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

Deliverable D2.4:

Annual report on phytoremediation performance and monitoring [M36]

for:
European Commission
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presented by Phy2Climate project consortium

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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
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MoM	Minutes of Meeting	
MAN	Procedures and user manuals	
WOR	Working document, issued as preparatory documents to a Technical report	
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INDEX

1. EXECUTIVE SUMMARY

This report is a detailed overview about the phytoremediation performance in the second year of pilot field experiments and the corresponding activities of involved partners. With exception of Argentina (southern hemisphere), the second year (cycle) of field trials has been completed in Spain, Serbia, Lithuania before December 2023. Results and observations are being discussed in this first version of the deliverable D2.4. An update including all data from Argentina will be provided in M42 (June 2024).

This document has used a methodology already developed in the deliverable D2.3 of Phy2Climate project (Grant Agreement number 101006912), following EU recommendations. Ad hoc modifications were added to comply with this updated version presenting new activities and related results from the pilot sites in year 2023.

This deliverable D2.4 is prepared according to the previous WP2 partners activities and results gathered in the previous Phy2Climate project steps:

- Deliverable D2.1: Harmonized experimental plan design and monitoring plan.
- Deliverable D2.2: Report on plant growth and phytoremediation capacity optimization.
- Deliverable D2.3: Annual report on phytoremediation performance and monitoring [M24].

The deliverable provides information about progress of the field trials, observations from plant and weather monitoring, phytoremediation performance, as well as encountered challenges. Although each pilot site has its own characteristics (type of soil, type of contaminant, plant species, amendments, climatic conditions, etc.), the 4 pilot site leaders have assessed their progress by evaluating changes in soil parameters and by assessing the biomass output. The deliverable also 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 (TPH) and polycyclic aromatic hydrocarbons (PAH), 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 included landscape and soil preparation activities, seeding and planting, setting up monitoring programme, harvesting of biomass, drying activities and biomass pelletizing. The field trials in all pilot sites were then continued for another year following the same phytoremediation strategy. As it was defined in the Harmonized plan, a certain set of soil parameters must be analysed by every pilot site leader, every year after the end of field trials to evaluate phytoremediation performance and to enable representable comparison of phytoremediation strategies (Deliverable D2.1).

Detailed information of data sets and additional tables and figures, from Spain, Serbia and Argentina are represented in Annex.

For further information on all WPs and project partners the link to the project website: https://www.phy2climate.eu/

3. PHYTOREMEDIATION PERFORMANCE IN FIELD TRIALS

3.1 Objectives

The pilot site validation is 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 per plant variety and remediate the contaminated sites in a rate that results in <20 years for complete site remediation and its rejuvenation to 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. Mainly, the terrain delimitation, pilot site division, soil levelling was performed once in the first year of pilot site trials in 2022 and not repeated the following year. The soil preparation as well as the seeding were programmed and remained according to the local weather conditions and good 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 of 2023. Plant monitoring included such parameters as germination of the seeds, 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, if the station is in a close distance to pilot site. Weather monitoring included parameters that are listed in Table 3.1. However, for the interpretation of the obtained results, mainly air temperature, precipitation and hours of light 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

3.4 Description of harvesting campaign and biomass processing

Pilot sites harvesting campaigns were carried out according to plant species in each pilot site. The biomass harvesting, collection and processing (drying and pelletizing) was performed with the protocols and frequency described in the deliverable 2.1 by the partners involved in each pilot site. All processed biomass after pelletization was or will be sent to Fraunhofer (FRA) for WP3 activities.

The biomass after harvesting was left to dry in the field as much as possible. Later, biomass was carried out to each pilot site facilities for additional drying, but if no additional drying was needed, the biomass was shredded or milled instantly. After that, the biomass was pelletized.

The pellets of energy crops from the pilot sites leaders will be/were shipped to the FRA partner facilities in Sulzbach-Rosenberg (Germany), for biomass conversion in form of oil seeds and/or bulk biomass, according to each pilot site leader preferences. Later, the bulk biomass will be fed to the Thermo-Catalytic Reforming (TCR).

3.5 Description of phytoremediation performance M24-M36

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 Phy2Climate value chain economically valuable. It is important to consider, that biomass amounts directly correspond to biofuel production volumes in WP3.

Soil parameters obtained after the growth of season M24-M36 were compared with the initial characterisation performed in different soil depths at the beginning of the Phy2Climate project and with the 1st year of phytoremediation trials in pilot sites, during M24-M36. To evaluate the effect of phytoremediation, translocation factor for heavy metals, and phytoremediation potential for organic contaminants were calculated according to each pilot site contaminants origin. Finally, biomass output was evaluated and compared to the expectations calculated based on the pot experiments from year 2021 and the pilot site yields from the growth period M24-M36

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 at the first round. 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, some heavy metals) are included in the parameters considered to estimate it. Based on the Klimkowicz-Pawlas et al., 2019, minimal data set (MDS) was comprised of sand, N_{tot}, C_{mic}, C_{org}, humic acids (HU), nitrification potential and dehydrogenase activity. However, since some of the obtained values for MDS for some pilot sites were beyond the ranges given by Klimkowicz-Pawlas et al., 2019, the calculation of SQI was not possible. Therefore, in order to overcome this, main data set was updated by performing principal component analysis (PCA) on the data set obtained by the pilot sites. However, due to the low number of the data sets included in the PCA non representative MDS was obtained. More precisely, this approach non

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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.

justifiably favoured some of measured variables (i.e TPH vs heavy metals) which led to the non-meaningful and non-comparable SQI between the pilot sites. Therefore, the approach for SQI calculation was changed to using total data set (TDS - all available measured variables) based on the Qi et al., 2009[2]. The TDS included Cr, Mn, Fe, Cu, Zn, As, Cd, Pb, organic matter, humins, respiration potential, pH, conductivity, total nitrogen, available phosphorus and potassium, sand, silt, clay, TPH, Ca, Mg, Na, total microbial biomass. The authors established a soil quality grade division based on the total dataset that is presented in Table 3.2). Calculation of the SQI for the pilot sites is in progress.

Table 3.2. Criteria for soil quality grade divisions for TDS

Grade	I	II	III	IV
TDS	≥0.76	0.76-0.66	0.66-0.56	≤0.56

3.7 Summary

The pilot site leaders evaluated that the second year of field tests was carried out successfully, and without major drawbacks. Lessons learned in the first year allowed to achieve higher yields and more effective phytoremediation in the second year. Pot-experiments were also successfully completed, which provided even more knowledge and experience.
Furthermore, an excessive literature review was done towards defining a useful tool to evaluate the efficiency of phytoremediation – soil quality index.
It must be said that in none of the pilot sites has it been possible to achieve that the soil no longer exceeds the maximum permissible concentrations. A third year of field testing is expected to provide more information to predict soil cleanup.

Besides, due to climatic differences, Argentinian pilot site leader is unable to deliver laboratory testing results of soil physicochemical parameters and pollutants (metals and metalloids) from the second harvest by M36. In the Argentinian pilot site, the vegetation of plants for the second cycle of quinoa crop continued till M32 (August 2023) and soil sampling was carried out in M34 (October 2023). The required data of soil parameters from the second cycle of quinoa crop for the Deliverable D2.4 Annual report on phytoremediation performance and monitoring [M36] will be obtained in March-April 2024. The final numbers from Argentinian pilot site will be updated on M42.

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[2] Yanbing Qi, Jeremy L. Darilek, Biao Huang, Yongcun Zhao, Weixia Sun a, Zhiquan Gu in 2009, Evaluating soil quality indices in an agricultural region of Jiangsu Province, China. Geoderma, 149 (3-4), 325-334.

4. FIELD TRIALS ON THE SPANISH PILOT SITE

4.1 Soil preparation and seeding campaign

The Spanish site is located in the north-eastern part of Spain, within the autonomous community of Catalonia (Tarragona), and it belongs to the company Exolum, formerly known as Compañía Logística de Hidrocarburos S.A. (CLH), and a partner in the present project.

Landscape preparation, including excavation to relocate total petroleum hydrocarbon (TPH) contamination to the upper layers of the soil, installation of a geotextile fabric of PEAD to prevent contaminant leaching, and deep tillage, was carried out before the first growing season of *Sorghum* in 2022. The description of these activities is presented in the deliverable 2.3. Similar to 2022, during the period of M19-M36, a smaller excavation campaign was performed prior to the seeding of *Sorghum* in May 2023 (Figure 4.1), again, aiming to have TPH contamination in the upper layers. This time, the excavation vessel was smaller, according to the reduced experimental parcel area that was defined prior to the rapeseed seeding in September 2022 (see below), and it only covered the first meter deep, so it did not affect the geotextile fabric installed in the previous year.

Soil preparation activities before the first growing season are described in the deliverable 2.3. For the growing season of rapeseed, soil preparation activities, including tillage, were carried out in early September 2022, after harvesting the biomass from the first campaign of *Sorghum sp.* Subsequently, *Brassica napus* was sown on the 8th of September 2022 and harvested on the 12th of April, 2023. Preparation for the second growing season of *Sorghum sp.* started in late April 2023 according to good agricultural practices. Besides the small excavation mentioned above, and consequent tillage, no major levelling works were performed. No delays were faced.

Figure 4.1 Excavation works aiming to have TPH contamination in the upper layers (left) and tilling activities (right) perfomed prior to the second growing season of *Sorghum*

Regarding the experimental parcel setup (described in the deliverable 2.3), it was decided to reduce the plot area prior to the seeding of *Brassica napus*, only focusing on the most contaminated sub parcels, which were E1.1, E2.1, E2.2, E4.1, E4.2 (according to the soil characterization of the site before and after the first *Sorghum* campaign). Consequently, the control parcel was also reduced to two sub parcels (C1.1 and C1.2).

In addition, for the second growing season of *Sorghum*, an additional sub parcel, named K1, was implemented. K1 is a small parcel defined within the experimental plot (contaminated soil) but without plants, so that it represents a second control, but with contaminated soil.

The new configuration of the pilot site (5 experimental parcels: E1.1, E2.1, E2.2, E4.1, E4.2 and 3 control parcels: C1.1, C1.2 and K1) will be applied for the rest of the field campaigns within the project, thus, no more results will be obtained for the experimental parcels E1.2, E1.3, E1.4, E2.3, E2.4, E3.1, E3.2, E4.3, E4.4, and the control parcels C2.1 and C2.2 (Figure 4.2).

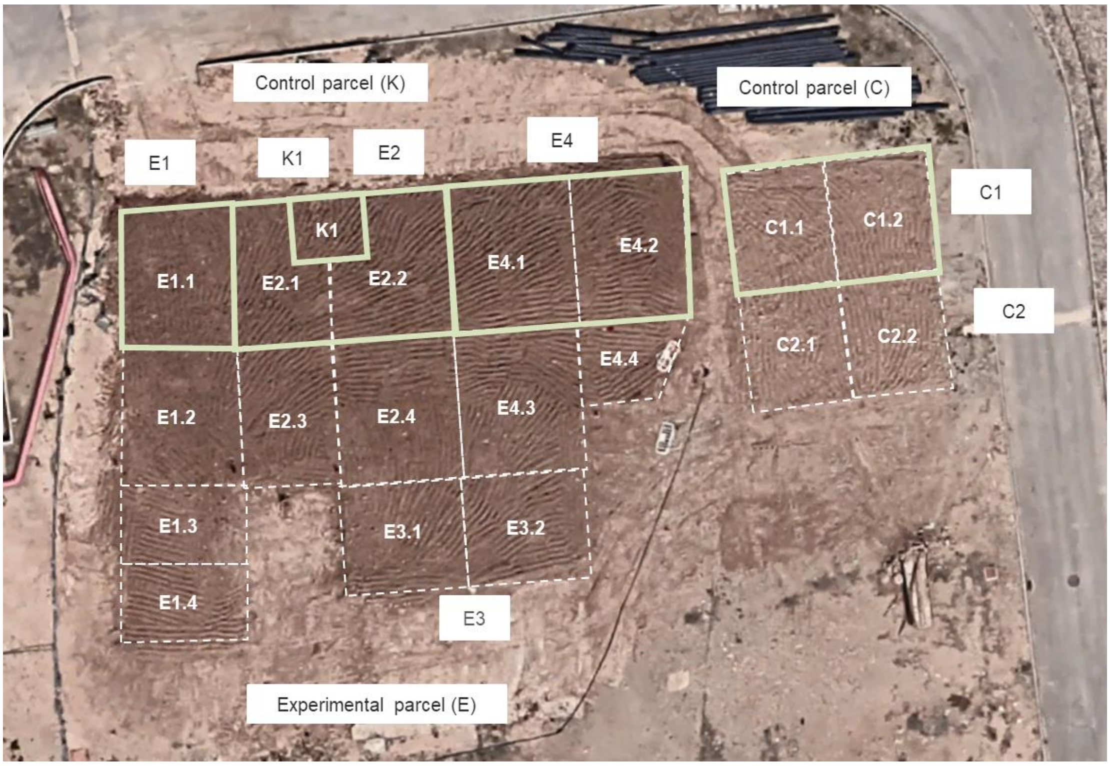


Figure 4.2. New configuration of the Spanish pilot site. The plot area (in green) is now defined by 5 experimental parcels (E1.1, E2.1, E2.2, E4.1, E4.2) and 3 control parcels (K1, C1.1 and C1.2). No more pilot site work will be performed at the other sub parcels

Concerning the application of amendments, the general criteria, that was used for the first growing season of *Sorghum,* has been maintained. Therefore, for the rapeseed (*Brassica napus*) growing season, the amount of compost to apply was determined based on the amount of N needed to reach the biomass production goal of 3000 kg/ha. Moreover, the compost calculation also considered the nutrient deficiency of the soil of the site, so that basic nutrient requirements were also provided by the added compost. The amount of biochar was calculated as 20% volume of the total amount of compost. Both compost and biochar were added and mixed directly on the field, during the tilling activities. Finally, plant growth promoting rhizobacteria (PGPR) was applied according to the fertilization program provided by the supplier (different applications during the 3 months of the phytoremediation strategy).

For the second growing season of *Sorghum*, the biochar dose was increased and the amendment was added also in depth (during the excavation works) in order to improve soil texture and reduce soil compaction. This was decided because, during the first season, it was observed that soil compaction was high. In the framework of the pilot test and the project goals, it was considered important to reduce soil compaction to allow greater vertical root growth (to greater depths) and to potentially increase the overall phytoremediation efficiency applied at the site. For this reason, during the excavation campaign in late April 2023, a biochar dose of 6,7 kg biochar/m^3 of soil was applied. Afterwards, more biochar and compost were applied only in the surface, during tillage, following the general criteria and procedure described above, and the same with PGPR.

4.2 Monitoring program

The monitoring program was defined before the first growing season in 2022 and has been implemented and continued during the second growing season in 2023 both for rapeseed and *Sorghum*. The monitoring program is composed of four parts:

- **Plant monitoring** was carried out every 15 days to evaluate the degree of lushness of plants (luxuriant plants), plant and stem height, nutritional deficiencies, and absence/presence of pests or fungus.
- **Weather monitoring** was carried out through the meteorological station located in Exolum facilities. The station provides hourly data, every day, on air temperature (ºC), relative humidity (%), amount of precipitation (mm), solar radiation (W/cm^2), vapor pressure (hPa), air pressure (hPa), daily global solar exposure (MJ/m^2) average wind speed (m/s), and wind direction (degrees). Also, this meteorological station records data related to water content of the soil (m^3/m^3), an important parameter to aid irrigation decisions, soil electrical conductivity (dS/m) and soil temperature (ºC).
- **Soil monitoring** was carried out via soil sampling at different times during the progress of the field trials. Several physicochemical and biological parameters are included in the monitoring program, such as the concentrations of TPH and metals in soil; soil pH, electrical conductivity, humidity, and organic matter; and microbial biomass in soil, among others. In 2023, the soil monitoring program was expanded to include different soil horizons (depths). These will be described in more detail further below.
- **Harvested biomass characterization** was carried out after the harvest of each crop, and includes the weight of the produced biomass and, for Sorghum, the analysis and characterization of the biomass and pellet produced. These will be described in more detail further below.

4.3 Plant development

The aim of the plant monitoring was to follow and register the height, lushness (values given in points, with a maximum of 9), and development stage (9 stages for *Sorghum*, 18 stages for rapeseed) of plants. Also, visual assessments were made to detect and/or prevent nutritional deficiencies, pests, or diseases.

For the current growing season, rapeseed was monitored once a month (Figure 4.3) for six months (September 2022 – May 2023), while *Sorghum* was monitored every 15 days also for six months (May 2023 – October 2023). The results are described below. Results of the first growing season of *Sorghum* in 2022 are included and described in the deliverable 2.3.

Rapeseed

This crop was sown in early September 2022 and, after several weeks, it was observed that germination was really poor and was not homogeneous throughout the pilot site. This was linked to heavy rainfall during the first days of the growing season, which is common in that geographical area and might have prevented the adequate germination of seeds.

After six months (Figure 4.3), it was observed that the specimens established in the control (C1) and E1.1, E2.1 and E2.2 experimental parcels were the most luxuriant (Figure 4.4), reaching maximum average heights of 68 cm (C1), 60 cm (E1.1), and 51 cm (E2.1 and E2.2). In parcels E4.1 and E4.2, the specimens were shorter than in the parcels mentioned above, with heights between 23 – 21.5 cm. Moreover, in parcels E4.1 and E4.2, most of the specimens were small and showed a medium lushness.

Figure 4.3. The development of rapeseed (*Brassica napus*) during the growing season of 2023

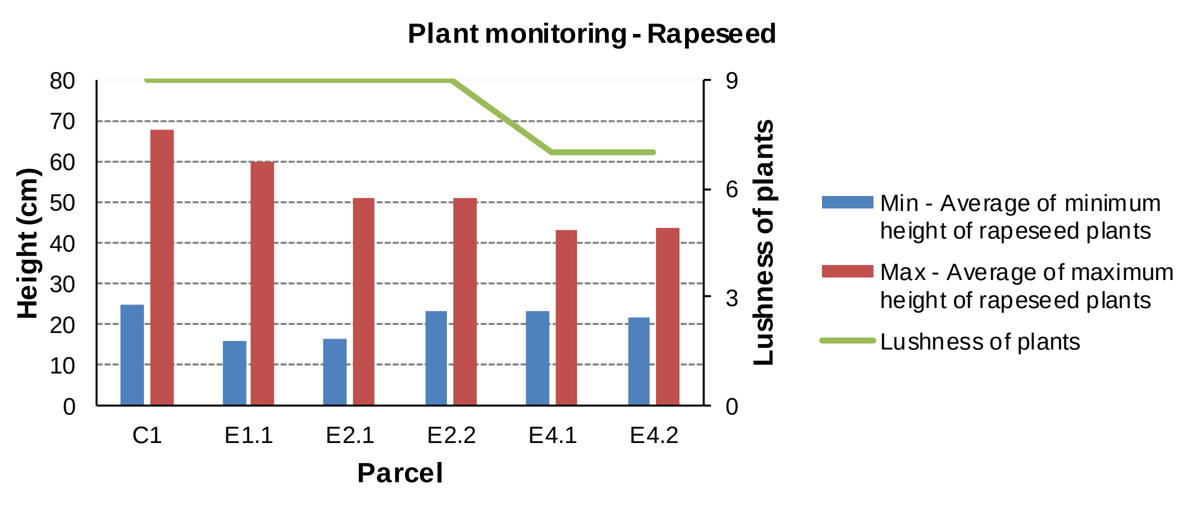


Figure 4.4. Height and lushness of rapeseed (*Brassica napus*) during the growing season of 2023

As it can be seen in the Figure 4.5, rapeseed specimens were able to grow, but they turned out to be small and showed a medium lushness of plants. Moreover, the roots did not grow into depth, which could be a sign of intolerance to TPH in soils and low phytoremediation efficiency.

Figure 4.5. Growth pattern of rapeseed (*Brassica napus*) roots during the growing season of 2023

Finally, given that the heavy rainfalls period at the site coincides with the sowing season of rapeseed, and that it is considered that such intense precipitation affects the germination, and that the amount of biomass required to reach the project objectives for each year is sufficiently fulfilled with the annual *Sorghum* harvest, it was decided that a rapeseed growing season after the harvest of *Sorghum* was not necessary anymore. Therefore, no more rapeseed will be grown for the pilot trials at the Spanish pilot site.

Sorghum

This crop was sown in early May 2023 and, due to the poor quality of the seeds used (which was detected later on), the pilot trial showed weak germination and poor biomass output in some parcels, compared to the previous growing season of 2022. Also, pesticides were used around the pilot site area, which resulted in plant damage, especially in the control parcel C1. For this reason, a second seeding campaign of *Sorghum*, only in the control parcel C1, was performed in mid-July 2023, aiming to increase biomass output at this parcel.

After six months of *Sorghum* monitoring, it was observed that the specimens established in E1.1 and E4.2 were the most luxuriant and all had panicles, reaching average heights of 147.1 cm, and 146.7 cm, respectively (Figure 4.6).

However, despite the poor quality of seeds mentioned above, the specimens established in E2.1, E2.2, and E4.1 were the most luxuriant and all had panicles, reaching similar heights values (between 141.5 and 151 cm) to those recorded in 2022. Finally, comparing both years, there is a clear trend towards increased growth and height in all parcels, except the control, due to pesticide damage to the plants. It is possible that the reason behind the increased growth and height observed in all experimental parcels is due to the combination of the following factors:

- The increased biochar presence in soil (improvement of soil texture, less soil compaction).

- The overall ecological richness of the soil rhizosphere (compared to the first growing season).
- The overall lower TPH concentration detected in soils even with the new excavation campaign (compared to the first growing season).

In terms of maximum values, in 2022, the parcels with the highest height values were C1 (205 cm) and E1.1 (220 cm), while, in 2023, the parcels with the highest height values were E1.1 (212 cm), E2.1 (205 cm) and E2.2 (224 cm). Comparing the data relative to the maximum average height in both years, in 2023 there was a tendency to exceed or establish a maximum height of around 200 cm in all the studied parcels except for the control C1, whereas in 2022 most of the specimens presented a height of around 150 cm (Figure 4.6). As mentioned above, it is possible that the reason behind the increased growth and height observed during the 2023 season is due to the increased biochar dose in soil, the overall ecological richness of the soil rhizosphere, and the overall lower TPH concentration detected in soils.

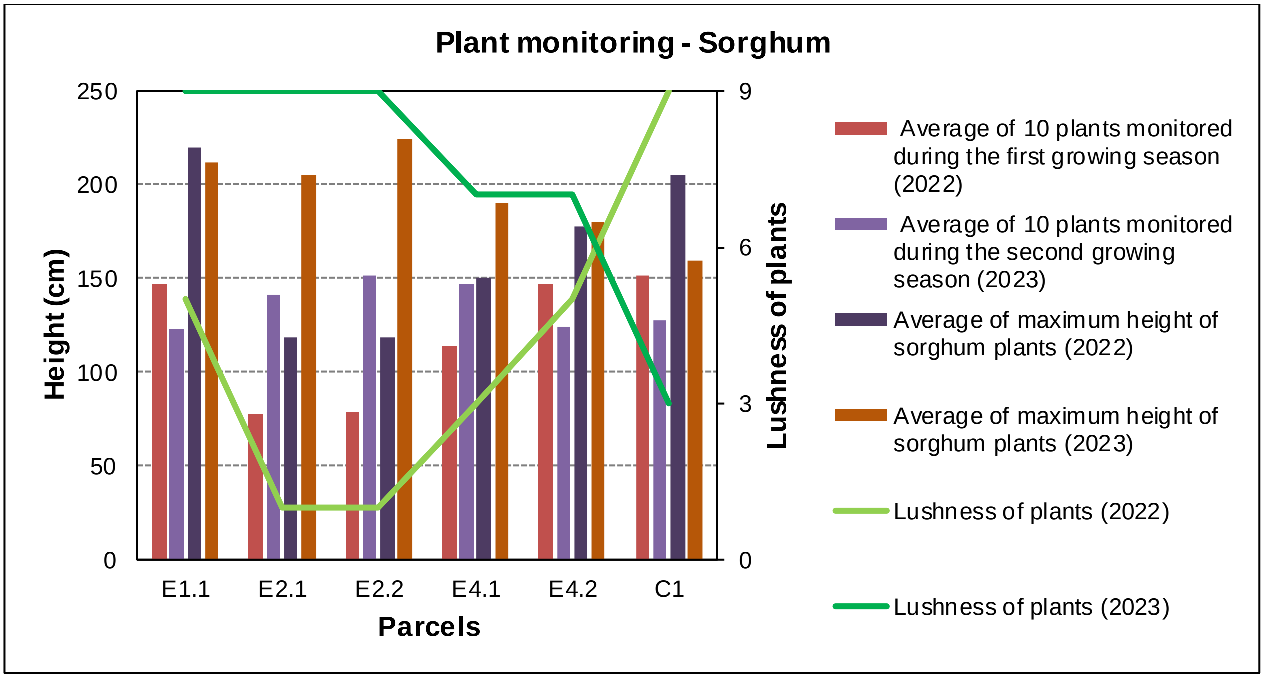


Figure 4.6 The average height and the lushness of *Sorghum* plants during the field trials in 2022 and 2023

In addition, signs of pest and/or disease were observed during the field trials, both in 2022 and 2023. During the vegetation phase, *Sorghum* was monitored carefully for pest occurrence. In mid-July 2022 and 2023, occurrence of aphids and whiteflies was detected, so the crops were treated with the phytosanitary products Tromin Oil (300 ml/100 L) and Bijap (500 ml/100 L).

Figure 4.7. The development of *Sorghum* during the second growing season in 2023

Lastly, it is important to notice that some *Sorghum* plants that were not harvested in September 2022 to test for possible re-germination, actually started to re-germinate during autumn, probably due to the unusually high temperatures observed during the months of September, October, and November 2022.

4.4 Environmental conditions

Concerning weather monitoring, a meteorological station collecting meteorological parameters established in the common framework (Deliverable 2.1, Table 3.8) is placed at the site together with sensors to detect water content, electrical conductivity, and temperature in soil. The data are collected hourly (then averaged on 10 days) and sent remotely to LEITAT's facilities.

Precipitation. According to the precipitation data, September of 2022 and February of 2023 were the months with the largest amounts of cumulative precipitation (189.2 mm and 95.3 mm, respectively), while the least rain fell during April 2023 (only one rainy day, 0.4 mm). Analysing the cumulative precipitation during 2022 and 2023, it can be concluded that both growing seasons (rapeseed and *Sorghum*) occurred under a dry period, with the exception of September of 2022 and February of 2023.

On the other hand, it is important to notice that the heavy rainfalls recorded in September 2022 coincided with the sowing season of rapeseed (Figure 4.8). It is considered that such intense precipitation affected the germination of *Brassica napus*. A replanting strategy was not considered here because, when the rainy season ended, the optimal time for rapeseed sowing had already passed. Moreover, the amount of biomass required to reach the project objectives for the season had been already obtained in the first growing season of *Sorghum*, so there was no need of extra rapeseed biomass.

