4830.00	362.00	6817.00
End, MIX 1-1y	5.00	45.67	900.67	127.47	1078.80
End, MIX 1-2y	5.00	20.50	528.50	74.20	628.20
End, MIX 2-1y	5.00	49.00	1010.00	154.67	1218.67
End, MIX 2-2y	5.00	29.67	701.67	105.20	841.53
End, MIX 3-1y	5.00	53.33	1031.00	161.67	1251.00
End, Mix 3-2y	5.00	40.50	849.50	121.50	1016.50
MPC for TPH	200.00	200.00	200.00	200.00	200.00

Figure 6-5. Average concentrations of petroleum hydrocarbon fractions in the contaminated soil before the pot experiment and after two consecutive years of testing (n=3). Note, the Y-scale is logarithmic

6.10 Overall summary of phytoremediation performance in M12-M24

The first growing season has been completed without major drawbacks. The soil on the Šiauliai site is contaminated with petroleum hydrocarbons. A complex, including specially selected plants, mineral and organic fertilizers as well as bacterial additive, was applied to the field. The effectiveness of this complex has been proved in the greenhouse experiment previous year. Two monocultures: Jerusalem artichoke and amaranth, and a mix of herbaceous plants was grown in the field trials, and it all cases the goal to produce 40 kg of dry material was reached. Furthermore, promising phytoremediation results regarding degradation of contaminants were obtained as in some places the contamination dropped almost 4 times.

7. FIELD TRIALS ON THE ARGENTINIAN PILOT SITE

7.1 Landscape preparation

Landscape preparation, surface levelling and debris removal were not needed in the Argentinian Pilot Site.

7.2 Soil preparation and seeding campaign

Soil preparation tasks were planned to be carried out with the help of members of the La Planta community that lives in the surroundings of the contaminated site. As a first step, soil samples were taken from two sites: contaminated (Site 1) and reference site (Site 2), as mentioned in Deliverable D2.1. As Site 1 presents different pH values and a heterogenic distribution of metal(loids) in soil, two sampling points were taken. An initial physicochemical characterisation of three sampling points was carried out in the contaminated site. The total fraction of metal(loid)s in soil is shown in Tables 7.1-7.3 because the physicochemical characterisation and the soluble fraction of metal(loids) data were presented in the Deliverable D2.1 (Updated version at M15).

Regarding sub-plot division, two 504 m^2-plots with different contamination degrees were built in the most contaminated areas (Figure 7-1).

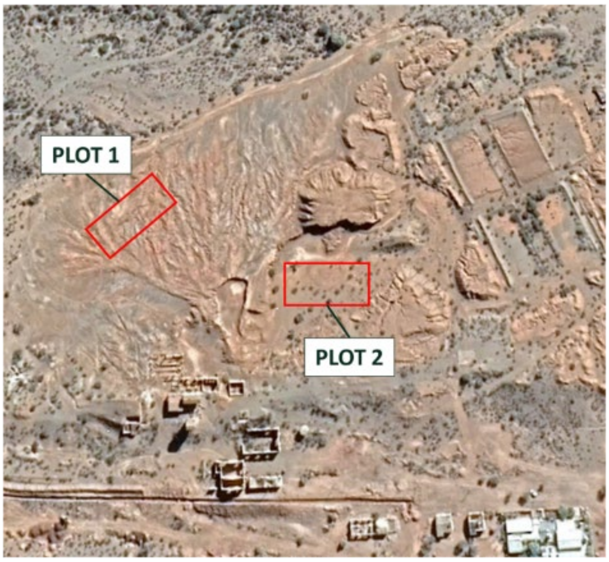


Figure 7-1. Google Earth image showing the location of the Plots in the Argentinian Pilot Site

High concentrations of metal(loid)s, acidic pH (2.7) and low organic matter content were detected in the soil. Therefore, two soil amendments were selected to be applied: compost (organic amendment) and dolomite (inorganic amendment). Compost is produced from municipal and agro-industrial waste in the city of San Juan, Argentina. It is a mixture of pruning remains, industrial tomato waste, garlic husk, cow and poultry manure, and mature compost. Dolomite is composed of 62% calcium carbonate and 26% magnesium carbonate.