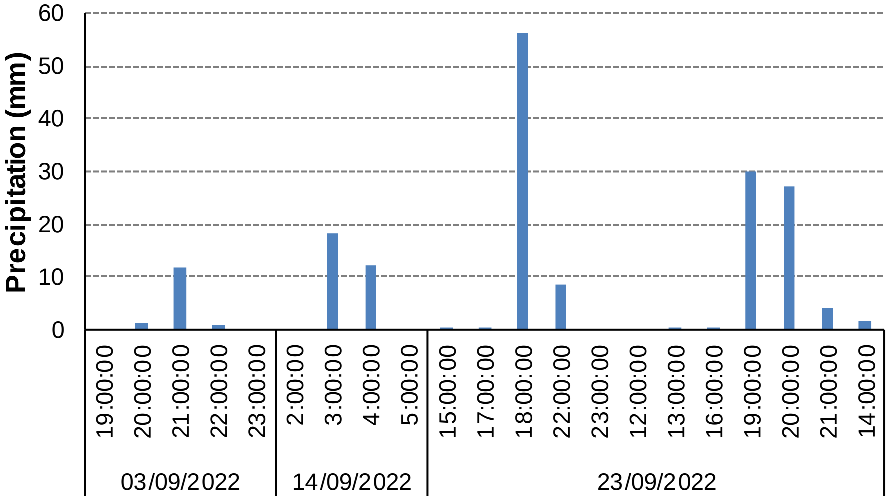


Figure 4.8. The abundance of precipitation registered by the weather station installed in the Exolum facilities during September 2022

Air temperature. According to the air temperature data (Figure 4.9), the warmest month was August (both in 2022 and 2023), with the average maximum daily air temperature of 30.1 ºC in august 2022 (14/08/22) and 29.5 ºC in August 2023 (02/08/23). On the other hand, regarding the minimum air temperature, the coldest month was recorded in January 2023, with an average minimum daily air temperature of 5.24 ºC (25/01/23).

It is important to notice that some *Sorghum* plants that were not harvested in September 2022 to test for possible re-germination, actually started to re-germinate during autumn 2022, probably due to the unusually high temperatures observed during the months of September, October, and November 2022.

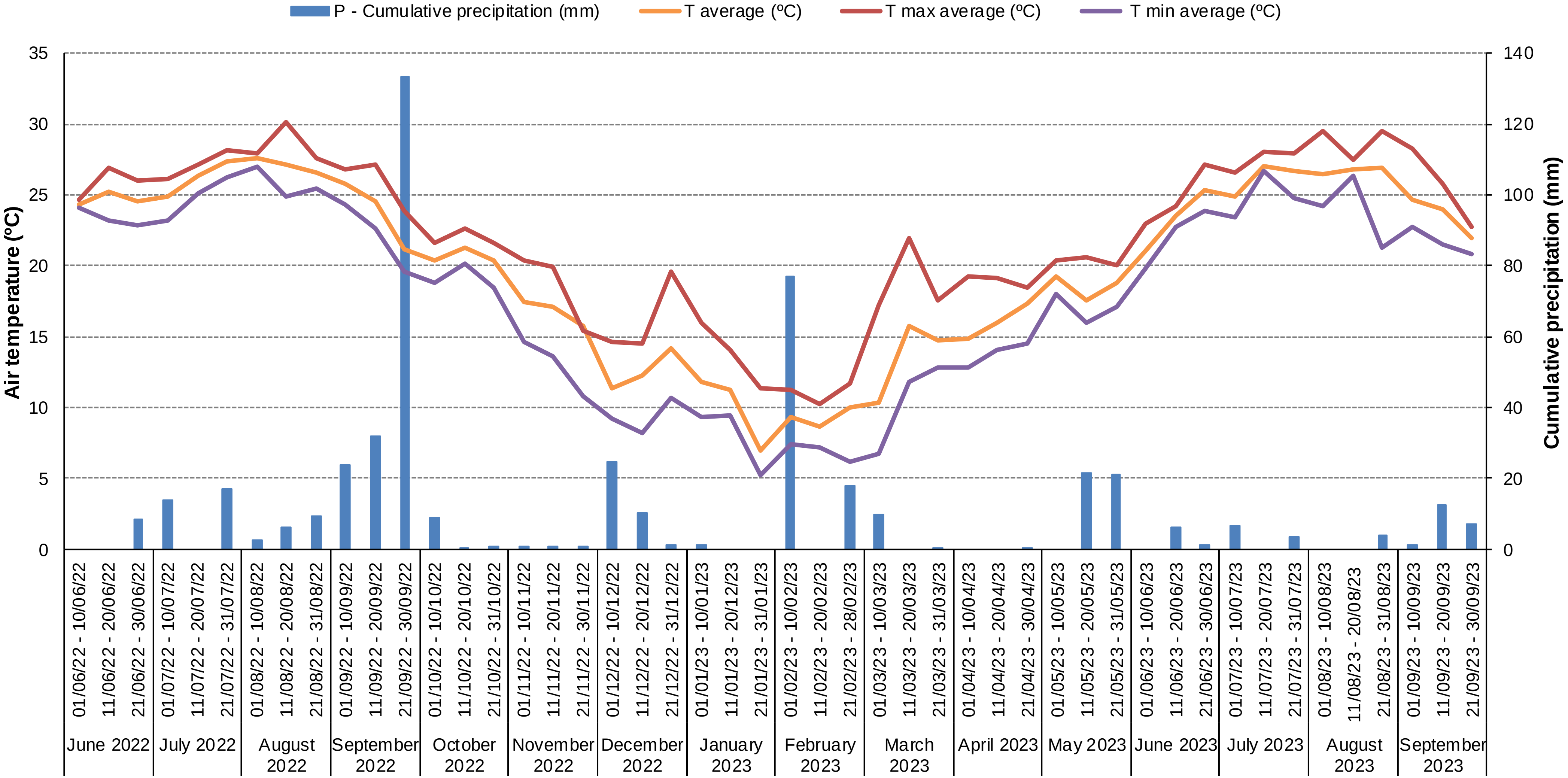


Figure 4.9 Average of maximum, minimum and cumulative precipitation during the second growing season

4.5 Harvest and pelletizing

Harvest: The first *Sorghum* harvesting campaign was carried out between 31/08/22 and 02/02/22, and the second *Sorghum* harvesting campaign was carried out between 09/1023 and 10/10/23, while the *Brassica napus* harvesting campaign was carried out on 04/05/234

Sorghum biomass was collected all at once (stems+leaves+roots+seeds), whereas the seeds of *Brassica napus* were collected manually first, and then, the rest of the aboveground and belowground biomass was collected (leaves, stems, and roots). In all three harvesting campaigns, the number of plants per parcel was counted, and plants were rolled into bales for faster drying. Almost all biomass was air-dried in the Exolum facilities.

Pelletization: *Sorghum* biomass (first growing season, 2022) was shipped for pelletization at an external facility. Prior to pelletizing, the biomass was completely dried and shredded to the size of 4-5 mm.

An initial biomass characterization was performed, in which water content, ash, elemental carbon, hydrogen and nitrogen, sulphur and chlorine, and oxygen content were analysed. Results for the *Sorghum* biomass characterization is presented in Table 4.1.

Table 4.1. Initial *Sorghum* biomass (2022) characterization prior to pelletizing

Parameter	Method	Value	Units
Total moisture	UNE-EN ISO 18134-1:2016	13.7±0.3	%m/m
Ash	UNE-EN ISO 18122:2016	23.9	%m/m dw
Carbon	UNE-EN ISO 16948:2015	31.4	%m/m dw
Hydrogen		3.9	%m/m dw
Nitrogen		0.56±0.08	%m/m dw
Sulphur	UNE-EN ISO 16994:2017	0.096±0.032	%m/m dw
Clorine		0.354	%m/m dw
Oxygen	Calculated	39.8	%m/m dw

Afterwards, several trials were performed to ensure the pellet quality requested by WP3. In particular, the investigated variable was the compression ratio of the matrix, with two compression ratios tested: 1:4 and 1:6 (Table 4.2 and Table 4.4). For each of those, mechanical durability, apparent density, total humidity, and size of the pellets obtained was determined (Table 4.3 and Table 4.5). The compression ratio that achieved the best results was later used to pelletize the total amount of dry *Sorghum* biomass, which was then shipped to WP3.

Table 4.2. Pelletizing conditions for the 1:4 compression ratio test for *Sorghum* biomass (2022)

Parameter	Description
Compression ratio	1:4
Pellet diameter	6 mm
Working pressure	20-25 bar
Matrix temperature	65-70 ºC

Sample moisture	14 % approx. (as received)

Table 4.3. Results obtained in the 1:4 compression ratio test for *Sorghum* biomass (2022)

Parameter	Method	Value	Units
Total moisture	UNE-EN ISO 18134-1:2016	11.9±0.3	%m/m
Bulk density	UNE-EN ISO 17828:2016	560±40	kg/m^3
Mechanical durability of pellets	UNE-EN ISO 17831:2016	94.4±0.8	%m/m
Diameter	-	6.2±0.3	mm
Length	-	18±5	mm

Table 4.4. Pelletizing conditions for the 1:6 compression ratio test for *Sorghum* biomass (2022)

Parameter	Description
Compression ratio	1:6
Pellet diameter	6 mm
Working pressure	20-25 bar
Matrix temperature	65-70 ºC
Sample moisture	20 % approx.

Table 4.5. Results obtained in the 1:6 compression ratio test for *Sorghum* biomass (2022)

Parameter	Method	Value	Units
Total moisture	UNE-EN ISO 18134-1:2016	15±0,3	%m/m
Bulk density	UNE-EN ISO 17828:2016	640±40	kg/m^3
Mechanical durability of pellets	UNE-EN ISO 17831:2016	98,1±0,4	%m/m
Diameter	-	6,2±0,3	mm
Length	-	20±2	mm

Figure 4.10 shows drying process of the biomass in the warehouse available at Exolum facilities, the pilot plant in which the biomass was treated and pelletized, and the results of the two tests performed with the different matrix compression ratios 1:4 and 1:6.

Figure 4.10. Drying process of biomass (upper left). Biomass treatment and pelletization plant (upper right). Sorghum biomass (2022) pellets obtained with a compression ratio of 1:4 (lower left). Sorghum biomass (2022) pellets obtained with a compression ratio of 1:6 (lower right).

Given the quality requirements set by WP3 (Table 4.6), the compression ratio that met all of them was 1:6, therefore, all the biomass was treated and pelletized following this methodology. In the table below, the quality requirements set by WP3 and the final results obtained through the pelletizing process are presented and compared.

Table 4.6. Quality requirements set and results obtained in the pelletization of *Sorghum* biomass (2022) with the compression ratio of 1:6

Parameter	Range (min-max)	Target value	Result
Diameter	4 – 10 mm	6 mm	6,2 mm
Length	4 – 50 mm	25 mm	20 mm
Moisture	10 – 30 wt%	15 wt%	15,0 %
Mechanical strength according to ISO 17831-1	>95m %	>98m %	98.1 %

Based on all of the above, the biomass obtained during the second growing season of Sorghum (2023) will be treated and pelletized following the above-described methodology to ensure the quality requirements of the pellets produced. This is expected to be completed in the coming weeks/months; therefore, these results will be presented in the subsequent versions of this document, or in the final deliverable of the project.

4.6 Phytoremediation performance

The phytoremediation performance and soil sampling protocol were described in detail in the deliverable 2.3.

For the second growing season of *Sorghum* (May – October 2023), soil monitoring was expanded to include soil samples at different depths (surface (0 cm), 30 cm and 60 cm). Similarly, the intermediate sampling campaigns were expanded as well, to also include the analysis of basic physicochemical parameters of soil (pH, electrical conductivity, humidity, and organic matter) and metal contamination in soil. Thus, the new soil monitoring program allows a better assessment of the phytoremediation performance with time, and at different depths. The expansion of the soil monitoring program also includes the performance of additional molecular analyses (microbial biomass and massive sequencing) in order to assess the effect of the phytoremediation strategy on the population of soil microorganisms. Such an expanded monitoring program was implemented in order to understand the different transformation processes affecting TPH content in soil

In all cases and sampling campaigns, composite-soil samples comprised of a minimum of 4 sub-samples were collected according to the harmonized plan. It should be noted that, in the non-contaminated soil control parcels, samples were also collected in order to compare the results with the contaminated plots.

In the current version of this document (Deliverable 2.4) only the results regarding the samples collected at a depth of 30 cm will be presented. The results associated to the samples collected at both depths 0 and 60 cm will be presented in the subsequent versions of this document, or in the final deliverable of the project.

4.6.1 Soil parameters

General soil parameters. As per the soil monitoring program, general soil physicochemical parameters were analysed during the field trials. Some parameters were only measured before (pre-sowing) and after (post-harvesting) the growing season of each crop, according to the harmonized plan agreed within the project, while other parameters were measured several times within a growing season, to have the evolution of such parameter with time.

The monitored parameters include texture, pH, electrical conductivity (EC), water content (humidity), organic matter (OM), humins, total C, total N, P available, K available, P total, K total, Mg, Ca, Cu, Mo, Zn, Cd, Cr, Pb, S, B, As, Na, Cu, Ni, Mn, Fe, and microbial biomass.

Rapeseed

For the only rapeseed growing season, the monitoring plan was not yet expanded, so physicochemical results are only available for the pre-sowing and post-harvesting sampling events.

In the Annex, Tables from 9.1 to 9.8 present the soil parameters before (pre-sowing) and after (post-harvesting) the field trial for the only growing season of rapeseed. Initial samples were collected in September 2022, while the other set of samples were collected in April 2023, right after the harvest of rapeseed.

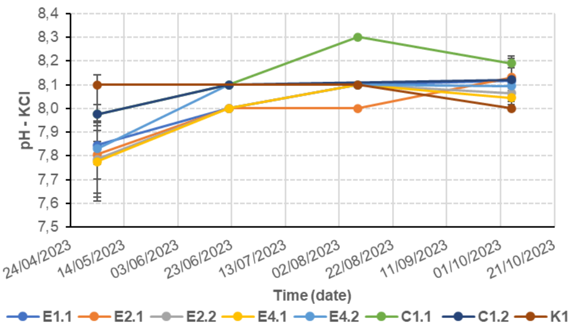
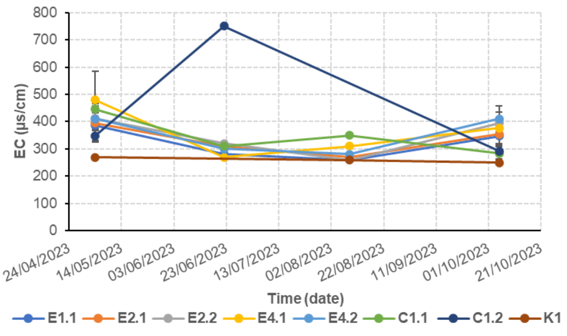
General physico-chemical parameters of contaminated soil did not have significant differences within different soil parcels, thus are described together. Analysis showed that before the first growing season of rapeseed compared to the end of the first growing season of rapeseed:

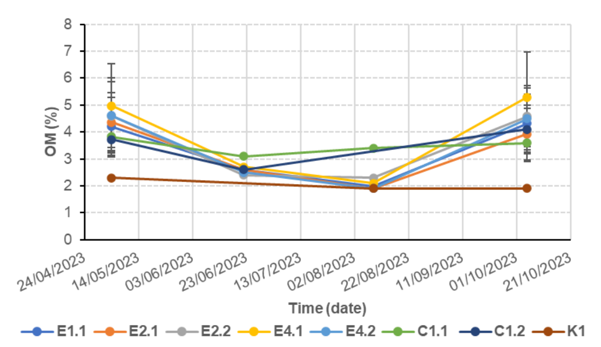
- pH$_{(H2O)}$, pH$_{(KCl)}$ and electrical conductivity (EC) mean values remained constant. pH average values remained around to 8.2 (basic values) while EC average values remained around to 400 µS/cm (non-saline soil).
- Water content (moisture) increased to 15%. This could be due to the increased volume of biochar applied in the field.
- Organic matter and total C average values remained constant at around 5%. However, regarding to total N averagevalues, a slight decrease was observed.
- Available P decreases from10 to 28 mg/kg dw while K available average values remained constant (between 109 to 174 mg/kg dw).
- Magnesium (Mg) and calcium (Ca) average values decreased. However, these are still high values and are normal in basic soils.

Sorghum

In the Annex, Tables from 9.9 to 9.18 present the soil parameters before (pre-sowing) and after (post-harvesting) the field trial for the second growing season of *Sorghum* (2023). Initial samples were collected in May 2023, while the other set of samples were collected in October 2023, right after the harvest of *Sorghum*. During the vegetative phase of *Sorghum*, the soil was also monitored by collecting intermediate samples at different depths (surface, 30 cm and 60 cm) as mentioned in the previous section. However, in the current version of the document, only the results at a depth of 30 cm are included.

Figure 4.11 presents the evolution with time of pH$_{(KCl)}$, electrical conductivity (EC) and organic matter (OM, in %).

<table>
<tr><th>Evolution of pH$_{(KCl)}$ at 30 cm – *Sorghum* 2023</th><th>Evolution of EC at 30 cm – *Sorghum* 2023</th></tr>
<tr><td>
</td><td>
</td></tr>
<tr><th>Evolution of OM at 30 cm – *Sorghum* 2023</th><td></td></tr>
</table>



Note: the initial (pre-sowing) and final (post-harvesting) results include several sample replicates, thus, data points show the error bars from replicate measurements. Intermediate results from the intermediate sampling events along the field trial correspond to single samples, thus, no error bars are shown. In all cases, analysed soil samples were composite-soil samples comprised of a minimum of 4 sub-samples collected from each studied parcel.

Figure 4.11. Results for the evolution with time of pH(KCl), electrical conductivity (EC) and organic matter (OM) at a depth of 30 cm during the second growing season of Sorghum (2023)

From the obtained results, it can be observed that the general physicochemical parameters of the studied parcels did not show significant differences among them, except for controls K1, C1.1 and C1.2. The most significant conclusions are summarized below:

- pH$_{(H_2O)}$ and pH$_{(KCl)}$ values remained generally the same, with pH$_{(KCl)}$ being between 7.8 and 8.3 (basic values). However, a general tendency of increase of pH$_{(KCl)}$ is observed in Figure 4.11, especially in the contaminated sub parcels, which generally started with a lower pH$_{(KCl)}$ value. The only parcel with a slight pH$_{(KCl)}$ decrease was K1, which did not have plants.
- Electrical conductivity (EC) average values also remained generally the same, with mean values around 400 µS/cm (non-saline soil). However, small variations were observed throughout the season, except for parcel K1 (without plants), which was the only one without changes.
- Organic matter (OM) also evidenced small variations throughout the season, with experimental parcels showing more changes than the controls. In general, experimental parcels show greater OM content, followed by non-contaminated control parcels, and, lastly, the contaminated control parcel K1 (without plants) is the only one without changes and the lowest OM content.
- Total C average values remained constant at around 5%. However, regarding total N mean values, a slight decrease was observed.
- Water content (moisture) increased to 15%. This could be due to the increased volume of biochar applied in the field.
- Available P decreased from 10 to 28 mg/kg dw, while K available average values remained constant (between 109 and 174 mg/kg dw).
- Magnesium (Mg) and calcium (Ca) average values decreased. However, these are still high values and are normal in basic soils.

In conclusion, the presence of plants increases the organic matter content in the soil. The fluctuation of the different soil parameters can be due to typical soil processes.

Soil contamination parameters

As per the soil monitoring program, soil contamination parameters were analysed during the field trials. In this case, all parameters were measured before (pre-sowing), during, and after (post-harvesting) the growing season of each crop, in order to have the evolution of each parameter with time. However, only the initial (pre-sowing) and final (post-harvesting) results include several sample replicates, and the intermediate results from the intermediate sampling events along the field trials correspond to single samples only. In all cases, analysed soil samples were composite-soil samples comprised of a minimum of 4 sub-samples collected from each studied parcel.

The monitored parameters include total petroleum hydrocarbons (TPH) and metals in soil and in plant tissue.

All samples analysed for TPH concentrations in soil, and the initial (pre-sowing) and final (post-harvesting) samples analysed for metals in soils, were analysed at the laboratory, according to the harmonized plan.

However, the evolution of metal contamination in soils with time was obtained through an XRF detector, which is an X-Ray fluorescence analyser, with a precision of ppm (equivalent to mg/kg). The analysis process of the selected metals is carried out in a single reading and is based on the USEPA method “SW-846 Test Method 6200: Field Portable X-Ray Fluorescence Spectrometry for the Determination of Elemental Concentrations in Soil and Sediment”.

As described earlier, for the second growing season of *Sorghum* (May – October 2023), soil monitoring was expanded to include soil samples at different depths (surface (0 cm), 30 cm and 60 cm). Similarly, the intermediate sampling campaigns were expanded as well, to also include the analysis of basic physicochemical parameters of soil and metal contamination in soil. In addition, in the current version of this document (Deliverable 2.4) only the results regarding the samples collected at a depth of 30 cm will be presented.

Therefore, due to the above, the second growing season of *Sorghum* has more results than the only growing season of rapeseed, and all of them are presented below.

Rapeseed

For the only rapeseed growing season, the monitoring plan was not yet expanded, so TPH results are only presented for the pre-sowing and post-harvesting sampling events, and the metal contamination evolution is not available. Furthermore, results are expressed per parcel (and not sub parcel).

Tables 4.7, 4.8 and 4.9 present the results for TPH concentrations before (pre-sowing) and after (post-harvesting) the field trial for the only growing season of rapeseed. Initial samples were collected in September 2022, while the other set of samples were collected in April 2023, right after the harvest of rapeseed.

Table 4.7. Pre-sowing TPH concentrations in soil at 30 cm (rapeseed)

Parcel	TPH C5-C10	TPH C10-C40	TPH C5-C35	BTEX	MTBE	ETBE	HAP 16 EPA
	mg/kg dw	*mg/kg dw*	*mg/kg dw*	*mg/kg dw*	*mg/kg dw*	*mg/kg dw*	*mg/kg dw*
E1	17±6	114±36	131	2±2	<0,020	<0,050	0,3±0,2
E2	14±10	116±23	130	<0,25	<0,020	<0,050	<0,16
E4	10±5	128±11	138	<0,25	<0,020	<0,050	<0,16
C1	9±4	138±62	147	<0,25	<0,020	<0,050	4,0±0,6

Table 4.8. Post-harvesting TPH concentrations in soil at 30 cm (rapeseed)

Parcel	TPH C5-C10	TPH C10-C40	TPH C5-C35	BTEX	MTBE	ETBE	HAP 16 EPA
	mg/kg dw	*mg/kg dw*	*mg/kg dw*	*mg/kg dw*	*mg/kg dw*	*mg/kg dw*	*mg/kg dw*
E1	25±37	93±39	118	3±5	<0,020	<0,050	0,6±0,9
E2	80±104	279±140	359	12±24	<0,020	0,06±0,03	2±2
E4	42±45	250±208	293	4±5	0,022±0,004	0,06±0,02	2±2
C1	9±3	78±13	87	<0,25	<0,020	<0,050	5±2

Table 4.9. TPH removal in % during the only growing season of rapeseed.

Parcel	TPH removal
	%
E1	10
E2	*
E4	*
C1	41

Note: * indicates no TPH removal, but that an increase of TPH concentrations was detected in these parcels.

As it is shown in the tables above, the results obtained for TPH concentrations in soil show great variability and a high standard deviation between replicate samples, which evidences the heterogeneity of the soil and the uneven distribution of TPH in soils. Moreover, TPH concentrations in soil were low from the beginning of the season and significant changes were not observed after harvesting.

In order to assess metal contamination in soil, the results obtained at the laboratory for metals in soil for the rapeseed season can be compared with the General Reference values used to assess soil contamination in Catalonia and established in the DL 1/2009. Table 4.10 shows the comparison between the designated reference values for industrial, urban, agricultural and forestry use those obtained in the field.

Table 4.10. Metal and metalloid concentrations in soil at 30 cm (rapeseed)

Element	Industrial use	Urban use	Agricultural and forestry use	Pilot site maximum values
	mg/kg dw	*mg/kg dw*	*mg/kg dw*	*mg/kg dw*
Rapeseed 2022				
Arsenic (As)	30	30	30	8±1 (E4.2)
Cadmium (Cd)	55	5.5	2.5	<LQ
Copper (Cu)	1000	310	90	20±1 (E4.2)
Molybdenum (Mo)	70	7	3,5	<LQ
Nickel (Ni)	1000	470	45	15±1 (E2.2)
Lead (Pb)	550	60	60	80±7 (E4.2)
Zinc (Zn)	1000	650	170	62±4 (E2.2)

Currently the study area is designated for an 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, the studied soils do not exceed the limits established for the protection of ecosystems or health except lead (Pb). Pb exceeds the limit concentrations for health protection for urban and agricultural and forestry use (60 mg/kg) in all parcels with a maximum value of 80±7 mg/kg in parcel E4.2.

Given the above results, and also considering the field observations gathered for rapeseed, that the heavy rainfalls period at the site coincides with the sowing season of rapeseed, that it is considered that such intense precipitation affects its germination, and that the amount of biomass required to reach the project objectives for each year is sufficiently fulfilled with the annual Sorghum harvest, it was decided that a rapeseed growing season after the harvest of *Sorghum* was not necessary anymore. Therefore, no more rapeseeds will be used for the pilot trials at the Spanish pilot site.

Sorghum

For the second growing season of *Sorghum*, the monitoring plan was already expanded, so both TPH and metal contamination results are presented as an evolution with time. Furthermore, results are expressed per sub parcel and the new control K1 is also included.

Figures 4.12* and 4.13* present the results for the evolution with time of TPH and metal concentrations, respectively. Initial samples were collected in May 2023, while the final set of samples were collected in October 2023, on the same week of harvest.

*Note: initial (pre-sowing) and final (post-harvesting) results include several sample replicates, thus, data points show the error bars from replicate measurements. Intermediate results from the intermediate sampling events along the field trial correspond to single samples, thus, no error bars are shown. In all cases, analysed soil samples were composite-soil samples comprised of a minimum of 4 sub-samples collected from each studied parcel.