Table 7.1. Initial total fraction of metals and metalloids in Site 1, Plot 2 (P2) (Mean ± SD)

SP	DEPTH	Mg	Ca	S	B	Cu	Fe	Mn	Mo	Zn	Cd	Cr	Pb	As	Na
-	*m*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*
P2	0-0.2	1740±2040	5177±1185	ND	n.d.	131±65	90213±23534	433±175	n.d.	5880±3759	53±33	n.d	634±437	4789±2040	58584±0
	0.2-0.4	3354±4166	6606±1354	ND	n.d.	159±76	91914±39228	561±145	n.d.	6606±4270	40±24	n.d	518±431	3933±2764	115094±0
	0.4-0.6	5716±3735	7242±1740	ND	n.d.	156±72	63367±50279	595±167	n.d.	5709±4635	35±21	n.d	404±351	2821±2983	115046±45839
	0.6-0.8	9813±1257	7799±1903	ND	n.d.	75±25	29151±2827	715±193	n.d.	3954±2454	25±16	n.d	28±32	358±248	134225±17786
	0.8-1	11951±427	10945±2163	ND	n.d.	62±36	30765±1689	739±85	n.d.	2744±1618	19±11	n.d	8±8	113±46	158722±22825

n.d.: not detected; ND: no data.

Table 7.2. Initial total fraction of metals and metalloids in Site 1, Plot 1 (P1) (Mean ± SD)

SP	DEPTH	Mg	Ca	S	B	Cu	Fe	Mn	Mo	Zn	Cd	Cr	Pb	As	Na
-	*m*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*
P1	0-0.2	6670±3357	10713±3955	ND	n.d.	826±327	116238±14635	1423±718	n.d.	2227±1284	19±9	n.d	450±214	2383±2061	57551±27714
	0.2-0.4	8335±2255	10752±3394	ND	n.d.	1035±251	114673±17523	1617±445	n.d.	2728±1273	21±9	n.d	545±213	2228±1823	71144±26559
	0.4-0.6	8268±1932	12173±2056	ND	n.d.	808±376	97555±36690	1376±271	n.d.	2035±1376	16±8	n.d	338±275	1046±1043	77312±45424
	0.6-0.8	9090±1763	10053±3420	ND	n.d.	544±388	67861±25192	1111±181	n.d.	1450±1053	13±6	n.d	192±154	651±577	120943±41590
	0.8-1	9244±2018	8992±3844	ND	n.d.	435±316	54442±16041	1132±418	n.d.	1708±1269	16±10	n.d	139±137	373±299	119112±36263

n.d.: not detected; ND: no data.

Table 7.3. Initial total fraction of metals and metalloids in Site 2, Reference Site (R) (Mean ± SD)

SP	DEPTH	Mg	Ca	S	B	Cu	Fe	Mn	Mo	Zn	Cd	Cr	Pb	As	Na
-	*m*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*
R	0-0.2	6014±1062	6816±198	ND	n.d.	205±9	16453±2943	380710±152441	n.d.	879±159	50±9	n.d.	109±15	771±28	6530±411
	0.2-0.4	7597±2831	7051±1136	ND	n.d.	203±15	20627±7886	304355±382886	n.d.	817±32	52±3	n.d.	106±11	767±20	7610±1902
	0.4-0.6	7638±3208	6719±1111	ND	n.d.	197±16	22869±10297	473150±349120	n.d.	805±64	48±10	n.d.	103±11	762±50	8076±3291
	0.6-0.8	6440±4714	6567±2116	ND	n.d.	191±30	19797±12141	477278±318497	n.d.	789±122	43±26	n.d.	102±16	734±77	6314±4506
	0.8-1	5640±1882	6422±1140	ND	n.d.	181±32	22531±858	505308±42800	n.d.	721±103	33±38	n.d.	94±14	691±55	5223±421

n.d.: not detected; ND: no data.

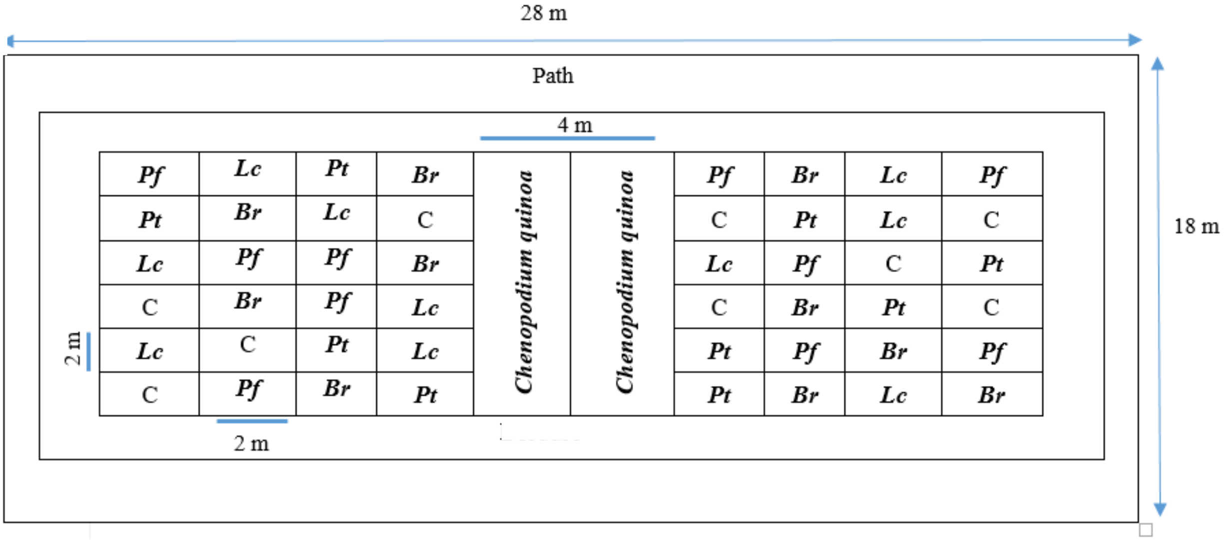
Because the pH-level needed to be regulated in Plot 1, dolomite was added to the soil as an amendment. In addition, compost was added to both plots in order to incorporate organic matter and nutrients. Based on results obtained in pot tests, 5% compost and 18% dolomite were added in Plot 1, and only 5% compost was added in Plot 2. Amendments were incorporated in the first 50 cm of soil using horizontal and vertical tillage with agricultural machinery (tractor) and implements (chisel, disc harrow). Then, topsoil was tilled using a motocultivator as shown in Figure 7-2.

Figure 7-2. Soil ploughing and tilling in the Argentinian Pilot Site

Five plant species were selected by their metal(loid) bioaccumulation capacity. The four native shrubs and trees selected were *Plectrocarpa tetracantha*, *Bulnesia retama*, *Larrea cuneifolia* and *Prosopis flexuosa* (see Deliverable D2.2), and the quinoa crop (*Chenopodium quinoa*) is been used as an herbaceous annual plant to increase the phytoextraction rate of metal(loids).

With respect to seeding and planting strategy, seeds of the four native species were collected from plants present in the study area. Seeds were cleaned and a pre-germination treatment was applied to ensure the emergence of the seedlings. This treatment consisted of a mechanical scarification through a cut with pliers or scraping with sandpaper, depending on the type of seed. The seeds were immediately placed in germination trays and incubated in a dark chamber at 25 °C (see Deliverable D2.1). Once the seedlings emerged, the trays were transferred to a temperature-controlled greenhouse, where they remained for 3 months (Fitotec Company). After this period, the seedlings were transplanted into 1 L nylon pots and kept under controlled conditions in a greenhouse (San Juan, INTA). Once the plants reached a minimum of 30 cm in height, they were placed outdoors for 3 months for acclimatization prior to transplanting.

Experimental design consists of 6 treatments randomly distributed within each plot. The size of each plot has a total of 504 m^2, including a border zone as a buffer (Figure 7-3). After the acclimatization period, shrubs and trees were transferred to the study site and planted in the experimental plots. In the case of the quinoa crop, seeds were directly sown in the experimental plots.