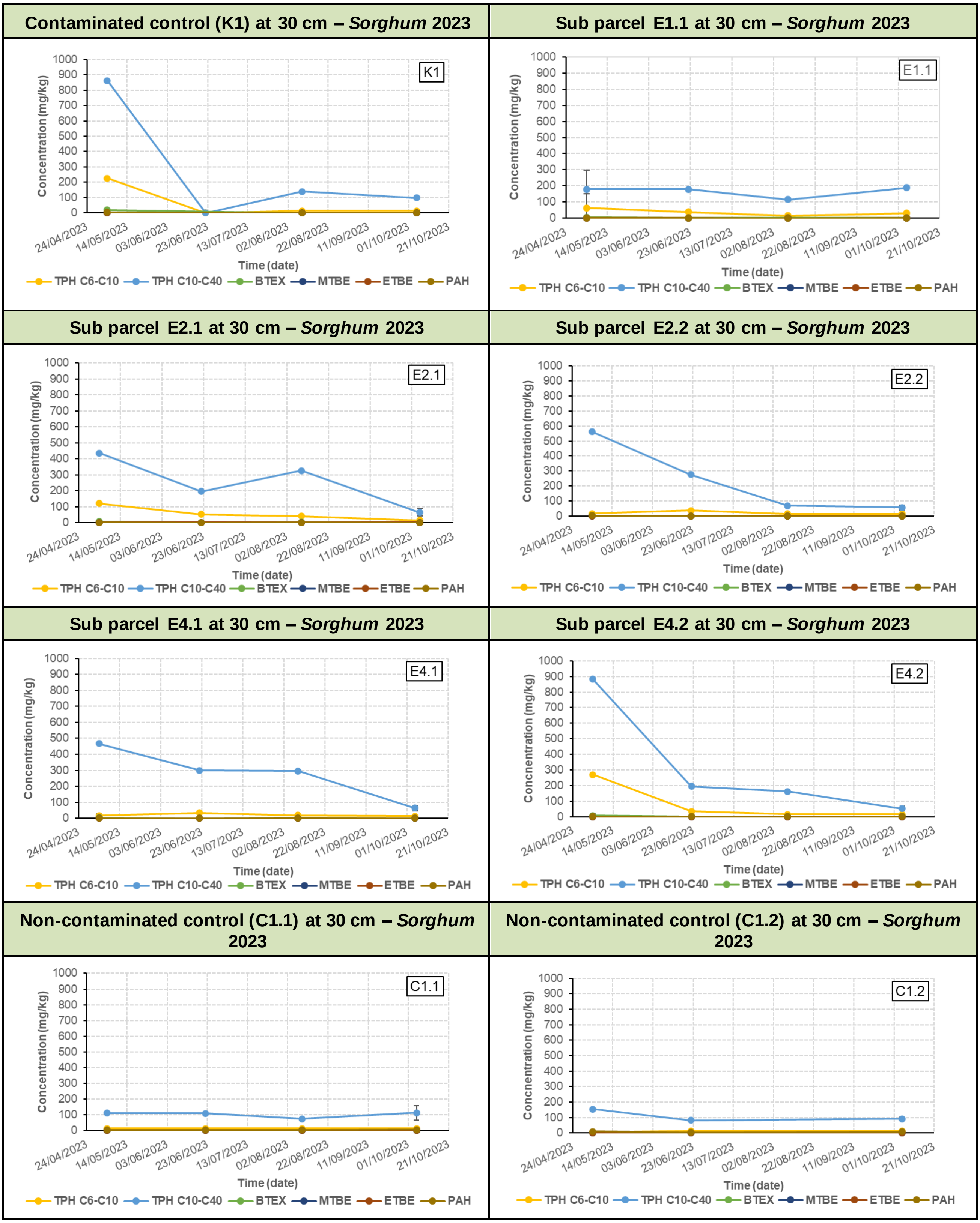
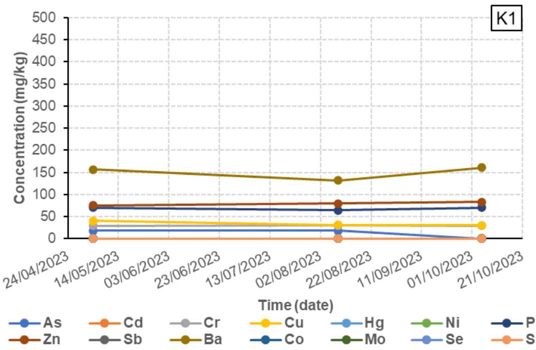
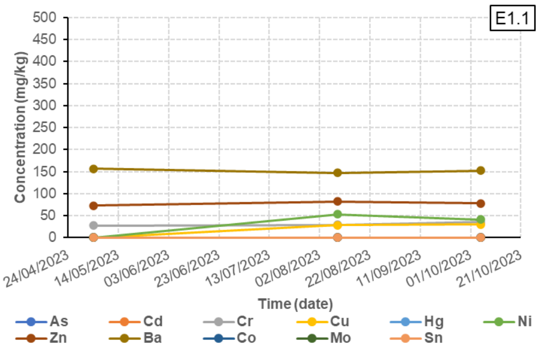

Contaminated control (K1) at 30 cm – *Sorghum* 2023
Sub parcel E1.1 at 30 cm – *Sorghum* 2023
Sub parcel E2.1 at 30 cm – *Sorghum* 2023
Sub parcel E2.2 at 30 cm – *Sorghum* 2023
Sub parcel E4.1 at 30 cm – *Sorghum* 2023
Sub parcel E4.2 at 30 cm – *Sorghum* 2023
Non-contaminated control (C1.1) at 30 cm – *Sorghum* 2023
Non-contaminated control (C1.2) at 30 cm – *Sorghum* 2023

Figure 4.12*. Evolution with time of TPH concentrations in soil (at 30 cm depth) during the second growing season of Sorghum (2023)

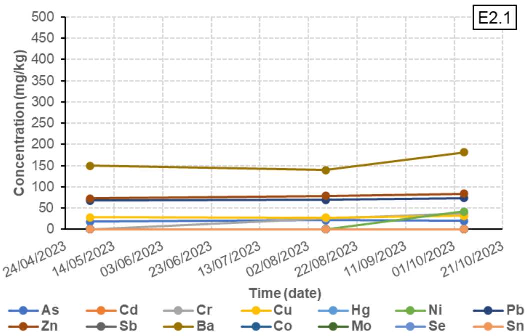
Contaminated control (K1) at 30 cm – *Sorghum* 2023



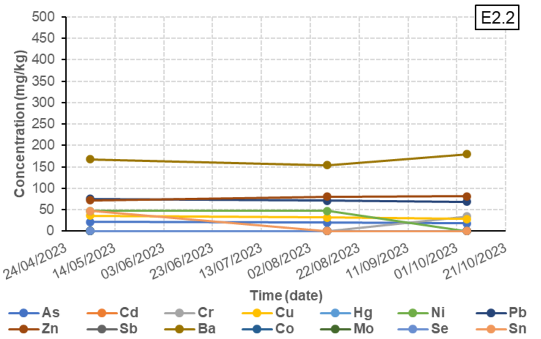
Sub parcel E1.1 at 30 cm – *Sorghum* 2023



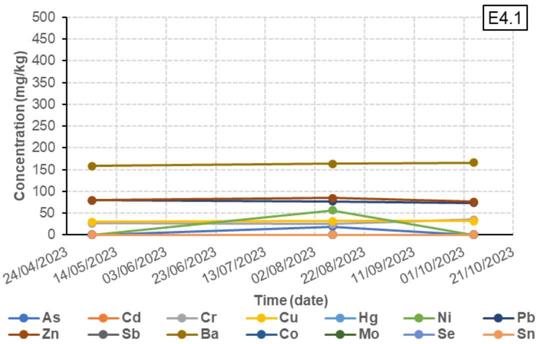
Sub parcel E2.1 at 30 cm – *Sorghum* 2023



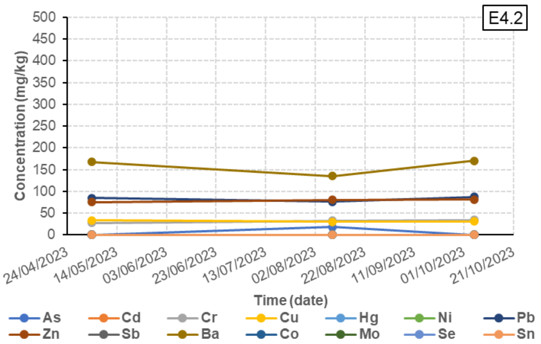
Sub parcel E2.2 at 30 cm – *Sorghum* 2023



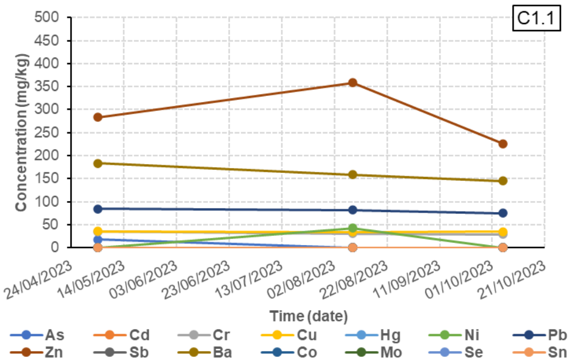
Sub parcel E4.1 at 30 cm – *Sorghum* 2023



Sub parcel E4.2 at 30 cm – *Sorghum* 2023



Non-contaminated control (C1.1) at 30 cm – *Sorghum* 2023



Non-contaminated control (C1.2) at 30 cm – *Sorghum* 2023

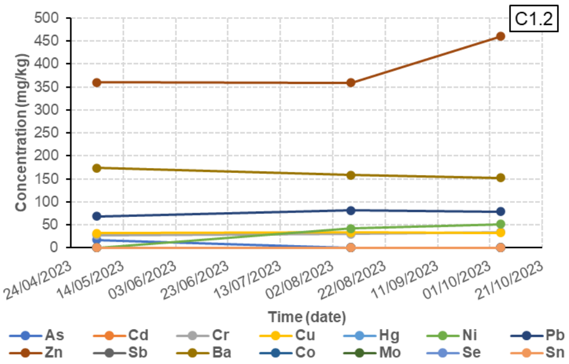


Figure 4.13*. Evolution with time of metal concentrations in soil (at 30 cm depth) during the second growing season of Sorghum (2023)

Based on the results presented in Figures 4.12, it can be seen that the TPH concentrations at a depth of 30 cm decreased drastically with the progression of the *Sorghum* season. The most significant conclusions are summarized below:

- The excavation performed just before the seeding of *Sorghum* allowed for a generally higher TPH concentration in the soil of the experimental sub parcels, as the pre-sowing TPH values are higher than the post-harvesting TPH values recorded within the rapeseed season.
- TPH contamination in soil was unevenly distributed throughout the experimental sub parcels, with sub parcels K1, E2.1, E2.2, E4.1 and E4.2 being the most contaminated.
- The non-contaminated control sub parcels C1.1 and C1.2 show a constant TPH contamination of around 100 mg/kg. And the same is observed for the experimental parcel E1.1, which is the less contaminated one.
- The contaminated control sub parcel K1, but without *Sorghum* plants, shows a sharp TPH decrease within the first 2 months and, after that, contamination remains constant at around 100 mg/kg. These results would suggest that TPH remediation could be a result of either bioremediation driven by soil bacteria or volatilization. This will be further studied and investigated in the following months.
- Similar to K1, results for the experimental parcels E2.1, E2.2, E4.1 and E4.2 also show a sharp TPH decrease with time, all with final TPH concentrations of around 100 mg/kg.

Regarding metals and metalloids, based on the XRF detector results presented in Figure 4.13 above, the metal contamination at a depth of 30 cm remained constant throughout the *Sorghum* season, and not significant changes were observed.

To assess metal contamination in soil, the results obtained at the laboratory for metals in soil for the *Sorghum* season can be compared with the General Reference values used to assess soil contamination in Catalonia and established in the DL 1/2009. Table 4.11 below shows the comparison between the designated reference values for industrial, urban, agricultural and forestry use those obtained in the field.

Table 4.11. metal and metalloids concentrations in soil at 30 cm (Sorghum)

Element	**Industrial use**	**Urban use**	**Agricultural and forestry use**	**Pilot site maximum values**
	mg/kg dw	*mg/kg dw*	*mg/kg dw*	*mg/kg dw*
Sorghum 2023				
Arsenic (As)	30	30	30	<LQ
Cadmium (Cd)	55	5.5	2.5	<LQ
Copper (Cu)	1000	310	90	22±1 (E2.2)
Molybdenum (Mo)	70	7	3.5	<LQ
Nickel (Ni)	1000	470	45	15±3 (E2.1)
Lead (Pb)	550	60	60	85±4 (E4.2)
Zinc (Zn)	1000	650	170	58±4 (E2.2)

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, the studied soils do not exceed the limits established for the protection of ecosystems or health except lead (Pb). Pb exceeds the limit concentrations for health protection for urban and agricultural and forestry use (60 mg/kg) in all parcels with a maximum value of 85±4 mg/kg dw in parcel E4.2.

Results for the analyses of TPH and metals in plant tissue are still not available, so they are not included in the present document. This is expected to be completed in the coming months; therefore, these results will be presented in the subsequent versions of this document, or in the final deliverable of the project.

4.7 Biomass output

All harvested biomass (aboveground and belowground) was measured gravimetrically at the end of each season, using both wet and dry weights. To determine dry weight, the biomass was dried by leaving it in a warehouse.

Biomass output for the first growing season of *Sorghum* is described in the deliverable 2.3. Results of wet and dry weight of biomass (collected as a bulk sample) for the only growing season of rapeseed, and the second growing season of *Sorghum*, are presented in Table 4.12 below.

Table 4.12. Biomass production determined for each of the experimental (E1, E2, E4) and control (C1) parcels

Rapeseed (*Brassica napus*) biomass 2022				
Parcel	**Number of plants harvested**	**Wet weight (kg)**	**Dry weight (kg)**	**Biomass production (t_{dw}/ha)**
E1	-	20.5	18	8
E2	-	34.5	26	2
E4	-	33.5	30	3
C1	-	33.5	23	2
***Sorghum* biomass 2023**				
Parcel	**Number of plants harvested**	**Wet weight (kg)**	**Semi-dry weight (kg)***	**Biomass production (t_{dw}/ha)**
E1	570	135	45	7
E2	810	275	129	11
E4	625	191	78	7
C1	65	20	7	1

*It is considered a semi-dry weight because the biomass was not completely dry when weighted. The drying process could not be completed in the original warehouse due to issues external to the project.

Currently, *Sorghum* biomass obtained in 2023 is undergoing drying. By the end of November 2023, the biomass will be shipped to the pelletization centre. The only rapeseed biomass obtained in this project (May 2023) will not be pelletized as it is not necessary to fulfil the project goals.

4.8 Encountered problems and amendments.

The main problems that were encountered in the field trials in 2022 and in 2023, and the solutions provided to alleviate the situation are listed below:

- A modification of Spanish site activities compared to the initially proposed was found to be necessary in 2022. 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+platform construction+platform dismantling.
- In 2022 and 2023, some pests (aphids, caterpillars and whiteflies) attacked the crops, and this negatively affected the development of plants → 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 in both years.
- In 2023 poor quality seed of *Sorghum sp.* was purchased and that resulted in a weak germination and poor biomass output → The seed quality will be checked before the third growing season in 2024.
- The use of herbicide against spontaneous weed species in May-June 2023 caused damage to the *Sorghum sp*. crop. Germination in the control plot was affected and plants were growing very poorly. This year, 2023, the amount of biomass in this parcel has decreased drastically compared to 2022.

4.9 Other information

II set of pot test:

The initial setup of the pot experiments is described in the deliverable 2.2. A second setup of pot tests was performed between 19/05/23 and 19/09/23. The pot tests remained in a temperature-controlled chamber (MP control, MP-1200-STAB) under constant temperature (25ºC), humidity (50%) and light conditions (16h light/8h dark, 250 lux). The temperature fluctuation was controlled within ± 2ºC. Control pots did not receive fertilization. The pots were watered weekly.

The overall objective of the additional pot trials was to evaluate the effect and contribution of volatilization, anaerobic transformations, and other physicochemical processes in the decrease of petroleum hydrocarbons' (TPH) concentrations in soil. This was deemed necessary to understand the results obtained in the first pot tests, where TPH decreased in all scenarios considered (included the controls), and in the field trials.

The additional pot trials were prepared with contaminated soil and the design of the experiment included a combination of scenarios and amendments (Table 4.13).

Table 4.13. Experimental design of the II set of pot tests

II set of pot test					
Soil	Only contaminated soil				Contaminated soil + sorghum seeds
Amendments	Without amendments (only contaminated soil)		Compost	Mix of compost, biochar and PGPR	Mix of compost, biochar and PGPR
Abiotic conditions	X	X	X	X	X

Anaerobic conditions	X		X	X	X
Natural conditions					X

In the current version of this document (Deliverable 2.4) only the experimental design and objectives of the II set of pot test are presented. The results associated to the samples of II set of pot test will be presented in the subsequent versions of this document, or in the final deliverable of the project because samples and data are being proceeded at this moment.

4.10 Overall summary of phytoremediation performance in M12-M36

The plots established for 6 months have shown good development. Sorghum has established and developed in all the plots, except in the control plots due to damage caused by the application of herbicide. The plants have reached height of up to 150 cm, with leafy specimens and the presence of panicles. Regarding the rapeseed crop, it has been decided not to sow it again in the following seasons due to pests and low biomass production.

The objectives of biomass production during the time of execution were met, obtaining in some parcel's quantities similar to last year.

Total TPHs concentration of the experimental parcels has been reduced by the establishment of the phytoremediation technique. Regarding the concentrations of metal(loid)s, special attention will be paid to the concentrations of Pb for future actions.

5. FIELD TRIALS ON THE SERBIAN PILOT SITE

5.1 Soil preparation and seeding campaign

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). The sediments that were dredged during 2017 was moved to Landfill 1 by the Project partner PWMCVV. Landfill 2 was prepared to accommodate fresh canal sediments also by the PWMCVV. Dredging of fresh sediments from the canal was finalised by the end of 2021. Therefore, the first growing season was done only on the Landfill 1, while the second growing season was done both on the Landfill 1 and 2. The first growing season for Serbian pilot site was finished in June 2022, results regarding the biomass output, plant monitoring and energy crop characterization was included in the deliverable 2.3. The soil characterization after the first growing season and all results from the second growing season are included in the current deliverable D2.4.

Figure 5.1: Position of landfills and piezometers

Soil preparation and seeding campaign. Landfill 1 and Landfill 2 were prepared for seeding only by ploughing and tilling using agricultural machinery. Since there was no significant presence of the weeds in the Landfills, herbicides were not used. Pre-sowing fertilization was performed using ammonium-nitrate in a dose of 25 kg of N/ha. Preparation of both landfills was performed during the first week of September 2022. (Figure 5.2).

Figure 5.2. Landfill 2 before ploughing and tilling, 05/09/2022

Based on the results from the first growing season, rapeseed (*Brassica napus*) winter variety Zlatna owned by Institute of Field and Vegetable Crops, Serbia was selected for seeding in the second growing season both at Landfill 1 and Landfill 2. Sowing of rapeseed was performed on 08/09/2022. Seeding rate was approximately 60-80 seeds per m^2.

5.2 Monitoring program

Soil monitoring. Sediment/soil sampling program was performed by UNSPMF and IFVCNS. For the monitoring purpose, Landfill 1 was divided into 10 experimental (1-10) and 2 control (11,12) sections, while Landfill 2 was divided into 10 experimental sections (Figure 5.3). 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, 88 soil samples from the pilot site were collected. Physical and chemical characterisation was done in accordance with the defined Harmonized plan, including parameters defined for Soil Quality Index (Deliverable 2.1). The soil samples were collected right before each sowing season, and after harvest.

Figure 5.3. Landfill 1 and 2 division into subplots

Monitoring of energy crops. a) Visual inspection of the energy crops on the site was done at regular intervals (IFVCNS). b) Sampling was performed by UNSPMF and IFVCNS. Five plants per section (minimum 50% of the sub plots as shown in Figure 5.3 was collected). Plant sampling points included stems and leaves; flowers/seeds-aboveground (composite); and roots-belowground (composite).

Monitoring of groundwater was carried out by UNSPMF. The groundwater samples from the 4 installed piezometers on the pilot site were collected. Samples were taken initially before first sowing season (as described in the Deliverable D2.3), during the first growing season, after the first harvesting season, during and after the second growing season.

Weather conditions were monitored by UNSPMF through AgroSense digital platform (https://agrosens.rs) and through portal of the Republic Hydrometeorological Service of Serbia (RHMZ).

5.3 Plant development

Progress of the in-situ phytoremediation is presented in Figure 5.4. Germination and growth of rapeseed before winter hibernation phase was satisfactory, with high rate of germinated seeds (approximately 90% based on visual inspection). Even though in September 2022 significant part of the Landfill 1 was covered in water due to heavy rains and inadequate water drainage which caused inhibition of plant growth, the overall plant growth at the whole pilot site was satisfactory.

In October 2022, rapeseed was treated with herbicide Chlopiralid (120 ml per Landfill) to suppress broadleaf weeds. In the mid of February 2023, rapeseed was fertilized using ammonium sulphate in a rate of 50 kg of N/ha.

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 (40 ml per Landfill), Boscalid (40 ml per Landfill) and Dimoxystrobin (40 ml per Landfill). Plant growth on Landfill 2 was satisfactory while rapeseed plants grown on Landfill 1 had lower biomass due to presence of water on the surface of the field during several months.

For the purpose of Bioaccumulation factor (BAF) and Translocation factor (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 as aboveground (composite); and roots as belowground (composite). Composite samples from 5 collected plants (separated by plant parts) were obtained for each sampling section. Sampling was performed after plant emergence (March 2023), during the flowering phase (May 2023), and just before the harvest (June 2023). 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).

30/09/2022, Landfill 1

20/04/2023, Landfill 1.

30/09/2022, Landfill 2

20/04/2023, Landfill 2.

30/06/2023, Landfill 1. 30/06/2023, Landfill 2.

Figure 5.4 Progress of the in-situ phytoremediation

5.4 Environmental conditions

Precipitation. According to the data on the amount of precipitation (Figure 5.5), August 2023 was the month with the largest amount of rain (126 mm), while the least rain fell during August 2022 (only 1.9 mm). It should be noted that in the period of August 2023 there were two unusual storms ("supercell storm") with a large daily amount of precipitation (39.3 and 34 mm/day).

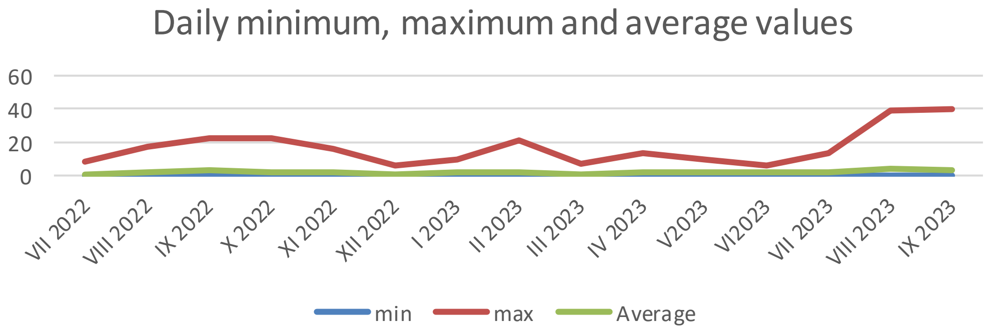


Figure 5.5. Precipitation daily minimum, maximum and average values (July 2022 – September 2023)

Temperature. The warmest month was July 2023 with the highest maximum temperature of 38.3°C, while the minimum temperature of -10.7°C was recorded in February 2023.

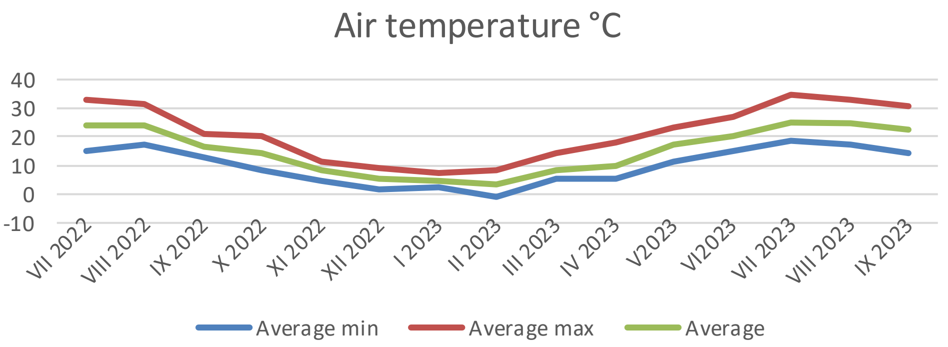


Figure 5.6. Minimum (average), maximum (average), and average air temperature values (July 2022 – September 2023)

Wind speed. The most common winds in this part of the country are northerly and *košava*. *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. As expected, higher values were detected in the winter period in the range of 76-91% (as an average value), while in the summer period the humidity was significantly lower 45-66%.

Light regime. The lowest average value for light regime was recorded in the month of January 2023 and increased from month to month, so that the highest values were recorded in the months of June and July 2023 (43.71 and 43.97 lux respectively). It should be noted that higher values were detected in 2023 compared to the same period in 2022 (e.g. May, June and August).

5.5 Harvest and pelletizing

Harvest was performed by IFVCNS on July 10th, 2023. 180 kg of seeds from Landfill 1 was collected, and 380 kg of seeds was harvested from Landfill 2. It is estimated that approximately 1.445 kg of dry harvest residues was produced at Landfill 1 (based on number of planted seeds per m^2, emergence rate and its average mass at the moment of harvest). Biomass which was harvested from Landfill 2 was left on a field for seven days to dry out and then it was collected and baled. A total of 230 bales were produced on Landfill 2 and the average mass of one bale was approximately 12 kg. Based on number of bales and their average mass it was calculated that 2760 kg of dry harvest residues was produced at Landfill 2. Approximately 120 kg of dried and baled harvest residues from Landfill 2 were transported to Novi Sad for further processing and pelletization in a pelletizing facility. Pelletization was completed in September 2023 and pellets were shipped to Fraunhofer on October 17th 2023.

1st growing season, Landfil 1	Seeds	530 kg
	Harvest residues	2500 kg
2nd growing season, Landfil 1	Seeds	180 kg
	Harvest residues	1445 kg
2nd growing season, Landfil 2	Seeds	380 kg
	Harvest residues	2760 kg

Figure 5.6. Harvesting and palettization

5.6 Phytoremediation performance

5.6.1 Soil parameters

General parameters (physico-chemical and microbiological) and SQI monitoring. The general chemical and physical parameters of samples characterization are presented in Table 5.1. General parameters are expressed as the average value of all sample measurements for Landfill 1 and Landfill 2 (average values is calculated from 44 samples for Landfill 1 and 44 samples for Landfill 2). Based on the TOC content contaminated sample can be considered as rich in organic carbon. According to the CEC value samples before sowing and after harvesting can be classified as loams and silty clays. Both Landfills, at the start and after harvesting, can be considered as slightly alkaline. 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 nitrification potential were obtained value at the end was almost 2-3 times higher than in samples at the start.