Pf	*Lc*	*Pt*	*Br*	*Chenopodium quinoa*	*Chenopodium quinoa*	*Pf*	*Br*	*Lc*	*Pf*
Pt	*Br*	*Lc*	C			C	*Pt*	*Lc*	C
Lc	*Pf*	*Pf*	*Br*			*Lc*	*Pf*	C	*Pt*
C	*Br*	*Pf*	*Lc*			C	*Br*	*Pt*	C
Lc	C	*Pt*	*Lc*			*Pt*	*Pf*	*Br*	*Pf*
C	*Pf*	*Br*	*Pt*			*Pt*	*Br*	*Lc*	*Br*

Figure 7-3. Representative experimental design of each Plot. C: control, *Pf*: *Prosopis flexuosa*, *Br*: *Bulnesia retama*, *Pt: Plectrocarpa tetracantha, Lc*: *Larrea cuneifolia*

7.3 Monitoring program

Concerning the monitoring program, plots were fertilised with compost as organic amendment from the beginning of the field tests. Additionally, based on the N content in soil, an inorganic fertiliser (urea) was added at the beginning (November) and at the end (February) of the main net primary production season of the shrub and tree species. Also, urea was added 15 days after sowing the quinoa crop. This procedure was expected to increase the availability of nutrients at the beginning of the season and the reserve substances at the end of the growing period (see Deliverable D2.1).

Maintenance tasks planned during the field tests were: 1) Installation of perimeter fence around the two plots (Figure 7-4); 2) Installation of the irrigation system; 3) Maintenance of the plots that includes checking the operation and repair of the facilities; 4) Recording of climatic events, temperature, rainfall volume, and relative humidity; 5) Irrigation flow recording and adjustments; and 6) Recording possible pests and other observations that arise during the experimental period. All maintenance and surveillance tasks were carried out by the community of La Planta under the supervision of INTA staff.

Figure 7-4. Experimental plots were delimited with a perimeter fence with posts

Seedling survival and growth are strongly conditioned by the water availability; hence an adequate volume of water for each plot increases the possibility of success of the experiment.

Rainfall records in La Planta average 85 mm per year and are mainly concentrated in the summer period (December-March). The low volume of rains highly concentrated in a short period of time means that detailed planning is required to achieve maximum use of the water. To obtain the necessary volume of water, a combined strategy was proposed that consists of taking advantage of the rainfall in the area and incorporating water through an irrigation system.

A drip irrigation system was installed (Figure 7-5). Black 0.5-inch irrigation hoses were placed with 1 L/h self-compensating drippers for each tree and shrub plant, and 2 L/h drip irrigation tape for quinoa crop. Water supply has been taken from tap located 300 m from Site 1 and was brought to each plot with 0.75-inch irrigation hoses. Irrigation regime depends on season and plant water demand. The maximum water flow calculated for each plot is 574 L/h (40 L/h for tree and shrub species plus 534 L/h for quinoa crop), reaching a total of 3400 L every 20 days (200 L for tree and shrub species plus 3200 L for quinoa crop).

Figure 7-5. Irrigation system installed in experimental plots

7.4 Plant development

According to the monitoring results, an increase in the main stem height of the species of shrubs and trees was observed. During a period of 149 d, *Bulnesia retama*, *Larrea coneifolia*, *Prosopis flexuosa* and *Plectrocarpa tetracantha* increased their size by 17.44, 19.73, 10.92 and 11.89%, respectively.

Chenopodium quinoa was sown manually in August 2022 after minimum temperatures exceeded zero degrees Celsius. Quinoa crop was monitored and harvested at the end of December 2022. Figure 7-6 shows representative pictures of the plants that are growing in the experimental plots. Overall, there are a total of 134 plants of shrubs and trees, and 7200 plants of the quinoa crop growing in the Argentinian Pilot Site.

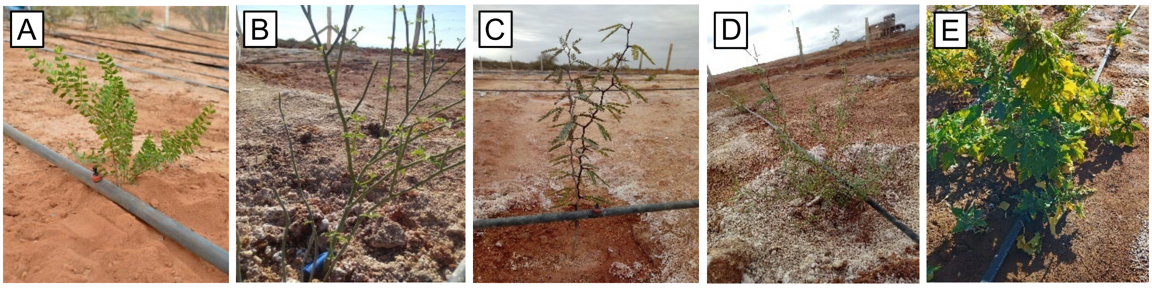


Figure 7-6. Representative pictures of plants growing in the experimental plots. A: *Larrea coneifolia*; B: *Bulnesia retama*; C: *Prosopis flexuosa*; D: *Plectrocarpa tetracantha*; E: *Chenopodium quinoa.*

7.5 Environmental conditions

The study area is characterised by an arid environment that corresponds to the "Monte" phytogeographic province. It has a dry and warm climate with mainly summer (December–March) rainfall of a torrential nature, ranging between 80 and 200 mm per year[9,10]. Temperatures are very high and reach an absolute maximum of 46°C[11]. Regarding geomorphology, the area is located in an extensive alluvial plain of the Bermejo River. Primary and secondary streams are often dry and only have water during certain seasons[12]. In this sense, the primary productivity of this kind of environment is limited.

All climatic events, rainfall, temperature and relative humidity have been recorded in the Argentinian Pilot Site. Table 7.4 shows the average, minimum and maximum temperatures between January 2022 and February 2023. Quinoa crop was sown after the winter season due to the minimum temperatures close to zero degrees Celsius that were recorded early between the end of March and the beginning of April. During this period, the recording of accumulated rainfall was 96.3 mm. The average relative humidity was 28.0 ± 6.2% (min: 6.0%; max: 92%) and ambient pressure was 1010.3 ± 10.1 hPa (min: 987.0 hPa; max: 1036.5 hPa).

Additionally, no pest problem were faced in the experimental plots.

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[10] Cabrera, A., 1994. Argentine Phytogeographic Regions. Argentine Encyclopedia of Agriculture and Gardening. First edition, T.II, F.1, ACME Editorial. Argentina (in Spanish).

[11] Dalmasso A, Anconetani J, 1993. Fruit productivity of *Prosopis flexuosa* (Leguminosae), Sweet Algarrobo, in Bermejo, San Juan. Multequina 2173-2181 (in Spanish).

Table 7.4. Average, minimum and maximum temperatures between January 2022 and February 2023

Year	Month	Average temperature (ºC)	Minimum temperature (ºC)	Maximum temperature (ºC)
2022	January	28.5	10.4	41.8
	February	26.2	13.9	38.4
	March	24.1	3.7	37.8
	April	17.0	0.1	34.5
	May	11.6	-1.3	25.2
	June	7.9	-3.5	22.5
	July	9.8	-3.9	28.1
	August	12.1	-2.9	29.2
	September	17.1	4.0	33.3
	October	21.4	5.5	39.6
	November	25.6	5.6	36.0
	December	29.4	14.9	45.7
2023	January	29.2	18.1	39.7
	February	28.1	7.6	41.4

7.6 Harvest and pelletizing

Biomass harvesting of the first cycle of quinoa crop was done manually between December 2022 and January 2023 (Figure 7-7). In the case of shrubs and trees, harvesting was done between October and November 2024. Then, biomass was dried, crushed and pelletized. After that, pellets were shipped to Germany (Fraunhofer – WP3).

Figure 7-7. *Chenopodium quinoa* dried and pelletized after harvesting between December 2022 and January 2023.

7.7 Phytoremediation performance

7.7.1 Soil parameters

The physicochemical characterisation of the plots was carried out after the first cycle of quinoa harvesting. Samples were taken in five different depths and the corresponding analyses were carried out (Tables 7.5-7.6).