Table 5.1. General chemical and physical parameters

Parameter	Unit	Landfill 1			Landfill 2	
		Before sowing	After 1st harvesting	After 2nd harvesting	Before sowing	After 1st harvesting
pH		7.44±0.21	7.21±0.31	7.13±0.27	7.28±0.09	7.18±0.51
Eh	µS/cm	441.5±37.4	421.6±70.0	390±61.7	852±61.7	615.2±22.1
TOC*	%	2.32±0.023	2.37±0.58	2.45±0.63	2.23±0.35	2.15±0.46
CEC	C_{molc}/kg DW	34.6±5.54	30.2±2.43	60.6±5.71	31.06±7.73	54.2±5.41
OM	%	8.58±1.68	8.45±0.96	8.33±1.0	8.37±0.44	7.78±0.91

Total N	mg/kg	2210±124	2350±94.3	2555±124.1	2910±102.3	2598±105.4
Total P	mg/kg	1590±164	1270±240	1373±107.1	1527±121.3	1263±87.1
Available P	mgP_2O_5/100g	89.6±14.2	83.7±4.74	71.5±9.65	81.4±12.0	70.0±11.0
S	mg/kg	46.9±7.22	42.1±5.13	40.1±7.52	24.1±4.87	32.4±7.34
Na	mg/kg	717±190	412.8±48.1	584±31.2	385.5±17.4	423±31.7
K	mg/kg	7990±321	6790±698	2944±41.3	4944±185.1	2350±17.1
Available K	mgK_2O/100g	15.9±1.54	26.2±2.41	27.5±4.34	25.5±4.29	24.1±3.23
Mg	mg/kg	6898±607.1	7364.6±93.7	718±42.4	7391±1409	608±51.3
Ca	mg/kg	5951±340.8	410.9±50.8	3035±127	356.7±13.4	692±41.2
Texture	% 0.05-2	60.4±6.22	60.4±5.99	66.6±8.54	63.0±5.8	61.4±1.8
	%0.002-0.05	8.28±2.88	10.9±2.41	11.1±2.2	10.9±3.9	10.7±1.1
	0.002%	31.3±6.26	28.7±3.07	22.3±2.97	28.7±5.7	27.9±2.0
FA	g/kg	0.812±0.60	0.991±0.13	1.06±0.20	0.880±0.062	1.01±0.24
HA	g/kg	2.07±0.56	1.55±0.42	1.56±0.70	1.305±0.79	1.36±0.63
HU	g/kg	29.8±1.95	39.1±1.43	28.3±5.50	43.9±9.36	31.1±6.20
NIT	µgNO_2^-/g dw h	0.74±0.11	0.40±0.09	2.21±0.58	0.51±0.14	1.51±0.48
BR	µgCO_2/g dw h	8.12±1.14	9.75±1.07	8.31±2.35	10.61±3.58	9.54±3.37
C_{mic}	µg/g dw	276±27.6	460±21.7	354±18.1	437.6±12.8	315 ±13.8

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

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 Annex Table 9.17 as average value of 12 sampling points for each sampling depth for Landfill 1 and average 10 sampling point for Landfill 2. Generally, no significant difference was observed between the growing season and Landfill 1 and 2. But, it is important to notify that number of all group of microorganisms and DHA decrease with sampling depth, as a consequence lower amount of oxygen therefore lower activity of aerobic microorganisms.

Heavy metal monitoring. The content of the toxic elements Cr, Zn, Cd, Pb and Cd during the whole period of the project is presented in Figure 5.7. The concentration of those selected metals is presented as average value for each location (control, Landfill 1 and Landfill 2) for each sampling depth. The obtained results indicate high heterogeneity of the heavy metals concentration on the pilot site. Based on the obtained results there is no significant change in the total concentration of observed heavy metals. Other elements Ni, As, Mo, B, Fe and Mn are also measured, but since those are not considered toxic or not present in concentration above remediation level and there are no significant changes compared to the results presented in the deliverable 2.3, their content is not presented.

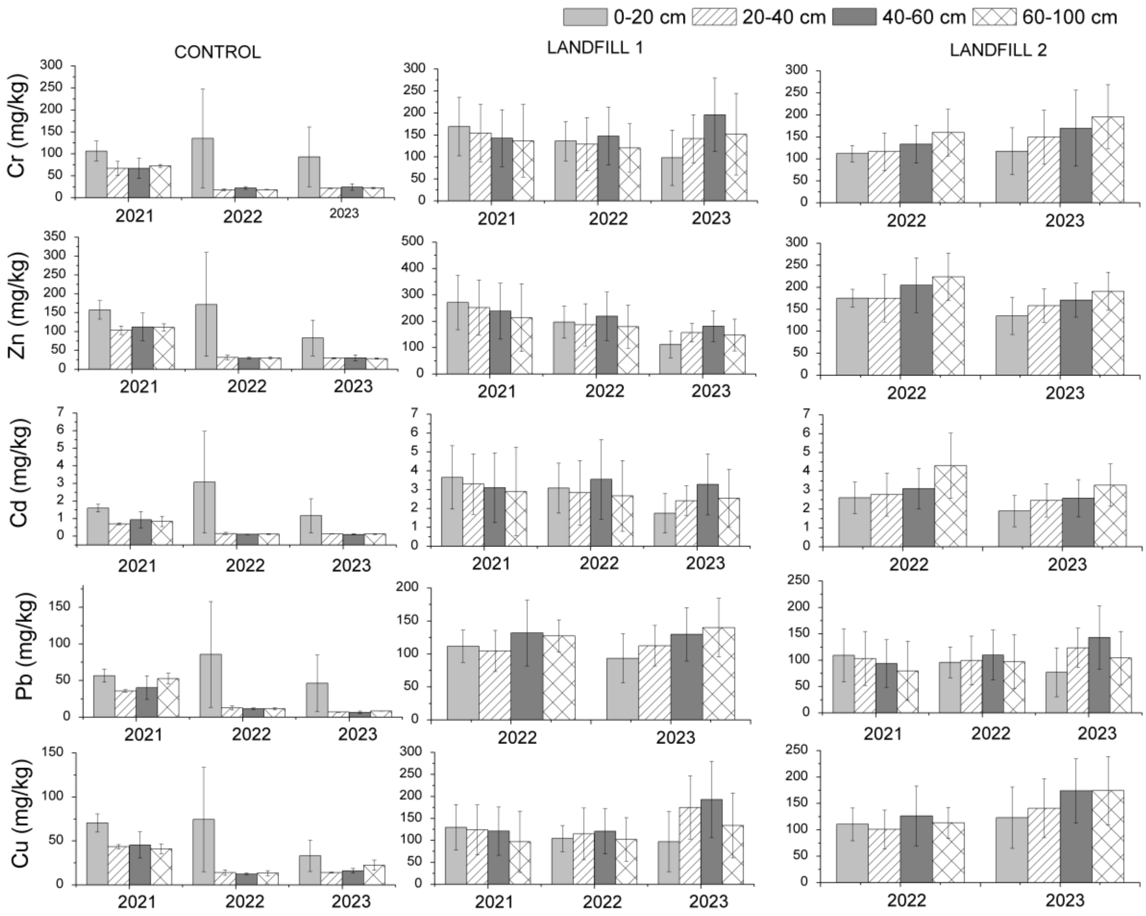


Figure 5.7. Heavy metal content in field experiments from year 2021-2023

The distribution of heavy metal speciation is just as important to heavy metal toxicity as the overall concentration of the metal. The environmental consequences of various forms are intimately related to the toxicity, migration, and natural cycle of heavy metals. The European Community Bureau of Reference introduced the BCR approach, which classified the heavy metals into four genera: exchangeable, reducible oxidizable and residual fraction. The two first portions correspond to the more mobilizable metals, which may be liberated by merely raising the ionic strength and making small pH adjustments. Relevant information on the potential metal content that the plants may bioaccumulate is provided by fractionation techniques.

The results of the BCR extraction for the year 2021-2023 are presented in Figure 5.8. Generally, change of the fraction distribution towards increasing the exchangeable and reducible fraction, over the growing season is observed. This can be consequence of the applied agricultural practices (tillage, degradation of harvest residues left on the site etc.), which lead to changes of oxidoreduction conditions in soil and consequently to the increasing the predominantly reducible fraction. Additionally, a higher amount of exchangeable and reducible fraction was observed in the Landfill 2, which can be attributed to the presence of fresh, not aged contamination, which is not undergone to the sequestration and weathering processes. Observed results are translated to the observed metal bioaccumulation in energy crops, described below.

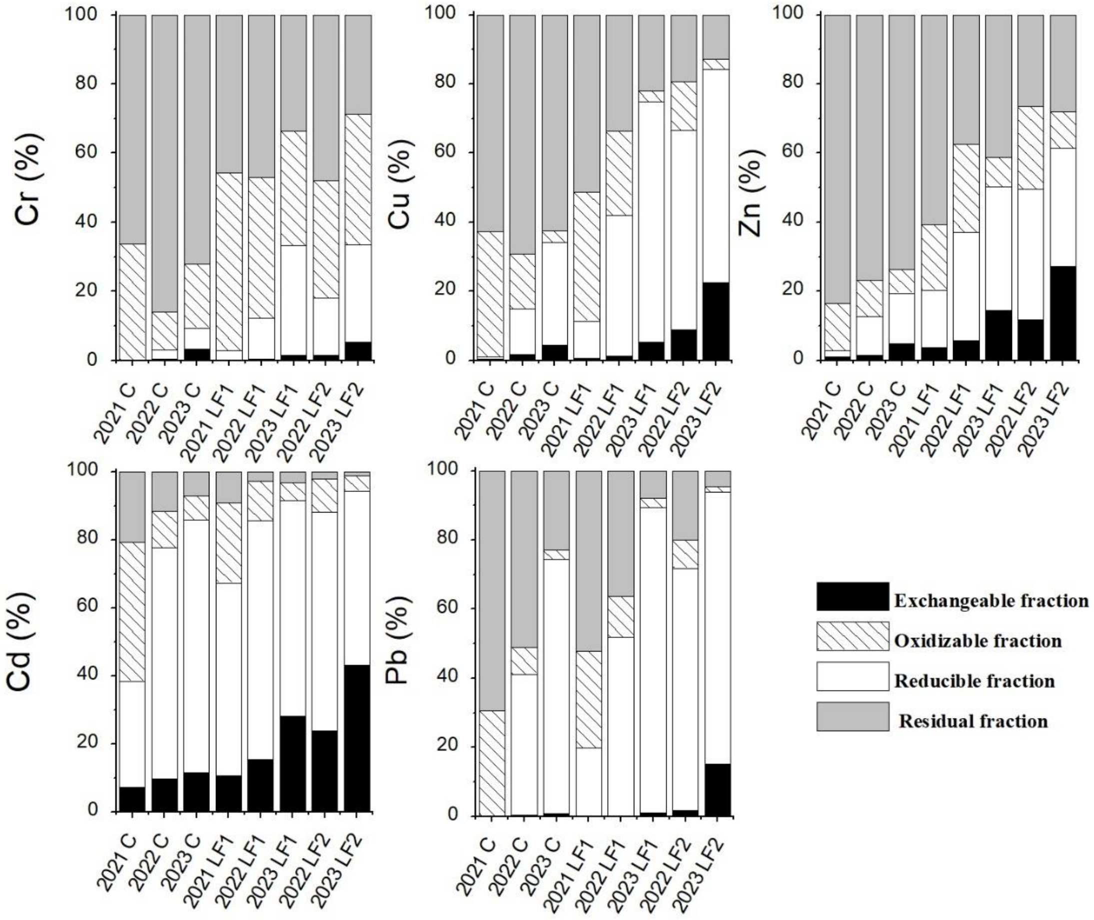


Figure 5.8. Results of the BCR extraction

Organic contaminants monitoring. The quantitative results of TPH and PAHs in soils for Landfll 1 and Landfill 2 are presented in Figures 5.9 and 5.10, respectively. For the Landfill 1 detected concentration are expressed for initial before harvesting, after the first growing seasons (GS I) and after the second growing season (GS II), while in the case of Field 2 detected concentrations are expressed for initial before harvesting and after the first growing season. The obtained results are expressed as median which presents the middle number of a group of numbers; that is, half the numbers have values that are greater than the median, and half the numbers have values that are less than the median. The initial and detected concentrations after the first and second growing seasons, expressed per sampling depth, are presented in Figures 9.1-9.4 in the Annex Landfill 1 and Landfill 2.

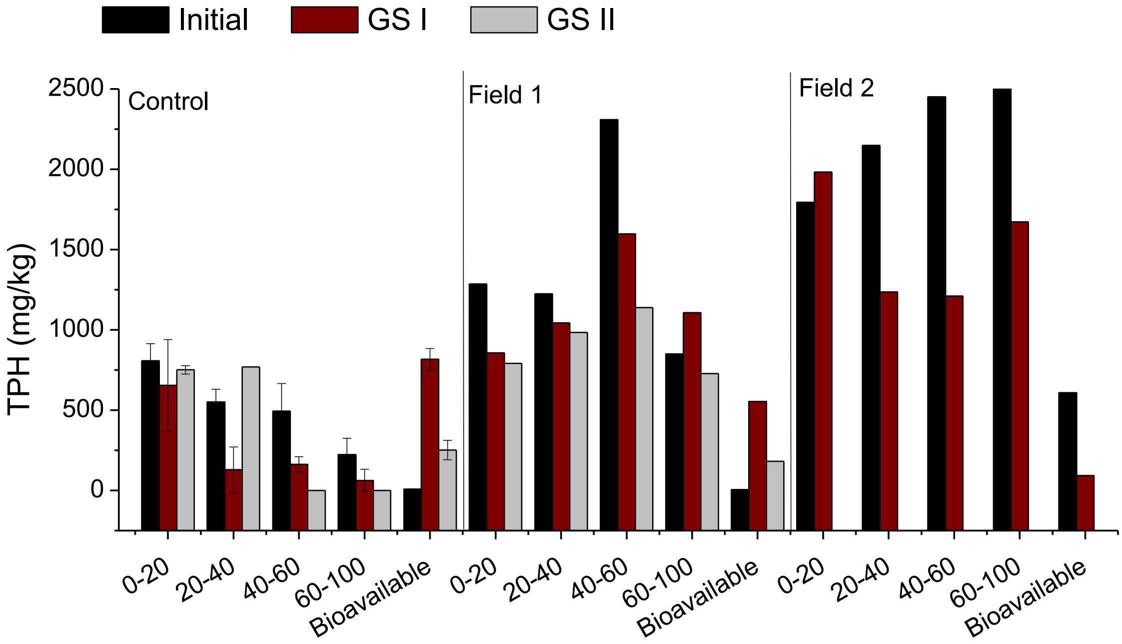


Figure 5.9. Detected concentrations expressed as the median values of TPHs in control samples, Landfill 1, and Landfill 2

The sum of median values showed a decreasing trend in the following order for Landfill 1: control samples (5700 mg/kg) > GS I (4602 mg/kg) > GS II (3641 mg/kg). In the case of Landfill 2, the sum of medians for initial concentrations was 8908 mg/kg, which decreased to 6099 mg/kg after the first growing season.

Analysing the data, after the first growing season, the concentration of TPHs decreased by approximately 19% compared to the initial concentration. Following the second growing season, there was an additional 17% reduction. Consequently, the overall removal of TPHs after the second growing season amounted to about 36% compared to the initial concentration. For Landfill 2, after the first growing season, TPH removal was approximately 31%.

The initial bioavailable fraction ranged from 0.49 to 149 mg/kg for Landfill 1. After the first growing season, this fraction demonstrated variability within the range of 21 to 865 mg/kg. In the second growing season, the bioavailable fraction ranged from 44 to 461 mg/kg. For the Landfill 2, initial bioavailable concentration was in range from 432 to 830 µg/kg, while after the first growing season bioavailable concentration highly decreased by 19-485 µg/kg. This data suggests a dynamic and fluctuating pattern in the bioavailable fraction across the two growing seasons, without a clear trend in alignment with the changes observed in the total concentration over time.

In summary, the TPH removal rates were similar for both fields, averaging around 33%. It's important to note that the results for Landfill 2, despite only one growing season, exceeded those of GS I for Landfill 1, indicating potentially effective remediation efforts in the shorter timeframe.

The median concentrations of detected PAHs in Landfill 1 followed the order: initial (5426 µg/kg) > GS II (1787 µg/kg) > GS I (563 µg/kg). As shown, the highest concentration was observed at the initial stage. Remarkably, after the first growing season, the PAH concentration showed a significant decrease of 90% compared to the initial level. After the second growing season, there was an increase in the median concentration compared to GS I but still lower than the initial stage. The percentage of removal was 67% less than the reduction observed in GS I (Figure 5.10).

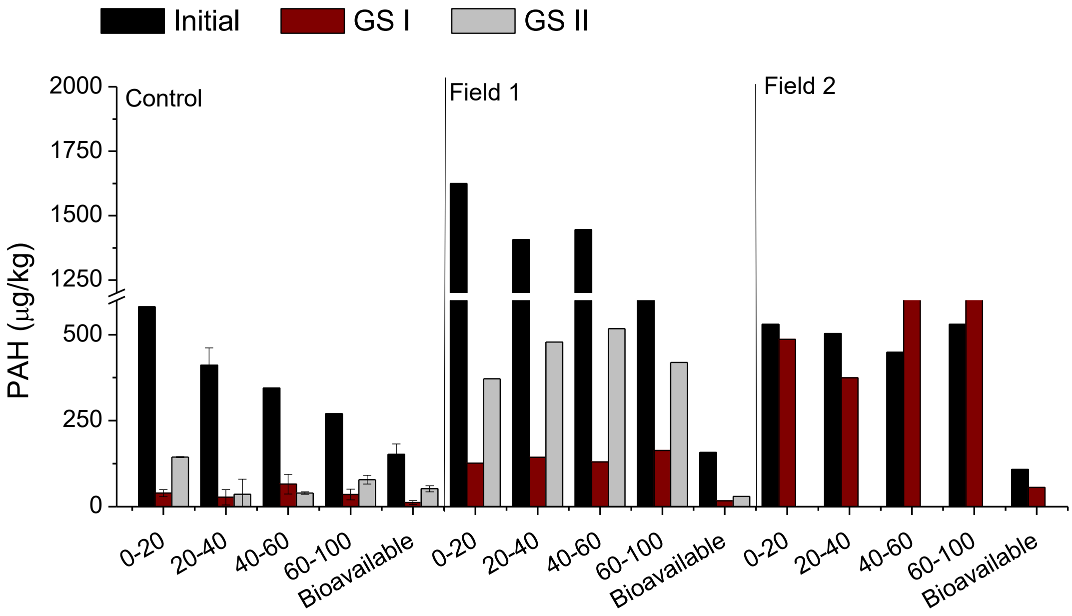


Figure 5.10. Detected concentrations expressed as the median values of PAHs in control samples, Landfill 1, and Landfill 2

The higher concentration detected after the second growing season could be attributed to microbial activity. Microorganisms play a crucial role in PAH degradation, and during the first growing season, microbial communities might have been more active or better adapted, resulting in a higher reduction. In the second season, challenges such as changes in environmental conditions, competition, or adaptation may have led to a somewhat less efficient degradation.

Additionally, the initial concentration of PAHs in the soil can impact overall reduction. It's plausible that the higher initial concentration in the first season contributed to a more substantial percentage reduction. Similarly, the median PAH values for Landfill 2 were higher for GS I in-depth layers (40-100 cm) compared to the start. This could be explained by microbial degradation of TPHs, which might be contributing to the higher concentration of detected PAHs in the soil.

In Landfill 1, the bioavailable fraction at the initial stage ranged from 4 to 157 µg/kg. Following the first growing season, it decreased to a range of up to 36 µg/kg, and further decreased to 8-58 µg/kg after the second growing season. Conversely, in Landfill 2, the bioavailable fraction ranged up to 457 µg/kg at the initial stage, reducing to 28-128 µg/kg after the first growing season. Generally, the bioavailable fraction was higher in Landfill 2 compared to the initial stage of Landfill 1.

As for pesticides, including organochlorine, atrazine, simazine, alachlor, chlorpyrifos, trifluralin, and other impurities such as pentachlorobenzene and hexachlorobenzene, as well as PCBs, the detected concentrations for both fields remained below 50 µg/kg. This was below the defined remediation value for soil and sediments as per Serbian legislation.

Ground water monitoring. Monitoring of groundwater was done after harvesting in March 2023 and July 2023. Results are presented in Table9.18 in the Annex. According to the results, 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 arsenic and zinc contamination in the two downstream samples, piezometer located next to Begej cannel, has exceeded the remediation threshold. However, those values were detected on these piezometers even before the pilot site during the period of monitoring from the 2003. Additionally, observed value low level of water in July in P2 and P4 could have caused waterflow from Begej cannel to the piezometer, indicating its impact to the groundwater. Also, arsenic in the surrounding area 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.

5.6.2 Biomass output

180 kg of seeds from Landfill 1 was collected, and 380 kg of seeds was harvested from Landfill2. It is estimated that approximately 1.445 kg of dry harvest residues was produced at Landfill 1 (based on number of planted seeds per m2, emergence rate and its average mass at the moment of harvest). Total mass of dry harvest residues produced at Landfill 2 was calculated on the basis of total number of produced bales and the bale average mass and is 2760 kg which corresponds to the yield of 13,8 t per ha. According to literature data (Ma et al. 2019[3]; Budzynski et al. 2015[4]) when grown on agricultural land, winter rapeseed biomass yield ranges between 3,5 and 10 t per ha which indicates that production of biomass at Landfill 2 was higher than at average agricultural land.

The significant difference in seed and biomass yields at Landfill 1 and 2 are probably due to presence of high surface water during autumn and early spring 2023 which inhibited growth and development of rapeseed roots. Although the yield at Landfill 1 was significantly lower than previous growing season, the aim to produce >40 kg (dry basis) of energy crops per growing season was achieved and exceeded.

The yield of rapeseed was 0,9 t per ha at Landfill 1 and 1,9 t at Landfill 2, yield on the uncontaminated agricultural soil was 2.5 to 3.5 t per ha. Low sees yield at Landfill 1 was

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[3] Ma, Y.; Fanf, S.; Peng, Y.; Gong, Y.; Wang, D. (2019). Remote Estimation of Biomass in Winter Oilseed Rape (Brassica napus L.) Using Canopy Hyperspectral Data at Different Growth Stages. Applied Sciences, 9, 545.

[4] Budzynski, W.S.; Jankowski, K.J.; Jarocki, M. (2015) An analysis of the energy efficiency of winter rapeseed biomass under different farming technologies. A case study of a large-scale farm in Poland. Energy, 90, 1271-1279.

probably due to to presence of high surface water during autumn and early spring 2023 which inhibited growth and development of rapeseed roots which prevented the plant from developing a high number of flowers and pods. Low seed yield at Landfill 2 was due to the fact that rapeseed plants were higher than average and some of the stalks bent during the rain a week before harvest which made collecting the seeds not possible.

Energy crop characterization. Translocation factor for Cu, Pb, Cd, and Zn reached values close or above 1 on both Landfills (Figure 5.11). Only the Cr's TF was below 1, this is due to the fact that, Cr is present in soil in form of its oxide which has high stability and low mobility. Significantly higher TF and metal concentration (Zn, Cd, Cu and Pb) observed in the Landfill 2, during the initial phase of plant growth. This can be attributed to the fact that pollution in the Landfill 2 can be considered relatively "fresh", and that it didn't undergo to the weathering and sequestrating processes which reduce pollutant mobility. This is confirmed by metal available fraction measurement (BCR methodology, Deliverable 2.3), where exchangeable and reducible fractions are significantly higher for Landfill 2, compared to the Landfill 1 which contain "old" weathered pollutants. After the initial plant growth phase, most of available metal fraction is uptaken by the plants, and TF and metal concentration in biomass is not significantly different to the Landfill 1.

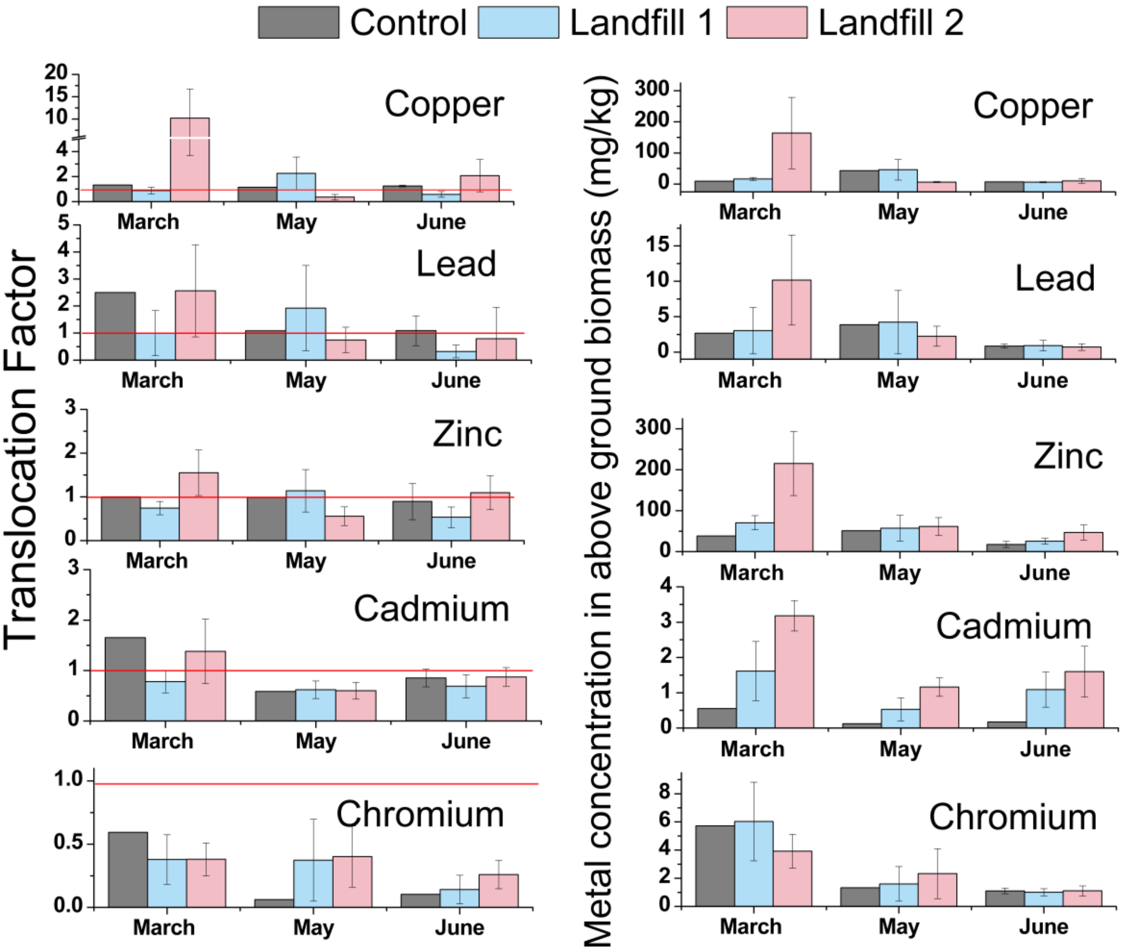

Figure 5.11. Translocation factors (left) and metal concentration in above ground biomass (mg/kg) during the 2nd growing season

Concentration of the metals in seeds are presented in Table 5.2. The obtained results for Landfill 1 for the 1st and 2nd growing seasons are not significantly different. Slightly higher concentration of all metals, especially in case of Landfill 2 was obtained compared to the control sample.

Table 5.2. Metal concentration in seeds

	Cr (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	Cd (mg/kg)	Pb (mg/kg)
			1st season		
Landfill 1	0.40±0.04	4.60±0.4	36.29±3.6	0.12±0.01	0.21±0.02
			2nd season		
Control	0.75±0.028	2.55±0.011	26.78±1.25	0.037±0.001	0.15±0.032
Landfill 1	0.85±0.29	5.87±02.34	37.01±5.75	0.12±0.053	0.71±1.03
Landfill 2	1.04±0.37	7.28±1.99	49.84±4.93	0.18±0.054	0.35±0.10

5.7 Encountered problems and amendments

Progress of the in-situ phytoremediation is presented in Figure 5.4. Germination and growth of rape seeds at Landfill 2 before winter hibernation phase was satisfactory, with a high rate of germinated seeds (approximately 90% based on visual inspection). However, due to heavy rains and high groundwater level which inhibited soil drainage, surface water at Landfill 1 was present from September 2022 to April 2023 on most of the surface of Landfill 1 which caused severe inhibition of plant growth. To avoid negative effects of surface water in autumn and spring, spring crop variety will be selected for the next planting season. Nutrient deficiency was observed in February 2023. Therefore, soil fertilization was done with ammonium sulphate 50 kg of N per ha.