Table 7.5. Second physicochemical characterisation of Site 1, Plot 1 (P1) (Mean ± SD) carried out after the first cycle of quinoa crop

SP	DEPTH	TEXTURE CLAY	TEXTURE SILT	TEXTURE SAND	Water content	pH	EC / Salinity	P available	K available	Mg	Ca	S	B	Cu	Fe
-	*m*	*% (m/m) ms*	*% (m/m) ms*	*% (m/m) ms*	*%*	*-*	*uS/cm*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*
P1	0-0.2	6±7	13±9	80±15	30±6	12±1	9144±3748	95±49	81±39	78717±24342	182787±46952	ND	n.d.	67±38	n.d.
	0.2-0.4	9±4	22±8	68±10	28±2	10±2	5729±1799	224±145	36±35	24116±15785	81928±53729	ND	n.d.	107±37	n.d.
	0.4-0.6	10±2	39±4	50±2	29±4	6±2	25879±13851	58±74	6±6	6229±3692	25886±20231	ND	n.d.	120±59	n.d.
	0.6-0.8	10±3	41±5	49±4	24±4	4±1	35262±11356	20±17	6±6	6038±923	20640±7136	ND	n.d.	106±16	n.d.
	0.8-1	16±9	42±13	43±6	23±3	3±1	37144±7009	9±9	5±4	8038±1170	19517±11896	ND	n.d.	149±10	n.d.

SP	DEPTH	Mn	Mo	Zn	Organic matter	Total C	Total N	Microbial biomass	TPH	PAH	Cd	Cr	Pb	As	Na
-	*m*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/g dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*CFU/ml*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*
P1	0-0.2	707±507	n.d.	2882±1211	3±1	1.5±0.3	874±92	ND	ND	ND	5±4	ND	166±160	6216±4118	2258±1514
	0.2-0.4	674±405	n.d.	3159±331	2±1	1±0.4	555±122	ND	ND	ND	16±21	ND	139±153	8623±2019	1434±355
	0.4-0.6	908±804	n.d.	4345±2068	1±0	0.7±0.2	375±42	ND	ND	ND	29±23	ND	279±119	8803±3356	1721±595
	0.6-0.8	875±469	n.d.	3670±556	1±0	0.5±0.2	402±45	ND	ND	ND	14±16	ND	413±179	6945±4598	6747±4896
	0.8-1	990±655	n.d.	4369±746	1±0	0.4±0.2	391±66	ND	ND	ND	20±13	ND	234±81	6564±5715	6662±6168

n.d.: not detected; ND: no data.

Table 7.6. Second physicochemical characterisation of Site 1, Plot 2 (P2) (Mean ± SD) carried out after the first cycle of quinoa crop

SP	DEPTH	TEXTURE CLAY	TEXTURE SILT	TEXTURE SAND	Water content	pH	EC / Salinity	P available	K available	Mg	Ca	S	B	Cu	Fe
-	*m*	*% (m/m) ms*	*% (m/m) ms*	*% (m/m) ms*	*%*	*-*	*uS/cm*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*
P2	0-0.2	18±7	39±7	42±13	12±6	5±1	18199±8464	19±14	14±12	11324±5133	19744±8194	ND	n.d.	785±306	n.d.
	0.2-0.4	11±11	33±11	55±3	10±2	5±1	14218±6456	13±9	16±17	13721±6356	29455±3677	ND	n.d.	880±343	n.d.
	0.4-0.6	11±2	28±3	59±4	10±2	5±1	11577±4164	8±4	18±18	11950±5419	30118±5865	ND	n.d.	921±351	n.d.
	0.6-0.8	10±3	21±9	67±8	8±3	6±0	9863±5002	9±5	14±13	11907±1660	27604±8036	ND	n.d.	558±273	n.d.
	0.8-1	9±6	25±5	66±3	6±1	6±1	9678±2275	10±6	8±5	13548±1215	34085±6395	ND	n.d.	743±47	n.d.