5.8 Other information

Third set of pot tests (UNSPMF, IFVCNS) - Increasing bioavailability of heavy metals

The aim of the III set of pot test was the investigation of phytoremediation potential of two additional plant species Sorghum and Hemp using the best performing treatment obtained in the II pot test from the aspect of plant yield, extracted amount of metals and plant health parameters. Soil (dredged aquatic sediment) used in pot tests was the same as used in the II set of pot tests. Experiments were performed in the open air under natural weather conditions, in triplicates. Sorghum and Hemp were used for the test. The seeding density was 5 seeds per pot. After plant germination, the pots were trimmed to 1 hemp plant and 2 sorghum plants per pot. The soil in pots was treated with 20 mmol/kg tartaric acid, glutamine acid, oxalic and malonic acids. The soil treatment was performed 6 and 8 weeks after seeding. 60 pots in total were set up (Figure 5.12). Plants were harvested one week after soil treatment with acids (after 7 and 9 weeks from the seeding).

Figure 5.12. Second season of pot test

Soil Characterization. The general chemical and physical characterization of soil/sediment used in POT experiments have been presented in Table 9.19 in the Annex. Initial and soil after the treatment have organic matter content around 10%. The texture of soil in all pots were similar, soil has around 78.4±0.7%of sand, 6.3±0.5% of silt and 15.2±0.4% of clay. According to the CEC value soil before treatment can be classified as organic soil, while after the treatment as dark-colored silty clay and loams and silty clay. Both, control soil and contaminated sediment, can be considered as slightly alkaline. The pH in all treatment marginally decreased at the end of the pot experiment. The content of the selected metal(loid)s at the beginning and end of the experiment is presented in Figure 9.5. in the Annex. According to national sediment legislation[5], a sediment is considered as highly contaminated, since heavy metals, such as Cu, Cr, exceeded remediation values. While concentrations of Zn, Cd and Pb exceed target value. Other heavy metal(loid)s content was below the target value. During the experiment, the reduction of all metals/metalloids (for all HEMP and SORGHUM samples) in all treatments was observed. There was no significant difference in metal reduction between Hemp and Sorghum in all treatments.

The results of a multi-step sequential extraction method for determination of potentially available metal fraction are presented in Figure 9.6 in the Annex. The more mobile 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 released into the environment. Based on the obtained results most of the present metal(loid)s are in non-available fraction (reducible and oxidizable). In general, the available fraction of all metals in the initial sample was higher than in the treated HEMP and SORGHUM samples. In the treatment SORGHUM OXA, after 6 and 8 weeks it has been observed a higher amount of some metals in available fraction, like Cu and Pb. In the initial sample the bioavailable fraction of almost all metals was below 5%. Only in the case of Cd, a higher amount of the available fraction is observed for the initial sample, around 15%.

During the 2023 growing season, the overall PAH concentration ranged from 300 to 924 µg/kg (Figures 9.7(1-3) in the Annex), except for the samples of Hemp that had been treated with glutamic acid after 8 weeks and citric acid after 8 weeks. In these specific samples, the PAH concentration was three times higher compared to the rest. In general, there were no notable discrepancies in the total PAH concentration between the two growing seasons. The bioavailable PAH concentration decreased within the range of 3-318 µg/kg, and it was lower than the initial concentration.

In the context of the 2023 growing season, the total concentration of TPHs ranged from 684 to 3070 mg/kg, with the initial concentration being below 2000 mg/kg. The highest TPH concentration was found in POT with hemp treated with glutamic acid after 6 weeks, while the lowest concentration was detected in the same crop treated with citric after 6 weeks. The bioavailable TPH fraction ranged from 15 to 741 mg/kg.

Energy crop characterization. Biomass output is presented in Figure 5.13, while Translocation factor is presented in Figure 5.14, and bioaccumulation factor in Figure 9.8 in the Annex. Biomass output for HEMP had no significant difference in all treatments compared to the control sample, only in the case of treatment with oxalic acid, a decreasing of biomass was observed. Generally, BAF of the below ground biomass was significantly higher for all investigated metal(loid)s except in the case of Cd. For Cd the BAF was approximately at the same level in above and below ground biomass which is in line with its high mobility explained above. Comparing sorghum and hemp treatments it can be concluded that higher BAF values were obtained for all used sorghum treatment.

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[5] 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)

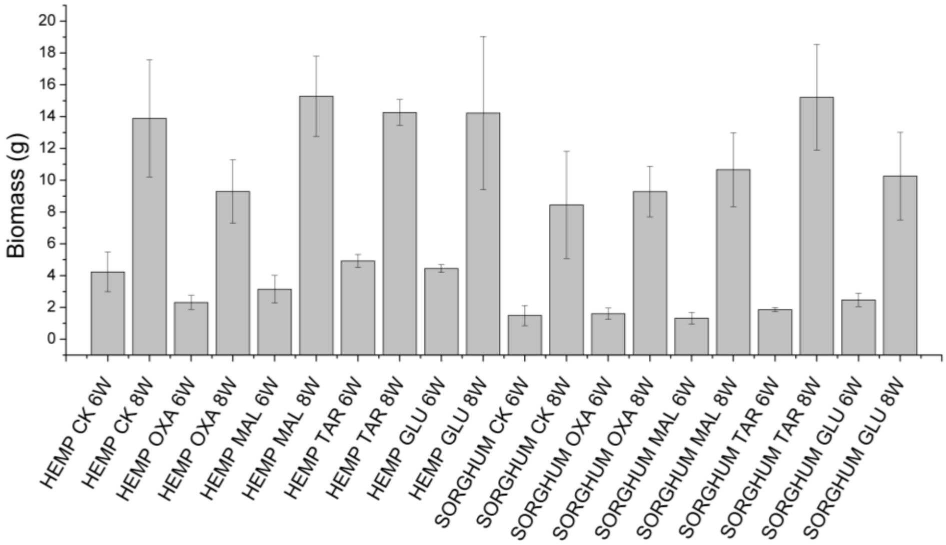

Figure 5.13. Biomass output during the POT experiment

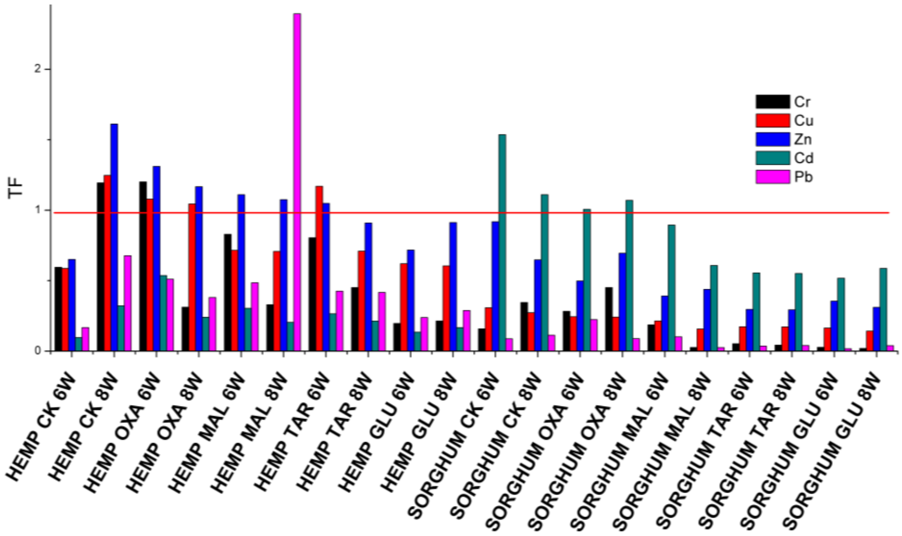

Figure 5.14. Translocation factor during the POT experiment

The TF was <1 which indicate that the main mechanism of the metal(loid) removal is phytostabilisation and not phytoextraction. TF above 1 was observed for treatment with oxalic acid for Cd in Sorghum samples and for Cr, Cu and Zn in Hemp, but as it was indicated above this plant generally accumulates low level of all metals.

5.9 Overall summary of phytoremediation performance in M12-M36

Second growing season include both Landfill 1 and Landfill 2. Based on the results from the first growing season, rapeseed (*Brassica napus*) winter variety Zlatna owned by Institute of Field and Vegetable Crops, Serbia was selected for seeding in the second growing season both at Landfill. Germination and growth of rapeseed before winter hibernation phase was satisfactory, with high rate of germinated seeds (approximately 90% based on visual inspection). Even though in September 2022 a significant part of the Landfill 1 was covered in water due to heavy rains and inadequate water drainage which caused inhibition of plant growth, the overall plant growth at the whole pilot site was satisfactory.

Harvest was performed in July 2023; it is estimated that enough biomass was produced. Approximately 180 kg of seeds from Landfill 1 were collected, and 380 kg of seeds were harvested from Landfill 2. It is estimated that approximately 1445 kg of dry harvest residues were produced at Landfill 1 and 15012 kg at Landfill 2.

Total concentration of the Cd, Cr, Cu, Pb and Zn didn't change significantly. But change of the fraction distribution towards increasing the exchangeable and reducible fraction, over the growing season is observed because of the applied agricultural practices. This is reflected in the higher concentration of all metals, especially in the case of Landfill 2 in the biomass collected. Translocation factor for Cu, Pb, Cd, and Zn reached values close to or above 1 on both Landfills. Only the Cr's TF was below 1, this is due to the fact that, Cr is present in soil in form of its oxide which has high stability and low mobility.

TPH removal rates were similar for both fields, averaging around 33%. Regarding the PAH the percentage of removal was about 70%in total Landfill 1 (1st and 2nd season). However slight degradation is observed for Landfill 2.

3rd set of POT test included testing Hemp and Sorghum plant species with and without additions of low molecular organic acids for increasing metal mobility. Based on the results of the 3rd set of POT test it was concluded that, both plants give similar biomass, but sorghum bioaccumulate higher amount of all metals of inters, higher BAF values were obtained. Therefore, sorghum was selected for the 3rd growing season on pilot site.

6. FIELD TRIALS ON THE LITHUANIAN PILOT SITE

6.1 Soil preparation and seeding campaign

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

Soil preparation and seeding campaign

The site was subdivided into 3 different size experimental parcels (squares). The colour of the parcel frame (Figure 6.2) indicates which plant species were sown/planted: green parcel - herbaceous plants mix; red parcel – amaranth (*Amaranthus caudatus*); yellow parcel – Jerusalem artichoke (*Helianthus tuberosus*). 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.

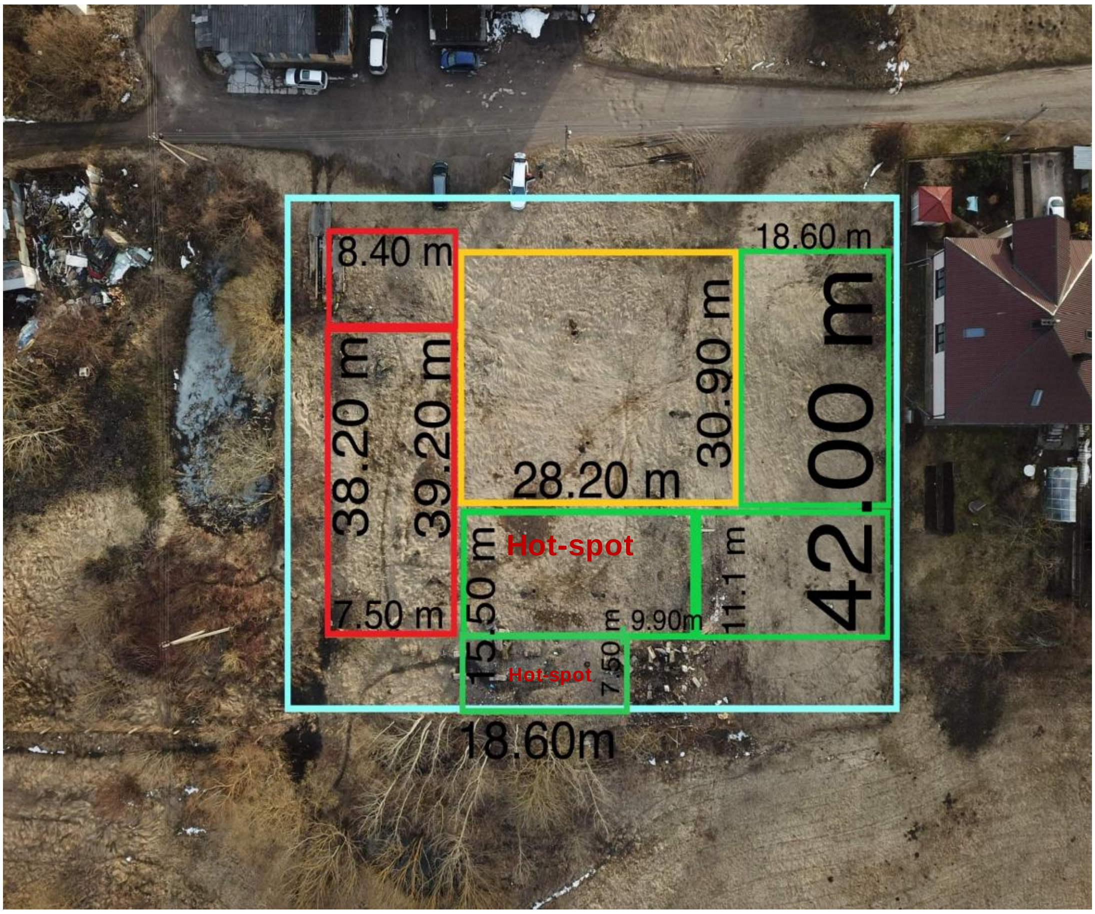

Figure 6.2. Subdivision and real-scale measurements of each parcel. Green parcels – herbaceous plants, red parcel – amaranth, yellow parcel – J. artichoke

Soil preparation, fertilizing and seeding campaign:

Herbaceous plants

- Mineral fertilizer – $(NH_4)_2SO_4$ 26-13 (%), 25 kg/parcel (0.20 t/ha); $(NH_4)_2SO_4$ 21-24 (%), 10 kg/parcel (0.08 t/ha).

Herbaceous plants hot spots

- Soil preparation – hot-spots (Figure 6.2), where poor development occurred last year, were re-harrowed and prepared for re-seeding.
- Compost – 200 kg wet weight (ww)/hot-spots (1.6 t ww/ha).
- Mineral fertilizer – NPK(S) 12-11-18 - 8S (%), 1 kg/hot-spots (0.05 t/ha).
- Reseeding - 1,7 kg of herbaceous plants mix were seeded with "Gardena" seeder. The seeded area was raked manually. 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

- Soil preparation – soil was power harrowed first. Then stones and small rubbish were collected with a special raking tool. After that, the 2nd power harrowing was performed. After fertilization, the soil was leveled with a towed leveler.
- Mineral fertilizer – NPK(S) 12-11-18 - 8S (%), 25 kg/parcel (0.80 t/ha); $NH_4)_2SO_4$ 21-24 (%), 8 kg/parcel (0.26 t/ha)
- Weed control – herbicide "Barbarian Biograde 360", 0.174 L/parcel (2 L/ha) was used. White goosefoot (*Chenopodium album*) weeds were pulled out manually.
- Seeding – 600 g/parcel were seeded with "Gardena" seeder (1 row on seeder scale, 3 times were driven through all parcel). After that, the area was raked manually.

Jerusalem artichoke

- Soil preparation – soil was power harrowed first. Then stones and small rubbish were collected with a special raking tool. After that, the 2nd power harrowing was performed, and rows for Jerusalem artichoke tubers were formed (Figure 6.3). Space between the rows was 55 cm.
- Mineral fertilizer was used: NPK(S) 12-11-18 - 8S (%), 50 kg/parcel (0.60 t/ha).
- Weed control – herbicide "Barbarian Biograde 360", 0.062 L/parcel (2 L/ha) was used.
- Seeding – about 150 kg ww of planting material was used in the parcel. Tubers were planted by hand in the preformed rows. Distance among the tubers in a row was ~0.4 m.

Control parcels were installed in 2022 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). 30 g of amaranth seed were seeded and about 7 kg ww was spread of compost into the designated parcel. Because herbaceous plants and Jerusalem artichokes are perennials, designated parcels have remained untouched since last year's season.

Figure 6.3. Rows for Jerusalem artichoke tuber seedings (10/05/2023)

6.2 Monitoring program

Monitoring of the plants was carried out every 10-14 days. Monitoring of the plants was carried out in three replicates in different 1 m^2 sub-plots. The following parameters were evaluated: germination rate, soil cover with plants, plant density, luxuriant (lushness of the plants), 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 was carried out through the Lithuanian Hydrometeorological Service Station. The station provided in fact hour data sets, each 10 days on air temperature, air humidity, amount of precipitation, sunny hours, average wind speed and wind direction.

6.3 Plant development

Plant development was monitored for 22 weeks throughout all vegetation periods. The trends are presented in Figure 6.4 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.

In general, the plant development in season M24-36 went successfully. The all 3 plant species gave sufficient amount of biomass and developed without main issues during the season Figure 6.5.

The main observations of the second pilot site growth season in M24-36 are as follows:

Germination

- The selected herbaceous plants are perennial, so their germination rate reached 100%.
- J. artichoke in the contaminated soil germinated (sprouted) slower than in the clean soil, nonetheless it reached 100%.
- At the beginning of the season, amaranth germinated similarly in both clean and contaminated soil. The germination of both groups was delayed due to the lack of moisture, but the situation changed at the end of May - beginning of June, the percentage of germination increased. Finally, the germination reached 75% in clean soil, 100% in contaminated soil.

Soil cover

- Herbaceous mix. As well as the last season, soil cover was higher in the control soil, where it reached 100%, and was very homogeneous. While the cover in the contaminated soil was, depending on the time of harvest, about 50-80%. In the contaminated soil, the soil coverage was non-homogeneous due to non-uniform distribution of TPH contamination.
- J. artichoke. As the control Jerusalem artichoke plants were not re-seeded in the spring, soil coverage remained around 75% throughout the season. After adopting new agrotechnical solutions, meaning that in the contaminated soil the plants were re-planted, the soil coverage until the middle of the season changed until it finally reached 80%.
- After changing the seed supplier, the amaranth growth situation improved significantly during the second season. The coverage of the contaminated soil reached 85%, when only 30% was achieved in the first season. However, the coverage of the control soil was very poor (1%). This could have been caused by poor growing conditions, lack of moisture and nutrients in the spring, when the plants became stressed and stopped developing.

Plant density

- Herbaceous mix. As well as last season, plant density was higher in the control soil, where it was always evaluated with the maximum score (9). While the plant density in the contaminated soil was, depending on the time of harvest, about 8 points.
- Final plant density was scored higher in the contaminated soil (8) than in the control soil (7). Compared to the last year, these results are a bit worse, when the plants reached maximum plant density (9 points) in both cases.
- Amaranth in control soil exhibited very low plant density. This coincides with other parameters, like low soil coverage and low score in luxuriant. In the second year, after

choosing better and more reliable seed, the plant density in the contaminated soil reached 8 points.

Luxuriant

- Herbaceous mix grown in the clean soil before harvesting was maximally lush (9 points). Plants grown on the contaminated soil were less luxuriant. The luxuriance of plants grown in contaminated soil fluctuated over time and before harvest was rated 6-8 points. Fluctuations in time were caused by changing weather conditions (higher temperature, lack of humidity). 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.
- Jerusalem artichokes growing in clean soil were lusher than those growing in contaminated soil until mid-July. However, at the end of July, plant luxuriance in both cases levelled off and was rated 8 points. Unlike last year, the maximum lushness score (9) was not achieved.
- As well as the density, the lushness of amaranth grown in the control soil was very poor. In the second year, after choosing a better and reliable seed, the plant luxuriance in the contaminated soil reached 7 points.

Plant height

- The height of herbaceous plants was similar at the beginning and end of the season, but the heights differed in the middle of the season. In the middle of the season (end of June-beginning of August), the height of plants grown in clean soil was about 10 cm higher than that grown in the contaminated soil.
- Until the beginning of August, Jerusalem artichokes growing in the clean soil were up to 30 cm taller than the ones growing in the contaminated soil. Later, in both cases, the height of the plants equalized.
- In regards of previously provided reasons, the amaranth plants growing in the contaminated soil were significantly taller than those growing in the clean soil and reached height of 0.9 m before harvest.

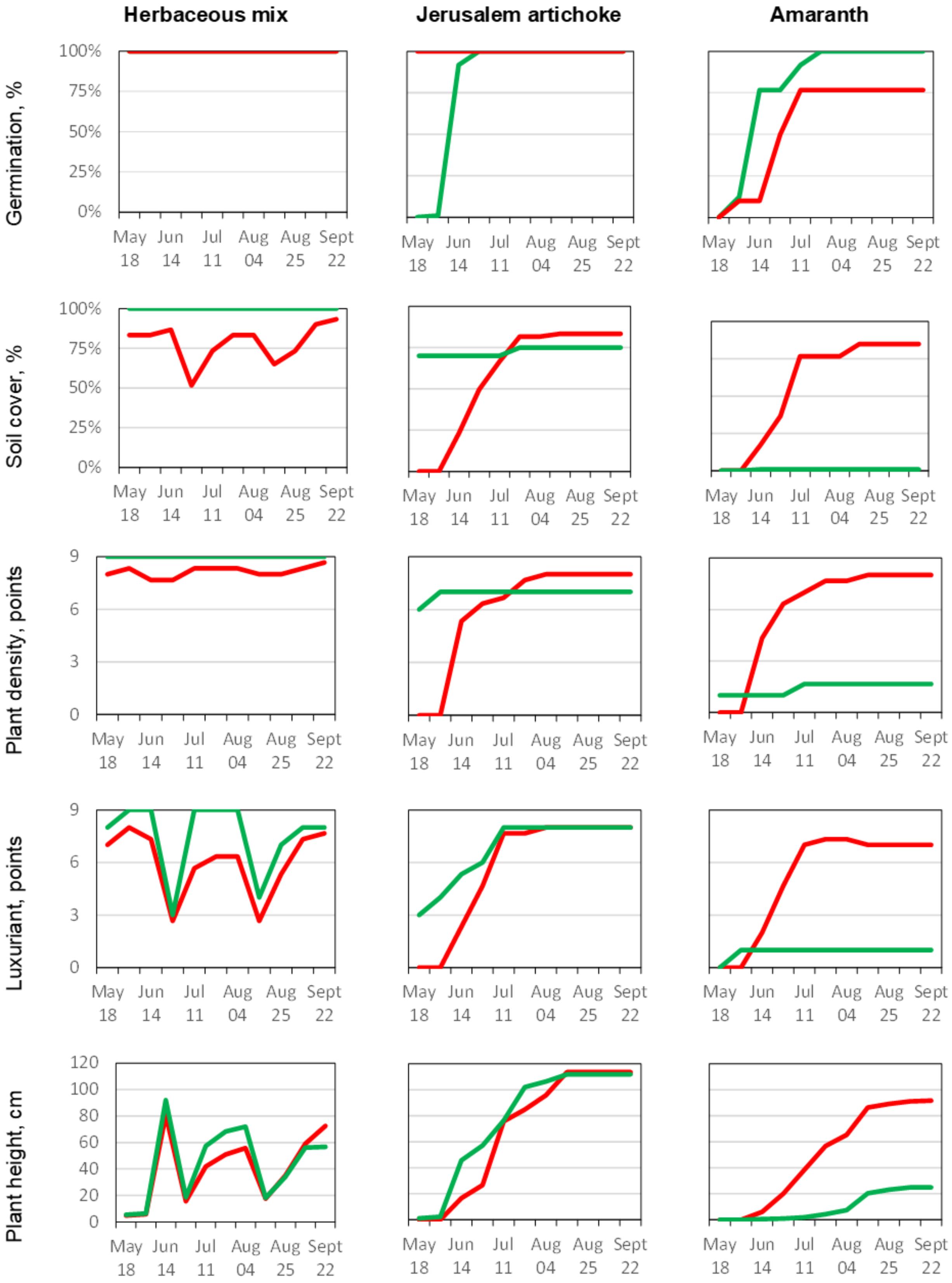

Figure 6.4. Plant development. Green line – plants grown on control (clean) soil; red line – plants grown on contaminated soil

Figure 6.5. LEFT: herbaceous plants grown on the contaminated soil (25/07/2023). MIDDLE: Jerusalem artichoke grown on the contaminated soil (14/08/2023) RIGHT: amaranth grown on the contaminated soil (11/09/2023)

6.4 Environmental conditions

Average 10 days air temperature and cumulative 10 days precipitation in the area, where the pilot site is located, during January 2023 - October 2023 are presented in Figures 6.6 and 6.7. Second growing season went without strong storms, heavy rainfalls or hailstorms which could have destroyed the plants. At the beginning of the second growing season in May, the amount of precipitation did not reach the optimal amount (40-60 mm) for the growth of most plants. This may have affected seed germination, vegetation initiation, plant development and nutrient uptake. Also, several episodes were identified in July and August when the temperature exceeded 20 °C and the amount of precipitation was lower than 10 mm per 10 days. During these episodes, the plants experienced drought stress, but luckily these conditions did not last long, and the plants did not suffer significant damage. Despite draught periods in May – June, the weather was typical to the climatic region where Lithuania is located. During the period total 521 mm of rainfall received, but it distributed very unequally. No artificial irrigation was applied to the plants.

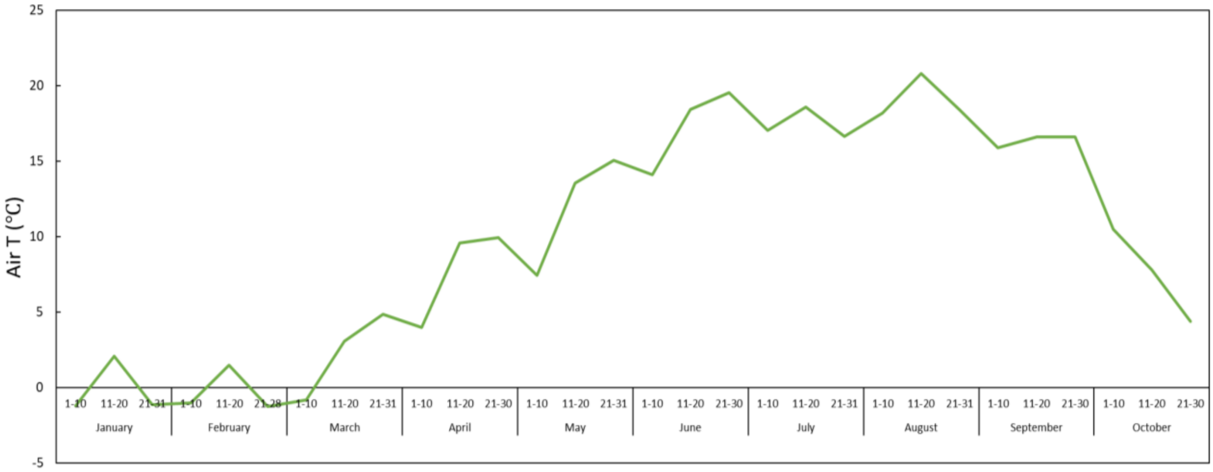

Figure 6.6. Average air temperature in the pilot site area during January-October 2023

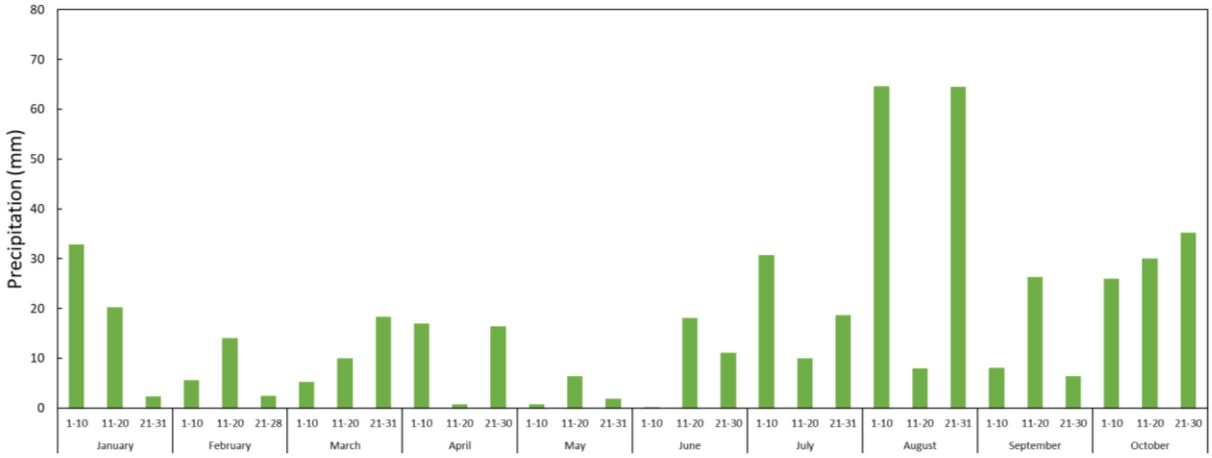

Figure 6.7. Cumulative 10 days precipitation in the pilot site area during January-October 2023

6.5 Harvest and pelletizing

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 – June 2023; August 2023; September 2023.
- Jerusalem artichoke harvest, amaranth harvest – September 2023.