SP	DEPTH	Mn	Mo	Zn	Organic matter	Total C	Total N	Microbial biomass	TPH	PAH	Cd	Cr	Pb	As	Na
-	*m*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/g dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*CFU/ml*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*	*mg/kg dry matter*
P2	0-0.2	1894±1261	ND	2071±458	0.6±0.3	0.3±0.1	658±116	ND	ND	ND	11±4	ND	243±195	5660±2358	3551±1474
	0.2-0.4	2385±1390	ND	2440±526	0.4±0.3	0.2±0.2	507±49	ND	ND	ND	13±4	ND	309±98	3671±2675	6531±0
	0.4-0.6	2683±1100	ND	2309±319	0.3±0.2	0.2±0.1	456±31	ND	ND	ND	6±3	ND	233±163	5337±3491	2979±3357
	0.6-0.8	2051±389	ND	1511±760	0.3±0.3	0.2±0.1	394±40	ND	ND	ND	3±3	ND	134±100	4865±3677	4293±3723
	0.8-1	2688±1195	ND	2041±99	0.4±0.3	0.2±0.2	461±116	ND	ND	ND	4±1	ND	194±145	5053±3613	3878±3033

n.d.: not detected; ND: no data.

Results show the heterogeneity in soil properties. The organic matter data in Plot 1 were overestimated due to interference in their determination. In fact, this technique does not allow to differentiate between organic carbon and inorganic carbon (e.g. carbonate from dolomite). Additionally, dolomite increased the pH value and the concentration of Mg and Ca in the upper 40 cm of the soil in Plot 1.

7.7.2 Biomass output

The global goal of the Phy2Climate project to achieve 40 kg of dry biomass from each pilot site was achieved in the first growing cycle of quinoa crop (Chenopodium quinoa). In addition, the shrubs and trees (*Bulnesia retama, Larrea coneifolia, Prosopis flexuosa* and *Plectrocarpa tetracantha*) that were planted in early 2022, they continue growing in both plots.

As the main findings, highlight that three varieties of quinoa crop were sown in the plots in order to test their yield under field conditions (Figure 7-8). In August 2022, quinoa crop was established on soil amended with compost and dolomite. Plant height (cm) and biomass production (kg/ha) were compared between the results obtained in the experimental plots and a control field located close to the Pilot Site (Table 7.7). After the first harvest, plants developed on the polluted plots showed worse growth, but they reached the biomass amount needed for the WP3 demand. All the varieties presented a reduction of 37-65% in plant height and 51-91% in biomass production.

Figure 7-8. Quinoa crop growing in Argentinian Pilot Site before the first harvesting.

Table 7.7. Reduction in quinoa yield expressed as difference between experimental plots and a control field in plant height and biomass production for each variety tested in field conditions

Variety of quinoa	Difference in plant height (%)	Difference in biomass production (%)
Morrillos	-36.92	-61.04
Hornillos	-64.66	-50.66
252	-58.13	-90.87

Regarding the native shrubs and trees, they have been monitored since planting in early 2022 (Figure 7-9). Plants present good growth rate and look healthy. All the plant species from Plot 2 are taller than those growing in Plot 1.