Harvesting herbaceous plants

Harvesting was done using disc trimmers. Then the wet biomass was laid into swaths for drying for 7 days. After drying on field, air-dried biomass was collected and transported to the drying facility. The completely dried biomass was rolled into bales. About 2.4 t of wet biomass was obtained from first harvest, from second and third harvest - about 1.4 t and 2 t, respectively.

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-September 2023. Jerusalem artichoke and amaranth plants were at the end of blooming phase, phenological stage BBCH 69, when stems of the plants start to lose its first leaves.

Jerusalem artichoke aboveground biomass and amaranth biomass were harvested using disc trimmers, biomass was laid into swaths, collected and transported to the drying facilities after 2 days on field pre-drying. For J. artichoke about 2.5 t of wet biomass was obtained from the parcel (870 m^2). While for amaranth, it was about 1.1 t 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 100 m^2 from the entire J. artichoke parcel area was harvested. 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 enough for further biomass processing.

Processing of the biomass

Biomass was milled with a regular feed-type grain mill, 6 kW of power through 6 mm sieve, and with the 50 kg/hour output.

Pelletization of the biomass

Milled biomass material was pelletized using pellet mill CPM-2000 series (California pellet mill). 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.

Figure 6.8. Pellets of the biomass obtained at Lithuania pilot site field trials in 2023

6.6 Phytoremediation performance

6.6.1 Soil parameters

General soil parameters. Tables 6.1 and 6.2 present soil parameters after 1st year field trial and after 2nd year field trial. In both cases, the sets of samples were collected in October, 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.

Table 6.1. Soil parameters in the contaminated soil determined after the 1st year (2022) field trial M12-24

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**	**P_2O_5**	**K_2O**	**Mg**
	cm	*%*	*%*		*mS/m*	*CFU/g*	*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	2300000	<50.0	1931	4.48	729	18.7	95.8	1020
	60-100	89.2	5.58	8.1	15.6	9300000	<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	5000000	<25.0	500	3.7	1033	38.0	158	292
	40-60	93.6	2.6	8.7	11.00	3000000	<25.0	494	4.00	816	19.1	105	123
	60-100	86.8	4.85	7.8	21.8	7300000	<50.0	1532	3.26	1840	56.4	154	188
Jerusalem artichoke	0-20	93.0	3.68	8.1	10.8	5000000	<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	5000000	<5.0	<100	3.41	430	54.9	306	142
	60-100	88.9	4.75	8.2	17.2	12000000	<5.0	<100	3.48	717	58.8	358	176

Table 6.2. Soil parameters in the contaminated soil determined after the 2^{nd} year (2023) field trial M24-36

AFTER field-trial (2^{nd} 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**	**P_2O_5**	**K_2O**	**Mg**
	cm	*%*	*%*		*mS/m*	*CFU/g*	*mg/kg*	*mg/kg*	*%*	*mg/kg*	*mg/kg*	*mg/kg*	*mg/kg*
Herbaceous plants	0-20	88.1	2.62	9.0	13.6	1900000	<5.0	287	4.49	413	<5.0	154	694
	20-40	88.7	3.65	9.0	13.1	1900000	<5.0	276	5.85	406	6.7	160	516
	40-60	89.6	3.08	8.9	19.2	1700000	<5.0	984	6.19	529	9.8	170	842
	60-100	84.0	3.11	7.8	17.5	1800000	15.9	1243	1.96	737	30.9	278	582
Amaranth	0-20	86.8	2.77	8.5	11.7	1600000	<5.0	437	3.53	545	19.1	242	250
	20-40	86.9	2.61	8.5	12.9	1600000	<5.0	527	4.20	386	19.1	215	254
	40-60	89.4	2.36	8.6	13.8	1700000	<25.0	1586	3.96	663	21.3	240	243
	60-100	86.6	3.65	8.1	21.4	1800000	<5.0	267	3.65	1490	39.5	276	369
Jerusalem artichoke	0-20	89.3	3.19	8.5	12.8	1800000	<5.0	146	3.65	1010	38.6	285	369
	20-40	87.8	3.35	8.5	10.6	1800000	<5.0	137	4.03	571	34.9	250	309
	40-60	86.6	2.85	8.5	13.5	1600000	<5.0	89	2.38	502	72.9	474	280
	60-100	88.4	1.84	8.6	14.0	1600000	<5.0	65	1.51	400	101	762	393

Analysis showed that after the first year of the field trials, several important soil parameters have improved due to complex of the applied remediation means: bacterial additive, fertilizers, compost and vegetation. Unfortunately, no such trends were identified in the second year of the trial. Comparing the first year of the field trials with the second, a decrease in important soil parameters was determined. This can be explained by the fact that the conditioning soil measures such as compost was not applied in the second year.

General parameters of the contaminated soil did not have significant differences within different soil (plant) parcels, thus are described together:

- organic matter, decreased by 0.92% on average;
- electrical conductivity, increased by 0.27 mS/m on average;
- microbial biomass, decreased by 2 500 000 CFU/g on average (by 2.5 times);
- total C, decreased by 0.09% on average;
- total N, decreased by 324 mg/kg on average (by 1.5 times);
- P_2O_5, decreased by 31.41 mg/kg on average (by 1.9 times);
- K_2O, increased by 106.73 mg/kg on average;
- Mg, increased by 118.58 mg/kg on average.
- In shallower layers, the pH increased by 0.3 in the parcel of herbaceous plants (0-40 cm), but in the deeper layers (40-100 cm) the pH decreased by 0.3-0.4. In the case of amaranth and Jerusalem artichoke, the pH change trends are the same - in the 0-20 cm layer, the pH increased by 0.3-0.4; in the 20-60 cm layer, pH decreased by 0.1-0.2; In the 60-100 layer, pH increased by 0.4. In general, the soil in Šiauliai site tends to become alkaline, in all cases the determined pH values are higher than optimal for the growth of selected plants (6.0-7.0). Acidifying means need to be applied to avoid further alkalisation.

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 following Figure 6.9, although data on it can be found in the Tables 6.1 and 6.2.

According to Lithuanian legislation, the limit value for petroleum hydrocarbons in the soil in areas with low sensitivity is 200 mg/kg (MPC). 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 pollution is found in the green plot in hot spots (Figure 6.2), at a depth of 40-60 cm (Figure 6.9).

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 tilled and well homogenized. It is likely that more contaminated soil was upturned and brought to the surface; ii) TPH contaminants can migrate in the vertical direction under gravity and under the action of capillary force, convection dispersion, dissolution, volatilization, adsorption, and desorption, they can migrate in the horizontal direction. Therefore, the possibility that pollutants migrated from more contaminated layers to a less contaminated soil layers cannot be ruled out; iii) soil contamination in the Šiauliai site is uneven.

Despite all these reasons, after the 2nd year field trial the most intensive remediation effect occurred in the most contaminated areas, where the contamination from the initial value dropped more than 13 times. Herbaceous plants were sown in this parcel. Unfortunately, the two-year cycle was insufficient to reach the limit values completely. However, it is expected that this limit will be reached in the following year, assuming that the perennial herbaceous plants which were replanted in the hot spots will be more strengthened and established.

Regarding the parcel where amaranth was growing, it is difficult to evaluate the real phytoremediation potential due to the above-named reasons. However, after evaluating the phytoremediation potential of the second year of field tests, it is possible that the existing TPH pollution tends to migrate from deeper soil layers to shallower ones. In shallower soil layers, pollution is more easily treated by phytoremediation methods, so better TPH degradation results are expected next year.

In the parcel where Jerusalem artichokes was growing, the situation looks very positive. It was possible to reduce TPH pollution (max 700 mg/kg) in all evaluated depths to the limit value 2 years after the applied soil remediation solutions. In the second year of the trial, the potential of phytoremediation was several times higher than in the first year. Such positive results in the second season could be due to the updated soil cultivation and preparation methodology.

Table 6.3. Phytoremediation potential for every sampling depth

Sampling depth, cm	Herbaceous plants			Amaranth			Jerusalem artichoke		
	1st year	2nd year	Total	1st year	2nd year	Total	1st year	2nd year	Total
0-20	0.54	4.39	2.48	0.53	1.06	0.56	1.37	3.49	4.77
20-40	2.25	6.04	13.61	1.58	0.95	1.50	0.66	4.75	3.15
40-60	3.69	1.96	7.25	2.08	0.31	0.65	1.00	1.12	1.12
60-100	1.29	1.56	2.00	0.36	5.74	2.09	1.00	1.55	1.55

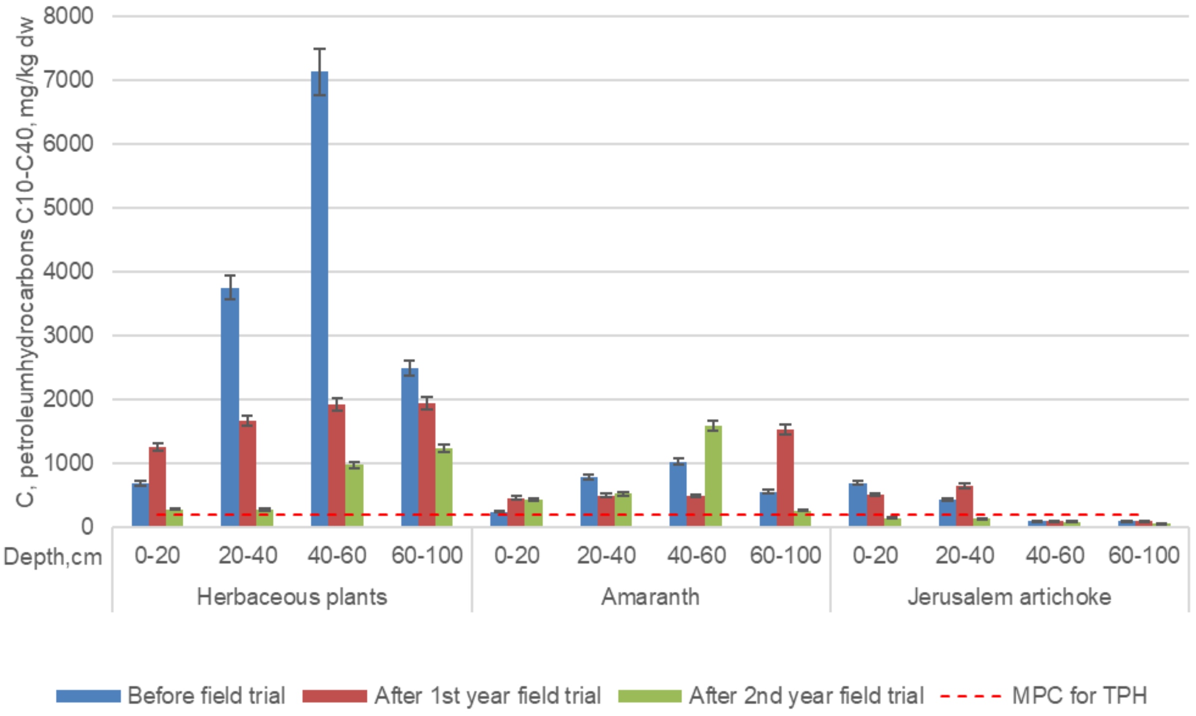

Figure 6.9. Concentration of heavy petroleum hydrocarbon fraction in the contaminated soil in Šiauliai site before and after field trials

6.6.2 Biomass output

Biomass output during the field trials was very important and closely monitored because it is an essential part for further biomass conversion to biofuels in WP3, by Fraunhofer.

Table 6.4 presents biomass output obtained in the Šiauliai site during the second year of field trials and a recalculated output for one hectare.

Herbaceous plants mix:

Compared to the last year's results (2022), year 2023 results are significantly better. Biomass yield increased by 12.5 times (from 1.30 to 16.23 t/ha dw) in the case of herbaceous plants. This is not surprising, since the selected herbaceous plants are perennials. The first year of growth was a period of adaptation, especially considering that the plants were grown in poor and contaminated soil. In case, in year 2022 there was only 2 biomass harvests of herbaceous plants, despite very dry conditions on year 2023, it was managed to harvest 3 yields. Considering the yield of control plot of herbaceous plants mix, plants were very dense and were not affected by dry conditions. The plant monitoring shows that all parameters in control plot were better compared to experimental plots. The harvest of control plot was performed same time and same method like in experimental plots. Besides this, herbaceous plants control led to uncertain high biomass yield – 40300 kg/ha dry weight summing up 3 cuts total. For comparison to agricultural land in Lithuanian conditions, according to literature, forage production of Grass–

Legume Binary Mixtures or similar herbaceous plant mixes yield about up to 13,000 kg/ha biomass dry weight.

Amaranth:

In the case of amaranth, the biomass yield increased by 8 times compared to year 2022 from 1.48 to 11.10 t/ha dw in year 2023. Such an increase in biomass yield was due to the fact that last year's practices were taken into account and mistakes made were eliminated. Additional mechanical weed control was performed. In addition, another seed supplier was selected, and seeds germination was double checked in an independent laboratory. Considering the yield of control plot of amaranth, the germination due to dry weather conditions was very poor – less than 10%. It led to significantly low number of plants per square meter. And total biomass yield of amaranth in control plot was 500 kg/ha dw. For comparison to agricultural land in Lithuanian conditions, according to literature, amaranth yields about 1-6 tonnes of seeds per hectare. The yield of aboveground biomass (dry weight) can reach up to 20 tonnes per hectare.

Jerusalem artichoke:

In case of Jerusalem artichoke yield in year 2023, it was almost 5 times higher compared to year 2022, from 3.90 to 18.31 t/ha dw of total aboveground biomass and tubers. While alone, Jerusalem artichoke aboveground biomass yield in 2023 was 10,167 kg/ha dry weight and 8,144 kg/ha tubers dry weight. This increase in yields were led by better agrotechnical solutions were adopted and row cultivation used to help deal with the increase of plant density and weed problems.
Compared to the control plot of Jerusalem artichoke in 2023, the aboveground biomass yield was 9,600 kg/ha dry weight, and tubers yield was 24,030 kg/ha dw. For comparison to agricultural land in Lithuanian conditions, according to literature, Jerusalem artichoke aboveground biomass yields about 10,000 kg/ha dry weight. The yield of tubers biomass can reach 10,100 kg/ha dry weight.

Table 6.4. Biomass output in Šiauliai site after the second year of field trials M36

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	5,980	2,003	16,233
J. artichoke aboveground	0.0870	2,465	885	10,167
J. artichoke tubers	0.0870	3,016	709	8,144
Amaranth	0.0310	1,085	344	11,100

6.7 Encountered problems and amendments

Considering the experience of the past year 2022, most of the problems were solved by adopting new agrotechnical and plant care solutions. In the case of amaranth, the seed supplier was changed and the germination of purchased seeds was re-checked in an independent laboratory.

However, even in the second year of field trials, we encountered a weed problem even though plant care products such as glyphosate were used. The selected plant care product was not effective in controlling white goosefoot (*Chenopodium album*), so these weeds had to be removed manually. Next growing season, this will be taken into account and appropriate measures will be taken to eliminate these weeds.

According to yield differences in Jerusalem artichoke compared to pot-tests and first pilot site year 2022, in the season of year 2023 the seeding rows were narrowed, from width of 75 cm in 2022, to 55 cm in 2023. This led to greater yield on the second year.

The soil in Šiauliai site tends to become alkaline, so in the next growing season this will be taken into account and certain measures will be taken to reduce the alkalinity of the soil. Sustainable agrochemical soil acidifiers can be used to achieve this goal.

As in the previous year (2022), this year (2023) there were problems with below ground Jerusalem artichoke biomass (tubers) pelletization. First, it demands a lot of energy for drying. Second, during the pelletization of biomass, the pelletizers were constantly clogging the equipment. Scientific literature considers that this cumbersome granulation process is caused by inulin polysaccharide that gives gummy-like consistency for milled Jerusalem artichoke tubers, which is very abundant in Jerusalem artichoke below ground biomass.

6.8 Other information

In addition to the main plant monitoring parameters, in the case of herbaceous plants, the composition of plants by families (*Fabaceae* and *Poaceae*) was evaluated. In hot spots, the species composition of plants according to families was divided in half - 50% *Fabaceae* and 50% *Poaceae* family plants. In the less polluted areas, the distribution of plants by family was equal at the beginning (50/50), but from the middle of the growing season, legumes such as alfalfa began to dominate (90/10).

6.9 Overall summary of phytoremediation performance in M24-M36

The second growing season has been completed without major drawbacks, with much better results than the first season in M12-24. The soil on the Šiauliai site is still contaminated with petroleum hydrocarbons. A complex, including specially selected plants, mineral and organic fertilizers, was applied to the field. Two monocultures: Jerusalem artichoke and amaranth, and a mix of herbaceous plants were grown in the field trials, and in most cases, it was possible to reach biomass outputs like in conventional agriculture. Furthermore, promising phytoremediation results regarding degradation of contaminants were obtained as in some places the contamination dropped almost 14 times from the initial value, which was measured before the start of the pilot site trials.

7. FIELD TRIALS ON THE ARGENTINIAN PILOT SITE

7.1 Soil preparation and seeding campaign

Landscape preparation, surface levelling and debris removal were not needed in the Argentinian Pilot Site. 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 (Figure 7.1).

Figure 7.1. Google Earth image showing the location of the Plots in the Argentinian Pilot Site

Different field activities were carried out in the Argentinian Pilot Site, as mentioned in the deliverable 2.3. For instance, field activities included soil preparation with agricultural machinery (tractor and motocultivator), amendment application (compost and dolomite), irrigation system installation, fence collocation, seedling planting (native plants), sowing (quinoa crop), and monitoring (weather conditions and plant growing).

The four native shrubs and trees (*Plectrocarpa tetracantha*, *Bulnesia retama*, *Larrea cuneifolia* and *Prosopis flexuosa*) selected by their metal(loid) bioaccumulation capacity were planted in March 2022 (Deliverable D2.2) and monitored up to the present moment. The native plants are scheduled to be harvested between October and November 2024.

In the case of the quinoa crop (*Chenopodium quinoa*), seeds were directly sown in the 64 m^2 experimental plots. In January 2023, biomass harvesting was carried out after the first quinoa cycle. In February 2023, soil preparation included compost application in Plot 1 and compost and dolomite application in Plot 2. Then, topsoil was tilled using a motocultivator. After soil irrigation for 3 weeks, quinoa sowing was conducted. Plants were monitored during the second quinoa cycle and then, harvesting and pelletising were carried out in July-August 2023.

7.2 Monitoring program

As mentioned in deliverable 2.3, maintenance tasks were: 1) Installation of perimeter fence around the two plots; 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.

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).

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 a drip irrigation system. Briefly, 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.

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 7.2 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.

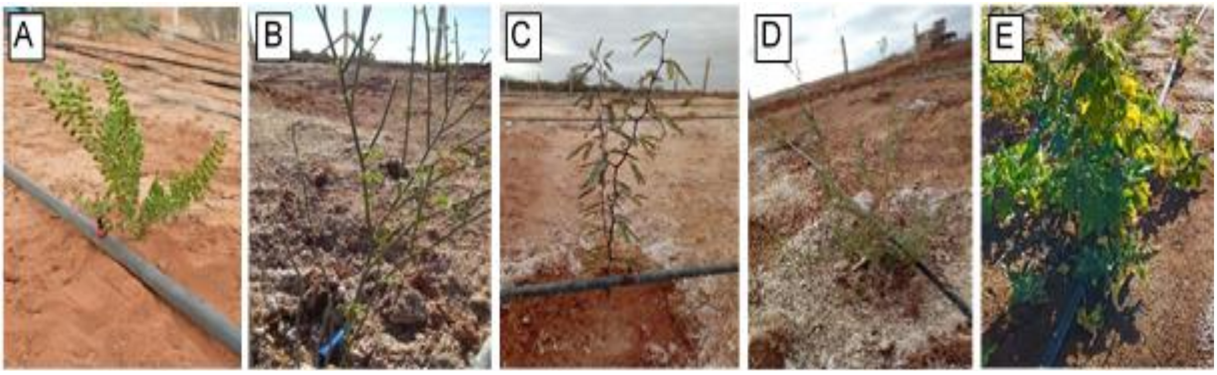

Figure 7-2. Representative pictures of plants growing in the experimental plots. A: *Larrea coneifolia*; B: *Bulnesia retama*; C: *Prosopis flexuosa*; D: *Plectrocarpa tetracantha*; E: *Chenopodium quinoa*

7.3 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[1],[2]. Temperatures are very high and reach an absolute maximum of 46 °C[3]. 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. Figure 7.3 shows the average (19.6 ºC), minimum (-3.9 ºC) and maximum temperatures (45.7 ºC) between January 2022 (M13) and October 2023 (M34). During this period, the recording of accumulated rainfall was 136.9 mm. The average relative humidity was 44.8 ± 13.2% (min: 6.0%; max: 92%), wind velocity was 10.9 ± 7.8 km/h, and ambient pressure was 1010.3 ± 10.1 hPa (min: 987.0 hPa; max: 1036.5 hPa).

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

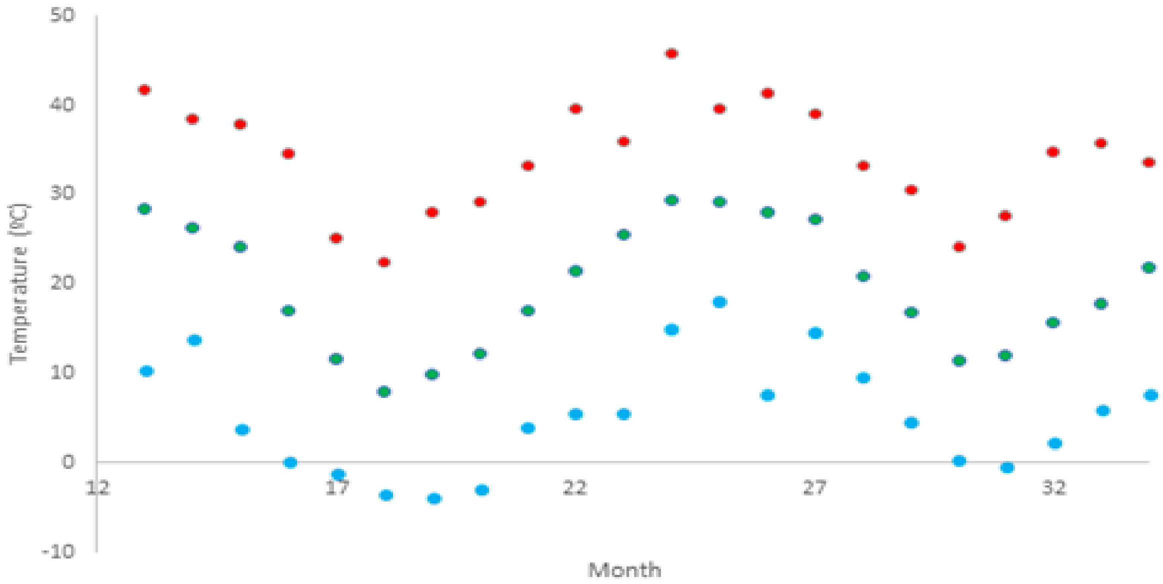

Figure 7.3. Average, minimum and maximum temperatures between January 2022 and October 2023

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[1] Poblete A, Minetti J, 1999. San Juan Climate Spatial Configuration. Synthesis of the Quaternary of the San Juan Province. Geology Institute Dr. Pedro Aparicio (INGEO). School of Exact, Physical and Natural Sciences. National University of San Juan (in Spanish).

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

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

7.4 Harvest and pelletizing

Biomass harvesting of the first and second cycle of quinoa crop was done manually in January 2023 and July 2023, respectively (Figure 7.4). In the case of shrubs and trees, harvesting was done between October and November 2023 Then, biomass was dried, crushed and pelletised. After that, pellets were shipped to Germany (Fraunhofer – WP3).

Figure 7.4. Chenopodium quinoa dried and pelletised after harvesting between December 2022 and January 2023

7.5 Phytoremediation performance

7.5.1 Soil parameters

The physicochemical characterisation of the plots was carried out after the first and second cycle of quinoa harvesting. Soil samples were taken in five different depths (0-100 cm) and the corresponding analyses were carried out. Tables 9.12 and 9.14 in the Annex show the physicochemical parameters after the first cycle of quinoa in Plot 1 and 2, while Table 9.15 in the Annex show the physicochemical parameters after the second cycle of quinoa in two sub-parcels of the Plot 2: quinoa, and control without quinoa. Plot 1 was not sampled due to quinoa biomass was completely harvested by ants twice (see section 7.6).

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.

Changes in soil quality parameters after the first growing season of quinoa crop are shown in Table 7.1.

Table 7.1. Changes in soil quality parameters after the first growing season of quinoa crop

Parameter	Plot 1	Plot 2
pH	pH increased from acidic to alkaline due to dolomite application (amendment)	pH value did not change
EC	EC decreased 79%	EC decreased 53%
OM	OM increased due to compost application (amendment)	
N	N content increased due to N fertiliser and compost application (amendment)	
Available P	P content increased due to compost application (amendment)	
Available K	K content increased	K content decreased
Texture	Sand % increased, while Clay and Silt % decreased	Clay % increased, while Silt % no changed and Sand % decreased
EC: electrical conductivity, OM: organic matter		

Data comparison of the main metal(loid)s in soil between the initial characterisation (before field activities) and the second characterisation (after first quinoa crop) are shown in Table 7.2.

Table 7.2. Metal(loid)s in soil of the initial characterisation (before field activities) and the second characterisation (after first quinoa crop)

Metal(loid)	Plot	Initial characterisation			Second characterisation		
		Mean	Min	Max	Mean	Min	Max
Cu	1	131.2	74.80	201.8	67.39	30.59	106.9
	2	826.1	454.4	1070	785.1	446.3	1041
Zn	1	5880	1731	9058	2882	1488	3674
	2	2227	1464	3710	2071	1616	2533
As	1	4789	3293	7113	6216	1955	10175

	2	2383	185.6	4273	5660	38716	83321.8
Cd	1	52.64	17.09	81.42	5.49	2.74	10.04
	2	19.34	12.35	29.62	11.07	7.13	15.07

Metal(loid) concentrations determined in soil total and soluble fractions of the quinoa rhizosphere are shown in Table 7.3.

Table 7.3. Metal(loid) concentrations in soil total and soluble fractions of the quinoa rhizosphere

Metal(loid)	Plot	Concentration in soil total fraction (mg/kg)		Concentration in soil soluble fraction (µg/kg)		Metal availability (%)	
		Mean	SD	Mean	SD	Mean	SD
Cu	1	53.12	7.93	24.05	12.93	0.046	0.026
	2	1398	235.6	37.53	3.91	0.003	0.000
Zn	1	2489	403.1	46.54	29.65	0.002	0.002
	2	3332	628.6	127.2	106.2	0.004	0.003
As	1	2120	827.7	773.7	356.2	0.047	0.043
	2	391.7	57.45	426.6	4.17	0.111	0.017
Cd	1	24.95	9.29	13.88	8.40	0.053	0.033
	2	19.88	7.11	44.51	12.93	0.229	0.043

7.5.2 Biomass output

The native shrubs and trees (*Bulnesia retama*, *Larrea coneifolia*, *Prosopis flexuosa* and *Plectrocarpa tetracantha*) that were planted in early 2022, continue growing in both plots. While it was already possible to harvest quinoa crop (*Chenopodium quinoa*).

As the main findings, highlight that three varieties of quinoa crop were sown in the plots to test their yield under field conditions (Figure 7.5). 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 7.4). 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 7.5. Quinoa crop growing in Argentinian Pilot Site before the first harvesting

Table 7.4. 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

Concentrations of Cu, Zn, As and Cd determined in quinoa biomass, Bioaccumulation Factors (BAF) and Translocation Factors (TF) are shown in Table 7.5. Quinoa has accumulated very high concentrations of these four metals(loid)s in its tissues. In particular, Zn was the element with highest values of TF, while Cd was the element with highest values of BAF. The highest TFs were found in Plot 2, where plants produced more biomass.

Table 7.5. Phytoextraction of metal(loid)s by quinoa crop after the first harvesting

Metal(loid)	Plot	Concentration in biomass (mg/kg)		BAF		TF	
		Mean	SD	Mean	SD	Mean	SD
Cu	1	164.5	46.82	3.20	1.12	1.89	0.85
	2	1140	402.4	0.87	0.42	2.26	0.72
Zn	1	3345	2738	1.44	1.14	11.52	0.00
	2	6633	5237	2.04	1.58	33.00	20.35
As	1	981.4	460.6	0.52	0.29	2.91	2.14
	2	517.6	69.51	1.35	0.28	4.58	1.09

Cd	1	104.3	105.4	4.08	3.81	2.41	2.11
	2	1103	566.8	62.19	42.10	8.28	3.21

BAF: Bioaccumulation Factor, TF: Translocation Factor.

Regarding the native shrubs and trees, they have been monitored since planting in early 2022 (Figure 7.6). Plants present good growth rate and look healthy. All the plant species from Plot 2 are taller than those growing in Plot 1.

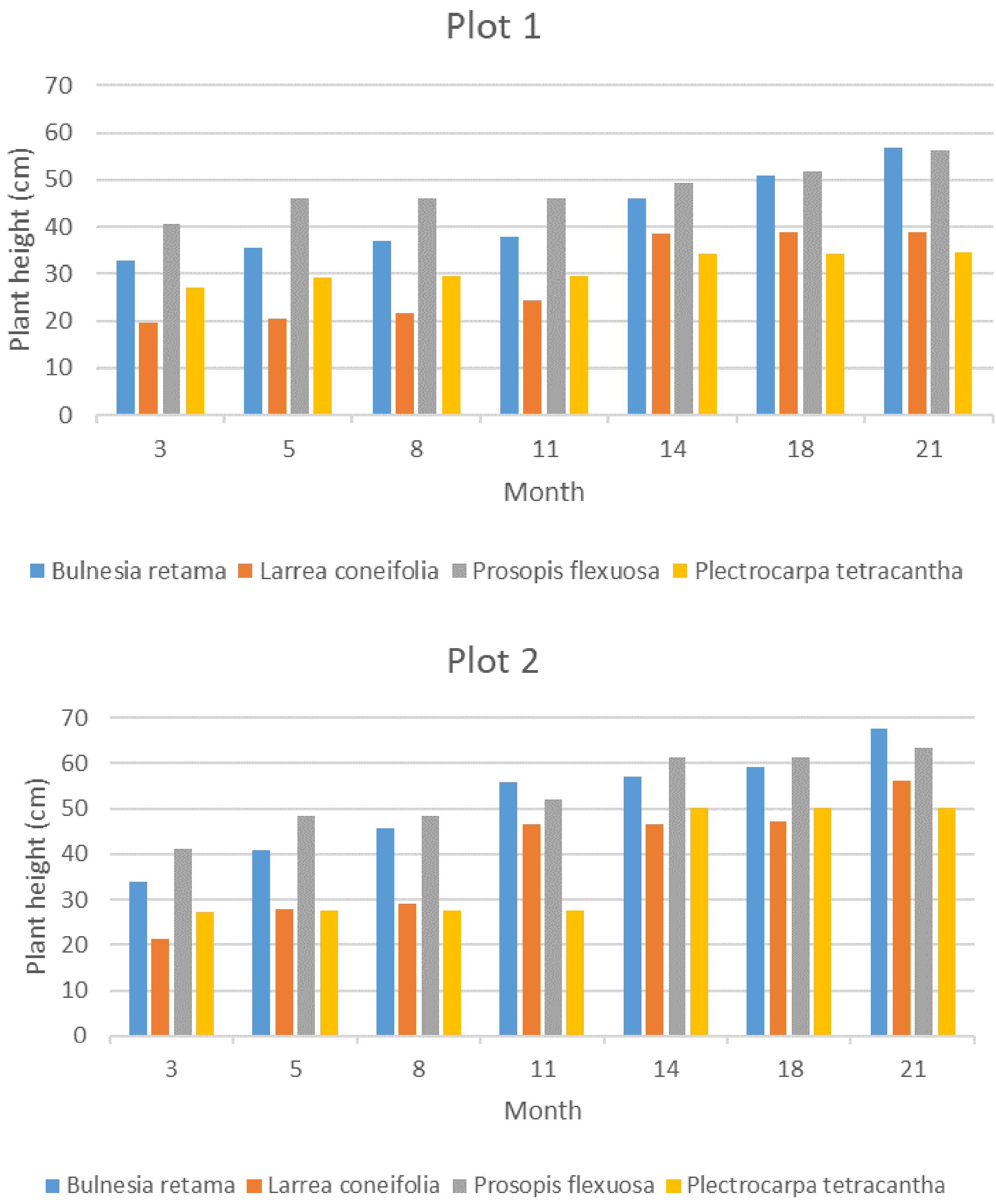

Figure 7.6. Native shrubs and trees height monitored since planting in early 2022

7.6 Encountered problems and amendments

No problem was encountered at the Argentinian Pilot Site during this period [M24-M36], except ants harvested all the quinoa biomass from the Plot 1 twice during the second quinoa cycle. However, it was no affect the biomass production for biofuel conversion (WP3) due to the biomass reached in Plot 2.

7.7 Other information

After acute exposure, seed germination of quinoa was not inhibited, but root elongation was strongly inhibited (Table 7.6). Regarding root elongation inhibition, a typical concentration-response curve was observed between 0.01% and 5%. However, a maximum of 90% inhibition was observed in the concentration range of 5% and 100%, as shown in Figure 7.7.

Table 7.6. Toxicological endpoints estimated in the acute toxicity test on quinoa (*Chenopodium quinoa*)

Seed germination	Root elongation	Phytotoxicity index	
IC_{50}	IC_{50}	$RGIC_{0.8}$	$GIC_{80\%}$
ND	2.29%	0.30%	0.22%

IC_{50}: Inhibitory Concentration 50%, RGIC: Relative Growth Index Concentration, GIC: Germination Index Concentration, ND: not determined because 50% inhibition was not reached.

Figure 7.7. Representative picture of the seedlings at the 100% concentration after acute exposure of quinoa (Chenopodium quinoa) to soil contaminated with mining waste

After the preliminary chronic exposure test, quinoa plants showed healthy growth in soil amended with compost and dolomite. The results showed a good growth in both treatments as shown in Table 7.7. A representative picture of quinoa plants is shown in Figure 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 days under normal conditions. An explanation of this effect could be attributed to stress.

Table 7.7. 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

After the chronic exposure test, native shrubs and trees (*Prosopis flexuosa*, *Plectrocarpa tetracantha*, *Bulnesia retama* and Larrea cuneifolia) showed healthy growth in soil amended with compost and dolomite. The Bioaccumulation and Translocation Factors of this pot test are shown in Table 9.17 in the Annex).

Figure 7.8. Representative picture of quinoa (Chenopodium quinoa) plants after a preliminary chronic exposure to contaminated soil amended with compost and dolomite

7.8 Overall summary of phytoremediation performance in M12-M36

Acute and chronic experiments showed 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 (quinoa) or planted (native trees and shrubs) 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, wind 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 and second cycle of the crop, quinoa growth variables were recorded and then harvest was carried out. Also, physicochemical characterisations were carried out after the first and second cycle of the quinoa crop. A high bioaccumulation of Cu, Zn, As and Cd was found in the quinoa biomass. Finally, quinoa crop was pelletised and sent to Fraunhofer (WP3) in order to assess the potential in biofuel production.

8. ACKNOWLEDGEMENTS

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9. ANNEX

Tables from the Spanish Pilot Site

Table 9.1. Pre-sowing physicochemical characterization of soil at 30 cm (*Brassica napus* 2022). Part 1

Parcel	Texture			Texture class	pH$_{(water)}$	pH$_{(KCl)}$	EC	Water content	Mg	Ca	Cu
	Clay	Silt	Sand								
	%	%	%	-	-	-	µS/cm	%	mg/kg dw	mg/kg dw	mg/kg dw
E1.1					8.3±0.03		288±6	0.88±0.68	21991±1098	210610±5654	17±1
E2.1					8.2±0.04		369±1	1.01±0.51	24172±1524	213726±19968	18±1
E2.2					8.1±0.02		313±15	1.01±0.51	24251±1499	228773±14847	20±1
E4.1					8.0±0.01		333±9	1.49±0.58	23631±1409	217478±4832	19±0
E4.2					8.1±0.01		342±15	0.99±0.29	22921±1012	210498±11334	19±1
C1.1					8.0±0.03		396±16	1.00±0.78	19226±1110	173270±14879	27±2
C1.2					8.0±0.03		288±6	1.47±0.57	18268±273	150892±4599	29±3

Table 9.2. Post-harvesting physicochemical characterization of soil at 30 cm (*Brassica napus* 2022). Part 2

Parcel	Texture			Texture class	pH$_{(water)}$	pH$_{(KCl)}$	EC	Water content	Mg	Ca	Cu
	Clay	Silt	Sand								
	%	%	%	-	-	-	µS/cm	%	mg/kg dw	mg/kg dw	mg/kg dw
E1	15	25	60	Sandy-loam	8.6 ± 0.01	±	390±8	2.1±0.25	31,676±1,338	218,755±2,337	21±1
E2	13	23	64	Sandy-loam	8.4 ± 0.02	±	357±13	1.6±0.28	31,996±545	223,327±1,506	21±0.2
E3	13	22	65	Sandy-loam	8.4 ± 0.1	±	418±20	0.9±0.01	30,385±747	218,701±5,928	25±3
E4	13	27	60	Sandy-loam	8.5 ± 0.02	±	380±12	1.4±0.44	31,426±267	220,521±6,377	37±26
C1	10	15	75	Sandy-loam	8.6 ± 0.1	±	383±27	0.9±0.43	28,605±1,706	177,404±6,928	30±1
C2	10	13	77	Sandy-loam	8.3 ± 0.03	±	578±16	1.2±0.54	29,260±2,793	205,391±1,121	33±1

Table 9.3. Pre-sowing physicochemical characterization of soil at 30 cm (*Brassica napus* 2022). Part 2.

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

Table 9.4. Post-harvesting physicochemical characterization of soil at 30 cm (*Brassica napus* 2022). Part 2.

Parcel	Organic matter	Humins	Mo	Zn	Total C	Total N	Cd	Cr	Pb
	%		mg/kg dw	mg/kg dw	mg/kg dw	mg/kg dw	mg/kg dw	mg/kg dw	mg/kg dw
E1.1	3.30±0.16		<LQ	58±2	5.47±0.43	0.069±0.022	<LQ	14±1	58±2
E2.1	3.73±0.28		<LQ	61±3	5.26±0.08	0.049±0.009	<LQ	15±1	60±6
E2.2	3.50±0.56		<LQ	62±4	5.89±0.36	0.041±0.003	<LQ	16±2	80±7
E4.1	3.88±0.17		<LQ	59±3	5.52±0.16	0.046±0.003	<LQ	15±0	75±2
E4.2	3.66±0.09		<LQ	56±3	5.28±0.60	0.038±0.015	<LQ	15±1	80±7
C1.1	3.67±0.40		<LQ	180±6	5.16±0.27	0.056±0.008	<LQ	15±0	83±5
C1.2	3.54±0.12		<LQ	689±154	4.92±1.02	0.043±0.009	<LQ	21±0	75±16

Table 9.5. Pre-sowing physicochemical characterization of soil at 30 cm (*Brassica napus* 2022). Part 3.

Parcel	P available	K available	S	B	As	Na	Microbial biomass
	mg/kg dw	*mg/kg dw*	*mg/kg dw*	*mg/kg dw*	*mg/kg dw*	*mg/kg dw*	*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	$1.83x10^{6}$

Table 9.6. Post-harvesting physicochemical characterization of soil at 30 cm (*Brassica napus* 2022). Part 3

Parcel	P available	K available	S	B	As	Na	Microbial biomass
	mg/kg dw	*mg/kg dw*	*mg/kg dw*	*mg/kg dw*	*mg/kg dw*	*mg/kg dw*	*CFU/ml*
E1.1	26±1	103±5	669±30	<LQ	8±0	212±81	$x10^{6}$
E2.1	29±5	109±4	754±173	<LQ	8±0	551±180	$x10^{6}$
E2.2	20±1	72±14	765±29	<LQ	9±1	267±30	$x10^{6}$
E4.1	24±2	56±8	758±19	<LQ	8±0	237±64	$x10^{7}$
E4.2	18±2	46±2	743±9	<LQ	8±1	247±34	$x10^{6}$
C1.1	52±9	90±18	765±107	<LQ	8±1	279±20	$x10^{6}$
C1.2	46±6	101±13	616±34	<LQ	8±1	208±27	$x10^{6}$

Table 9.7. Pre-sowing physicochemical characterization of soil at 30 cm (*Brassica napus* 2022). Part 4

Parcel	P total	K total	Respirometry	Ni	Mn	Fe
	mg/kg dw	*mg/kg dw*		*mg/kg dw*	*mg/kg dw*	*mg/kg dw*
E1	94±2	163±21		*	372±12	17.32±740
E2	99±5	147±10		*	398±3	18.08±379
E3	89±7	160±11		*	384±8	16.85±1.05
E4	95±4	117±15		*	386±11	16.73±504
C1	95±7	186±18		*	331±10	16.81±279
C2	124±7	269±7		*	353±10	15.51±280

Table 9.8. Post-harvesting physicochemical characterization of soil at 30 cm (*Brassica napus* 2022). Part 4

Parcel	P total	K total	Respirometry	Ni	Mn	Fe
	mg/kg dw	*mg/kg dw*		*mg/kg dw*	*mg/kg dw*	*mg/kg dw*
E1.1	439±26	3450±198		14±0	325±16	14477±1028
E2.1	534±56	3474±53		15±1	336±20	15376±750
E2.2	522±18	3824±425		15±1	338±24	15813±1210
E4.1	466±69	3216±81		15±1	344±15	14960±585
E4.2	426±90	3564±511		14±1	326±19	14915±804
C1.1	566±79	2911±104		14±1	288±19	15900±380
C1.2	439±8	2576±106		17±0	288±64	17252±2430

Table 9.9. Pre-sowing physicochemical characterization of soil at 30 cm (*Sorghum* 2023). Part 1

Parcel	Texture			Texture class	pH$_{(water)}$	pH$_{(KCl)}$	EC	Water content	Mg	Ca	Cu
	Clay	Silt	Sand								
	%	%	%	-	-	-	µS/cm	%	mg/kg dw	mg/kg dw	mg/kg dw
E1.1	18	26	56	Sandy loam	8.27±0.09	7.84±0.01	394±13	2.45±0.49	24709±1532	209220±3824	19±5
E2.1	17	29	54	Sandy loam	8.20±0.03	7.80±0.02	400±8	1.00±0.60	22332±1631	202891±10355	19±1
E2.2	18	33	49	Loam	8.10±0.04	7.78±0.01	414±3	1.48±0.57	21502±104	188603±8159	18±1
E4.1	16	35	49		8.00±0.02	7.77±0.01	482±6	0.98±0.76	22168±1008	193791±7429	18±2
E4.2	16	35	49		8.10±0.01	7.82±0.01	412±1	1.51±0.78	23499±588	203861±9420	18±1
C1.1	12	27	61		8.10±0.01	7.97±0.01	436±12	1.52±0.76	21321±6017	161274±43129	29±13
C1.2	12	27	61		8.10±0.03	7.97±0.01	338±19	1.50±0.50	20176±514	166743±16578	27±5

Table 9.10. Post-harvesting physicochemical characterization of soil at 30 cm (*Sorghum* 2023). Part 1

Parcel	Texture			Texture class	pH$_{(water)}$	pH $_{(KCl)}$	EC	Water content	Mg	Ca	Cu
	Clay	Silt	Sand								
	%	%	%	-	-	-	µS/cm	%	mg/kg dw	mg/kg dw	mg/kg dw
E1.1	17	41	42	Loam	8.23±0.02	8.13±0.02	348±1	13.20±1.34	21279±1095	162142±8943	19±1
E2.1	19	32	49	Loam	8.22±0.03	8.13±0.02	355±3	14.12±1.81	21952±652	173115±4781	20±1
E2.2	20	37	43	Loam	8.16±0.02	8.06±0.01	399±6	19.42±5.90	24262±1948	174643±13543	22±1
E4.1	17	40	43	Loam	8.17±0.01	8.04±0.01	379±4	10.49±2.86	22447±338	173980±1699	20±0
E4.2	17	40	43	Loam	8.16±0.02	8.09±0.01	414±8	11.58±1.14	21978±627	179146±2040	20±1
C1.1	14	30	56	Sandy loam	8.24±0.05	8.18±0.02	279±6	8.64±0.96	21969±1272	145653±9779	27±4
C1.2	14	30	56	Sandy loam	8.22±0.02	8.12±0.01	294±4	14.66±3.00	17286±911	148480±7172	30±2

Table 9.11. Pre-sowing physicochemical characterization of soil at 30 cm (*Sorghum* 2023). Part 2

Parcel	Organic matter	Humins	Mo	Zn	Total C	Total N	Cd	Cr	Pb
	%		mg/kg dw	mg/kg dw	mg/kg dw	mg/kg dw	mg/kg dw	mg/kg dw	mg/kg dw
E1.1	4.28±0.10		<LQ	57±2	5.36±0.66	0.047±0.003	<LQ	15±1	60±2
E2.1	4.45±0.18		<LQ	60±4	5.80±0.25	0.060±0.006	<LQ	15±1	64±5
E2.2	4.88±0.41		<LQ	56±3	6.04±0.48	0.061±0.005	<LQ	14±0	63±3
E4.1	5.01±0.06		<LQ	57±3	5.59±0.58	0.067±0.006	<LQ	14±0	70±3
E4.2	4.47±0.20		<LQ	58±2	5.88±0.15	0.055±0.005	<LQ	14±1	83±4
C1.1	4.03±0.34		<LQ	207±56	4.61±1.02	0.050±0.002	<LQ	15±4	67±33
C1.2	3.76±0.08		<LQ	570±32	5.57±1.10	0.061±0.009	<LQ	19±3	71±12

Table 9.12. Post-harvesting physicochemical characterization of soil at 30 cm (*Sorghum* 2023). Part 2

Parcel	Organic matter	Humins	Mo	Zn	Total C	Total N	Cd	Cr	Pb
	%		mg/kg dw	mg/kg dw	mg/kg dw	mg/kg dw	mg/kg dw	mg/kg dw	mg/kg dw
E1.1	4.41±0.10		<LQ	52±5	5.63±0.34	0.045±0.003	<LQ	13±1	67±4
E2.1	4.08±0.18		<LQ	53±4	5.44±0.20	0.042±0.006	<LQ	14±1	71±0
E2.2	4.62±0.13		<LQ	58±4	5.40±0.23	0.043±0.008	<LQ	15±11	75±6
E4.1	5.56±0.32		<LQ	57±2	5.83±0.33	0.065±0.010	<LQ	14±0	80±2
E4.2	4.49±0.27		<LQ	58±3	5.68±0.55	0.054±0.022	<LQ	15±1	85±4
C1.1	3.64±0.40		<LQ	216±32	5.29±0.61	0.075±0.028	<LQ	17±6	75±4
C1.2	4.10±0.01		<LQ	478±23	5.59±0.79	0.061±0.021	<LQ	17±2	76±4

Table 9.13. Pre-sowing physicochemical characterization of soil at 30 cm (*Sorghum* 2023). Part 3

Parcel	P available	K available	S	B	As	Na	Microbial biomass
	mg/kg dw	*mg/kg dw*	*mg/kg dw*	*mg/kg dw*	*mg/kg dw*	*mg/kg dw*	*CFU/ml*
E1.1	22±1	90±2	670±72	<LQ	8±0	236±15	x10^{6}
E2.1	21±0	153±24	593±43	<LQ	8±0	326±37	x10^{6}
E2.2	21±2	121±26	590±8	<LQ	8±0	386±139	x10^{6}
E4.1	23±2	119±4	651±48	<LQ	8±1	369±34	x10^{7}
E4.2	20±1	103±1	620±23	<LQ	8±0	283±29	x10^{6}
C1.1	39±2	127±15	727±197	<LQ	10±2	260±85	
C1.2	33±3	123±19	594±75	<LQ	8±3	197±20	

Table 9.14. Post-harvesting physicochemical characterization of soil at 30 cm (*Sorghum* 2023). Part 3

Parcel	P available	K available	S	B	As	Na	Microbial biomass
	mg/kg dw	*mg/kg dw*	*mg/kg dw*	*mg/kg dw*	*mg/kg dw*	*mg/kg dw*	*CFU/ml*
E1.1	11±3	109±5	498±55	<LQ	<LQ	259±39	x10^{6}
E2.1	10±3	109±5	546±52	<LQ	<LQ	281±14	x10^{6}
E2.2	14±1	120±2	631±58	<LQ	<LQ	324±30	x10^{6}
E4.1	23±2	174±13	662±19	<LQ	<LQ	275±8	x10^{7}
E4.2	16±1	130±5	582±69	<LQ	<LQ	281±11	
C1.1	28±6	121±5	517±6	<LQ	<LQ	260±12	x10^{6}
C1.2	36±1	162±3	626±81	<LQ	<LQ	243±14	

Table 9.15. Pre-sowing physicochemical characterization of soil at 30 cm (*Sorghum* 2023). Part 4

Parcel	P total	K total	Respirometry	Ni	Mn	Fe
	mg/kg dw	*mg/kg dw*	*mg/kg dw*	*mg/kg dw*	*mg/kg dw*	*mg/kg dw*
E1.1	501±31	3407±264		14±4	326±13	14788±466
E2.1	545±65	3393±249		15±1	331±13	15503±530
E2.2	509±83	3201±59		13±0	300±4	14183±431
E4.1	528±88	3192±116		13±1	313±9	14044±436
E4.2	473±24	3320±287		14±0	336±13	14986±405
C1.1	445±155	2740±773		13±3	263±75	15043±4544
C1.2	440±98	2661±398		15±3	280±40	16779±3276

Table 9.16. Post-harvesting physicochemical characterization of soil at 30 cm (*Sorghum* 2023). Part 4

Parcel	P total	K total	Respirometry	Ni	Mn	Fe
	mg/kg dw	*mg/kg dw*	*mg/kg dw*	*mg/kg dw*	*mg/kg dw*	*mg/kg dw*
E1.1	509±36	2253±171		13±1	317±15	13216±440
E2.1	466±65	2315±55		15±3	312±17	13960±465
E2.2	636±19	2522±2086		15±0	339±22	14360±465
E4.1	549±9	2755±50		13±0	338±4	15102±903
E4.2	490±34	2761±82		15±2	327±2	14694±568
C1.1	464±19	2463±89		13±1	278±39	16206±6285
C1.2	483±21	2311±84		14±1	277±3	16763±190

Tables and Figures from the Serbian Pilot Site

Table 9.17. Basic microbiological properties of soil

Layers (cm)	******Azotobacter* **sp. $\times 10^1$**	***Ammonifiers $\times 10^6$**	**The total number$\times 10^6$**	***Oligonitrophiles $\times 10^5$**	***Fungus $\times 10^3$**	***Actinomycetes $\times 10^3$**	**Dehydrogenase activity (DHA)**
	Number of microorganisms(CFU g^{-1} absolutely dry soil)						mU g^{-1} dry soil
Landfill 1 1st initial characterisation							
0-20	290±41	216±89	341±73	204±52.6	40.8±17.7	76.6±34.2	7.17±3.04
20-40	250±33	172±66	281±91	155±44	30.8±14.5	61.8±37.7	5.85±1.98
40-60	236±48	123±20	205±96	106±50	21.5±9.3	41.8±36.9	4.31±1.19
60-100	175±62	85.6±27.6	143±74	81.7±29.3	13.2±3.62	21.1±24.1	3.20±1.26
Landfill 1 1st harvesting							
0-20	179±29	187±67	269±62	318±56	49.3±10.6	5.5±35.0	10.6±4.89
20-40	155±24	134±53	205±58	260±60	42.0±9.62	29.4±15.0	8.83±3.20
40-60	137±26	78.4±25.3	151±61	191±69	25.8±18.3	21.2±18.3	7.13±3.27
60-100	107±23	48.2±21.4	77.03±40.2	103±54.5	17.7±9.47	8.21±9.47	5.43±2.39
Landfill 1 2nd harvesting							
0-20	201±30	365±91	490±160	441±91	69.5±22.4	33.9±25.5	7.15±1.88
20-40	157±31	272±82	379±135	327±101	56.2±20.6	26.4±23.5	4.92±1.68
40-60	124±38	206±77	331±150	222±102	38.7±20.7	17.8±20.6	4.02±1.82
60-100	82±31	132±44	209±96	173±106	21.7±11.6	7.0±5.4	2.94±0.96
Landfill 2 1st initial characterisation							
0-20	93±54	388±48	327±47	340±37	67.4±12.7	76.1±24.7	8.5±2.65
20-40	72±44	241±74	255±73	282±43	48.0±14.0	55.0±24.2	6.88±1.75
40-60	64±42	183±58	197±66.9	234±54.0	35.2±11.6	37.1±20.9	6.43±1.89
60-100	42.6±30.9	118±58	115±53	133±49	27.0±13.3	25.4±24.6	5.07±1.41
Landfill 2 1st harvesting							
0-20	201±30	365±91	490±161	441±92	69.5±22.4	33.9±25.5	7.14±1.87
20-40	157±31	272±82	379±135	327±101	56.2±20.6	26.4±23.5	4.92±1.68
40-60	124±38	206±77	331±150	221±102	38.7±20.7	17.6±20.6	4.02±1.82
60-100	82±31	132±44	209±96	173±106	21.7±11.6	7.0±5.6	2.94±0.96