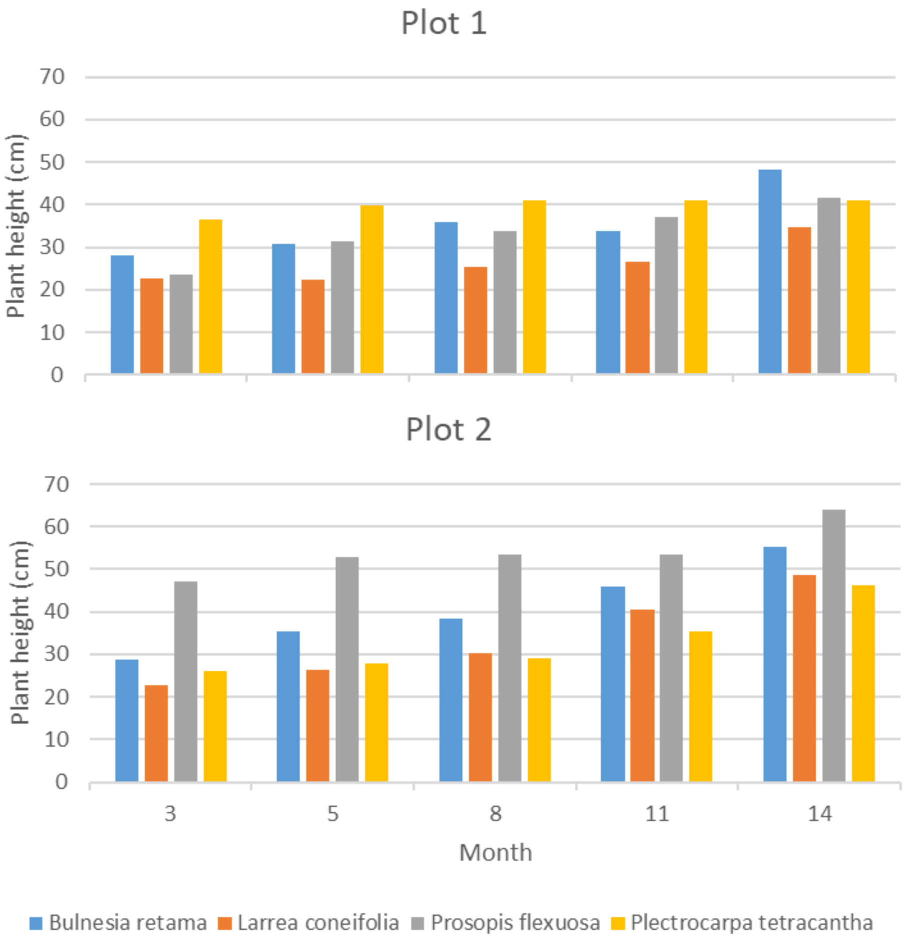

Figure 7-9. Native shrubs and trees height monitored since planting in early 2022.

7.8 Encountered problems and amendments

Initially, we selected four native species of trees and shrubs that grow around the contaminated site. Our idea was to promote the process of ecological succession, avoiding the use of commercial crops. However, their production of biomass is limited, although these species are adapted to this polluted environment. Therefore, quinoa crop was added to the Argentinian Pilot

Site to achieve the requirements of plant biomass for the biofuel production and metal recovery (amendment to the GA).

After soil preparation in the experimental plots, minimum temperatures close to zero degrees Celsius that were recorded early between the end of March and the beginning of April. Hence, quinoa crop was sown manually in August after minimum temperatures exceeded zero degrees Celsius. It was solved successfully.

7.9 Other information

A preliminary pot test was carried out exposing a variety of quinoa ("Morrillos") to contaminated soil with amendments (compost and dolomite) and a control group (reference soil) for 45 days. The results showed a good growth in both treatments as shown in Table 7.8. However, a reduction of 55.6% in seed yield (panicle size) was observed in the plants exposed to the contaminated soil with amendments. Panicle formation started before the expected time for this species, which is 65 d under normal conditions. An explanation of this effect could be attributed to stress.

Table 7.8. Average values of the main parameters measured as a response of a variety of quinoa ("Morrillos") exposed to contaminated soil with amendments (compost and dolomite) and a control group (reference soil) in a preliminary pot test

Parameter (cm)	Exposure time (d)	Treatment	
		Reference soil	Contaminated soil with amendments
Plant height	15	9.8	9.3
	30	21.0	21.0
	45	32.8	33.2
Stem diameter	15	0.17	0.27
	45	0.19	0.31
Panicle size	45	4.5	2.5

7.10 Overall summary of phytoremediation performance in M12-M24

Acute and chronic experiments show the toxicity caused by the contaminated soil. Also, the pot tests allowed defining the doses of dolomite and compost to use in the experimental site (Pilot Site). Two plots of 504 m^2 each were defined in the contaminated site. First, the perimeter fence with posts was installed in each plot. Subsequently, the application and incorporation of dolomite and compost was carried out using agricultural machinery (tractor, motor cultivator) and implements (chisel, disc harrow). After that, an irrigation system was installed. Five plant species were sown or planted in the soil of the experimental plots. People of the La Planta community help with maintenance and surveillance tasks, such as checking the operation and repair of the facilities, recording of climatic events, temperature, rainfall volume, and relative humidity, irrigation flow recording and adjustments, and recording possible pests and other observations. Plant growth variables measured in field conditions include plant height, crown diameter, and stem base diameter. At the end of the first cycle of the crop, quinoa growth variables were recorded and then harvest was carried out. Finally, quinoa crop was pelletized and sent to Fraunhofer (WP3) in order to assess the potential in biofuel production.

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