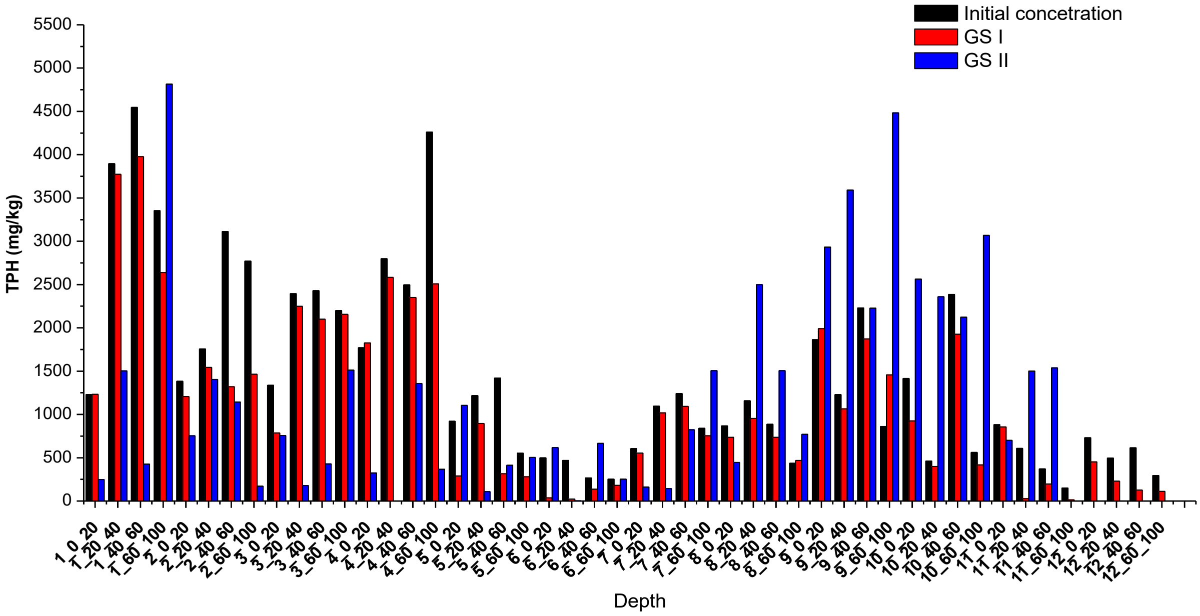

Figure 9.1. Detected concentrations of TPHs at the initial stage (black bars), after the first growing season (GS I, red bars), and after the second growing season (GS II, blue bars) in Landfill 1

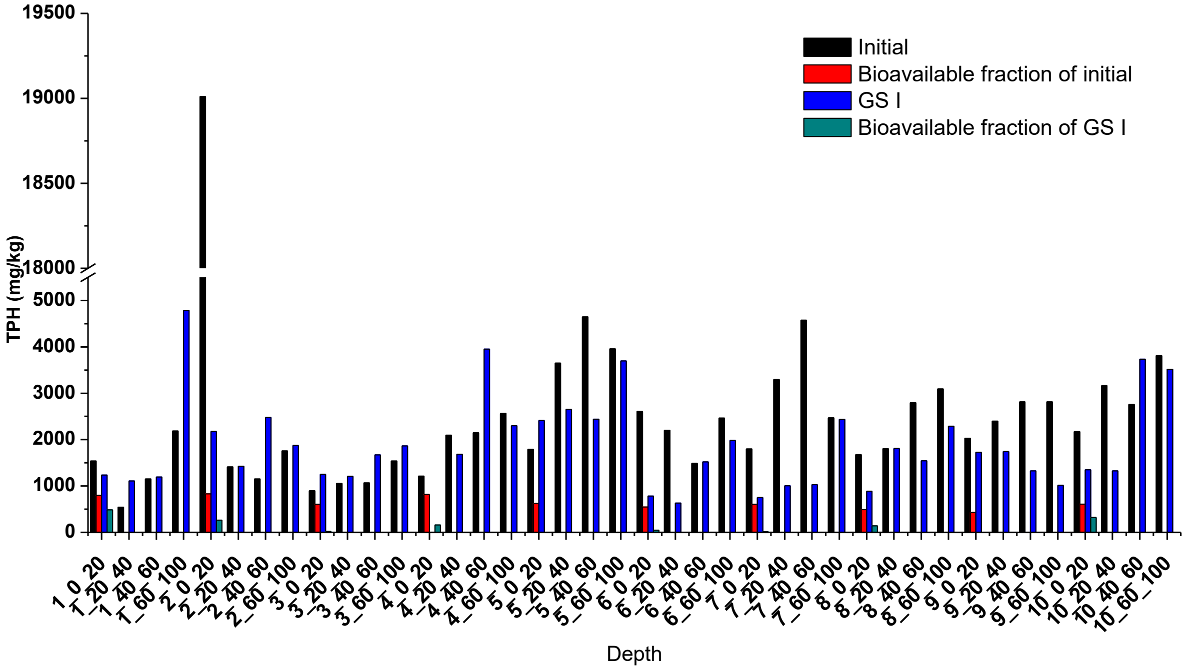

Figure 9.2. Detected concentrations of TPHs at the initial stage (showed by black bars), after the first growing season (GS I, represented by red bars), and the corresponding bioavailable fraction for Landfill 2

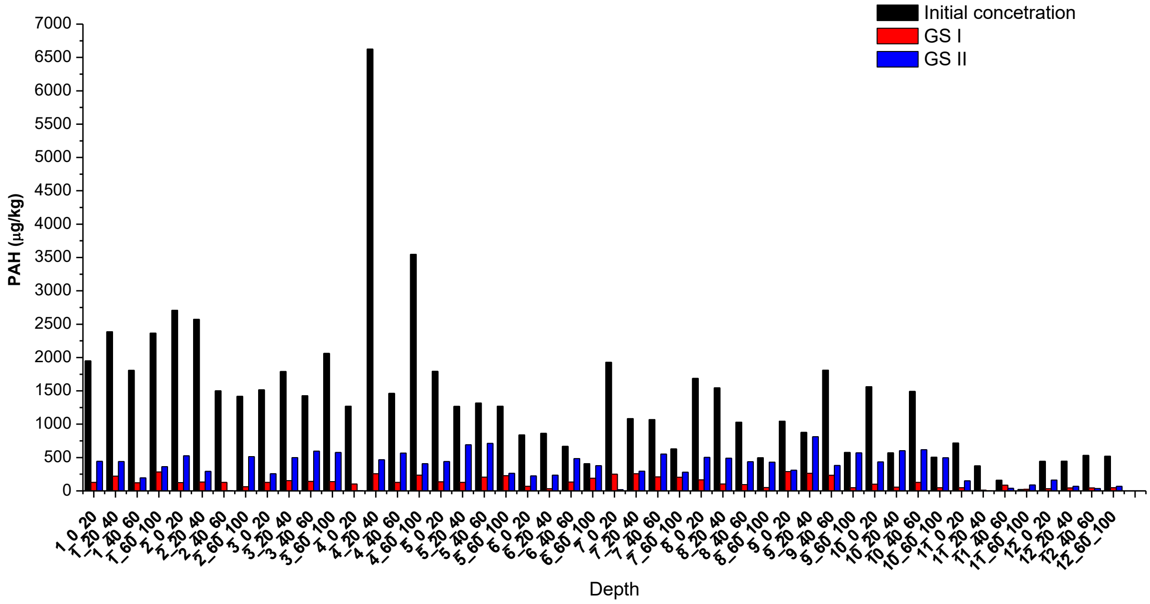

Figure 9.3. Detected concentrations of PAHs at the initial stage (black bars), after the first growing season (GS I, red bars), and after the second growing season (GS II, blue bars) in Landfill 1.

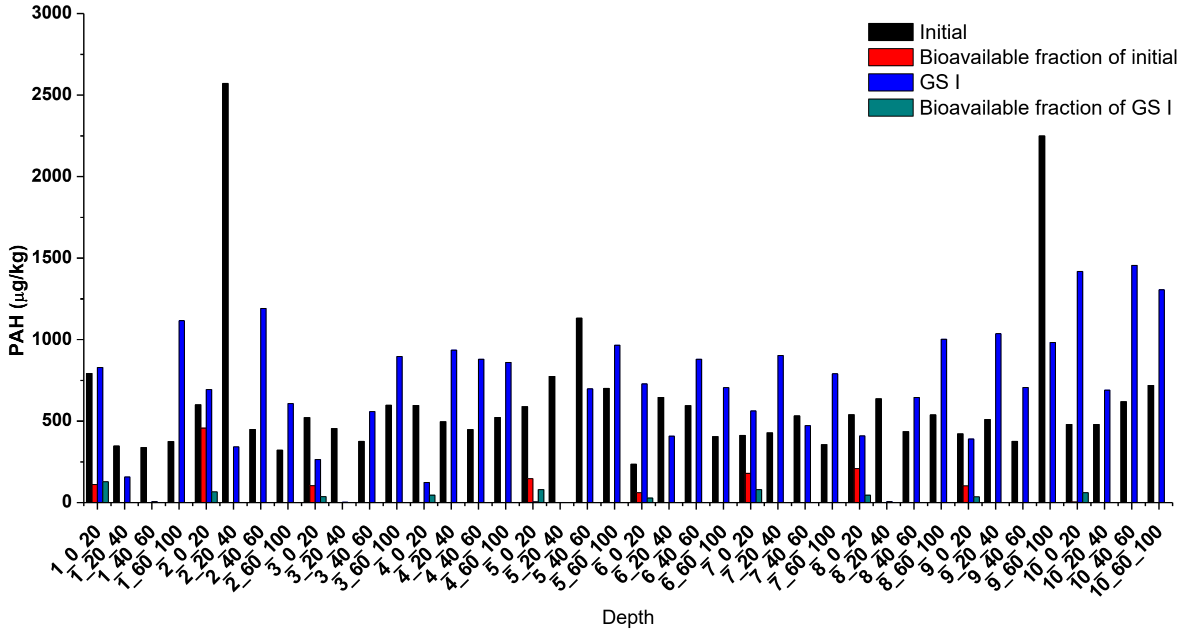

Figure 9.4. Detected concentrations of PAHs at the initial stage (black bars), after the first growing season (GS I, red bars), and after the second growing season (GS II, blue bars) in Landfill 2.

Table 9.18. Groundwater characterisation after harvesting in 2023. year

Parameters	Units	N 45° 34' 51" E 20° 45' 45"		N 45° 34' 46" E 20° 45' 43"		N 45° 34' 51" E 20° 45' 32"		N 45° 34' 50" E 20° 45' 27"	
		P1		P3		P2		P4	
Field parameters		March	July	March	July	March	July	March	July
Depth of sampling	m	2.20	3.80	3.72	3.0	4.25	4.40	4.52	8.40
Air temperature	ºC	17.9	20.2	15.8	20	15	20.3	14.8	20.8
Water temperature	ºC	16.8	17.5	16.8	17.6	16.6	17.4	16.5	17.4
pH	/	7.61	6.90	7.28	7.23	7.80	7.97	7.20	7.44
Conductivity	µS/cm	680	545	900	709	510	534	778	687
Dissolved oxygen	mgO$_2$/L	0.25	1.05	0.20	0.95	0.10	1.75	0.20	1.10
General parameters									
Total solids	mg/L	634	414	409	413	472	365	549	470
Chemical oxygen demand	mgO$_2$/L	<32	61	<32	<32	<32	<32	<32	<32
Biochemical oxygendemand	mgO$_2$/L	8	14.2	6	6	10	<4	5.4	<4
Ammonium ion	mg N/L	0.59	1.055	0.59	1.23	0.79	1.10	4.66	15.7
Nitrate	mg N/L	<0.02	0.27	0.022	0.55	<0.02	0.14	<0.02	0.07
Nitrite	mg N/L	<0.005	<0.005	0.017	<0.005	0.12	<0.005	0.025	<0.005
Chloride	mg Cl/L	15.5	17.3	19.3	18.5	19.3	16.3	18.5	14.8
Sulphate	mg SO$_4$/L	16.7	36.2	22.14	68.5	27.9	37.5	28.86	54.5
Phosphate	mg P/L	0.39	1.61	0.07	0.02	0.08	1.12	0.014	0.26
Fluoride	mg F/L	/	/	0.234	/	0.134	/	/	/
Metals									
Fe	mg/L	17.4	9.70	5.47	6.60	67.1	7.85	52.4	51.1
Mn	mg/L	0.038	0.11	0.039	0.14	0.039	0.12	0.039	0.095
Ni	µg/L	6.79	2.35	6.36	2.45	7.58	<2.2	6.82	2.92
Zn	mg/L	0.53	<0.023	0.22	0.060	0.64	2.39	2.19	3.56
Cd	µg/L	0.16	<0.15	<0.15	<0.15	1.42	<0.15	1.29	<0.15
Cr	µg/L	5.43	1.44	11.3	1.23	3.90	<0.90	5.91	1.1
Cu	µg/L	18.1	1.56	11.5	<0.90	23.2	5.1	37.2	3.90
Pb	µg/L	12.1	21.6	9.26	<5.9	63.5	32.3	35.4	<5.9
As	µg/L	7.43	7.29	17.9	5.7	104	95.8	109	5.26
Hg	µg/L	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50
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
Trichloretilene	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
Etilbenzene	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
Vinilchloride	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	<10.0	<10.0
Acenaphthylene	ng/L	<10.0	<10.0	<10.0	<10.0	<10.0	<10.0	<10.0	<10.0
Acenaphthene	ng/L	<10.3	<10.3	<10.3	<10.3	<10.3	<10.3	<10.3	<10.3
Fluorene	ng/L	<6.15	<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	<10.3	<10.3	<10.3	<10.3	<10.3	<10.3	<10.3	<10.3
Fluoranthene	ng/L	<10.3	<10.3	<10.3	<10.3	<10.3	<10.3	<10.3	<10.3
Pyrene	ng/L	<20.5	<20.5	<20.5	<20.5	<20.5	<20.5	<20.5	<20.5
Benzo(a)Anthracene	ng/L	<20.5	<20.5	<20.5	<20.5	<20.5	<20.5	<20.5	<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	<30.0	<30.0	<30.0	<30.0	<30.0	<30.0	<30.0	<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	10.5	<5.45	7.3	<5.45	<5.45	<5.45	<5.45	<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

Table 9.19. Basic physical and chemical characterization of soil/sediment used in POT experiments

Sample	OM	CEC (cmol/kg)	pH	Eh µS/cm	Total N mg/kg	Available P Mg P_2O_5/ 100g)	Na g/kg	K g/kg	Available K Mg K_2O/ 100g	Mg g/kg	Ca g/kg
Initial sample	7.39	60.7	7.46	503	0.264	62.15	0.29	3.03		3.91	1.24
HEMP CK 6W	8.96	21.0	8.08	424	0.268	74.24	0.34	4.44	15.00	5.54	0.72
HEMP CK 8W	12.7	31.8	8.16	355	0.265	62.24	0.23	2.92	14.50	4.85	0.83
HEMP OXA 6W	8.48	43.2	7.96	474	0.236	59.36	0.20	2.44	15.50	4.30	0.66
HEMP OXA 8W	8.63	36.2	8.13	388	0.245	65.69	0.24	2.95	14.50	5.06	0.60
HEMP MAL 6W	8.69	23.6	8.12	336	0.242	74.40.	0.22	3.17	15.50	4.79	0.54
HEMP MAL 8W	8.83	21.4	7.74	293	0.286	72.07	0.29	4.54	16.80	4.28	0.61
HEMP TAR 6W	8.59	19.8	8.03	335	0.260	72.89	0.27	3.92	16.40	4.05	0.426
HEMP TAR 8W	8.62	34.2	8.12	319	0.255	71.34	0.24	2.58	15.90	4.89	0.71
HEMP GLU 6W	9.12	58.5	7.7	1126	0.264	69.30	0.27	3.54	15.00	4.47	0.45
HEMP GLU 8W	8.97	40.5	7.97	435	0.239	67.30	0.30	4,24	14.50	5.39	0.60
SORGHUM CK 6W	8.58	39.6	7.86	567	0.242	68.18	0.38	5.67	18.20	4.56	0.47
SORGHUM CK 8W	4.66	27.4	8.07	334	0.255	67.79	0.30	3.94	15.50	5.27	0.77
SORGHUM OXA 6W	8.93	25.8	8.15	457	0.264	67.99	0.33	4.51	18.20	4.32	0.39
SORGHUM OXA 8W	9.46	35.1	8.05	289	0.242	70.16	0.29	3.59	15.50	5.36	0.72

SORGHUM MAL 6W	8.91	25.1	7.95	523	0.243	72.94	0.35	4.86	16.40	4.33	0.52
SORGHUM MAL 8W	8.59	25.6	7.97	457	0.217	72.51	0.42	5.88	15.90	4.22	0.31
SORGHUM TAR 6W	10.08	20.4	8.12	44	0.224	71.51	0.29	4.30	16.80	4.69	0.40
SORGHUM TAR 8W	8.53	22.4	8.18	243	0.264	64.59	0.39	5.85	15.90	4.54	0.37
SORGHUM GLU 6W	9.29	44.4	7.45	1189	0.253	65.53	0.29	3.32	17.30	5.14	0.50
SORGHUM GLU 8W	9.45	31.0	7.83	602	0.264	68.07	0.33	5.33	15.90	4.84	0.46

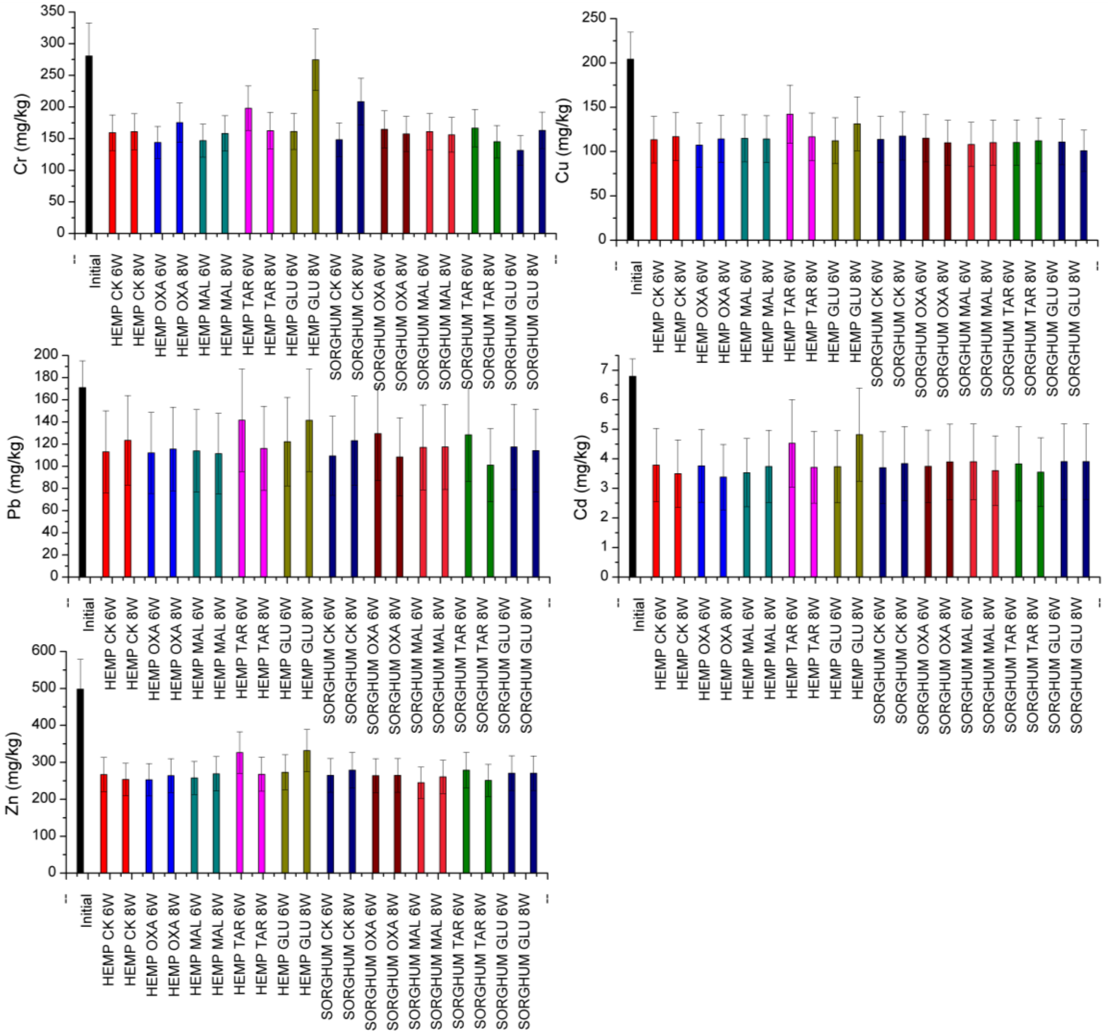

Figure 9.5. Metals and metalloids concentration in the soil during the port experiment

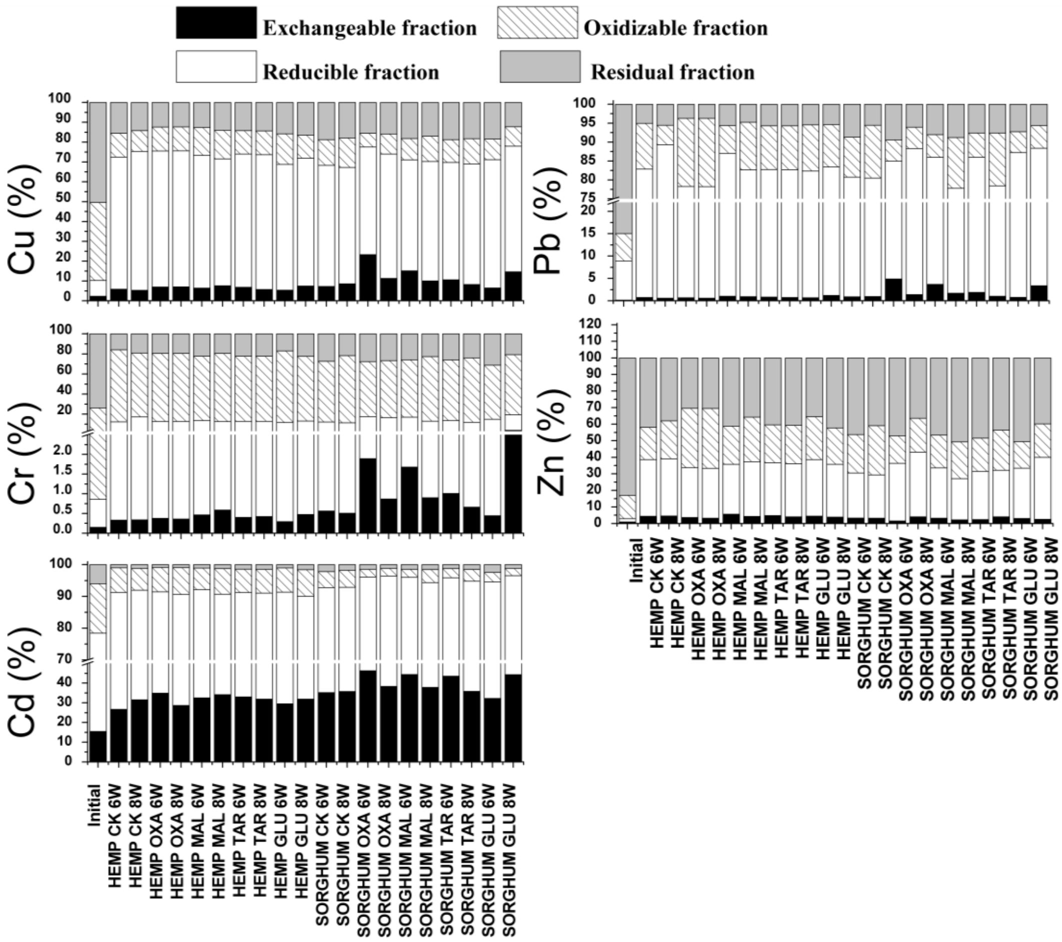

Figure 9.6. Results of the sequential metal(oid)s extraction – BCR

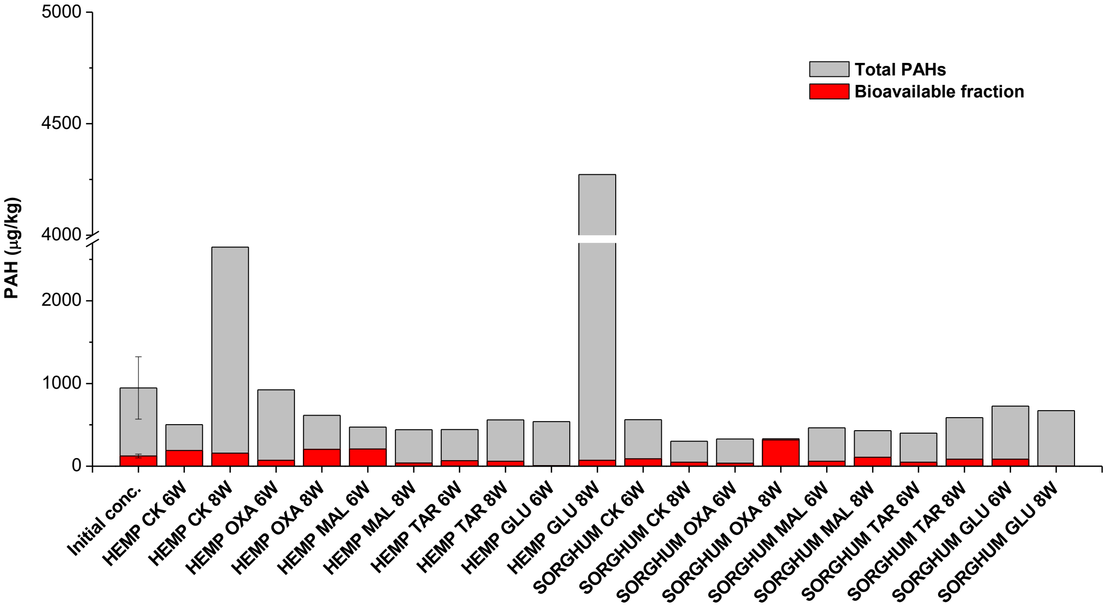

Figure 9.7-1. Total and bioavailable fraction of OCP, PCB, PAHs and TPH during different treatments in 2023 growing season

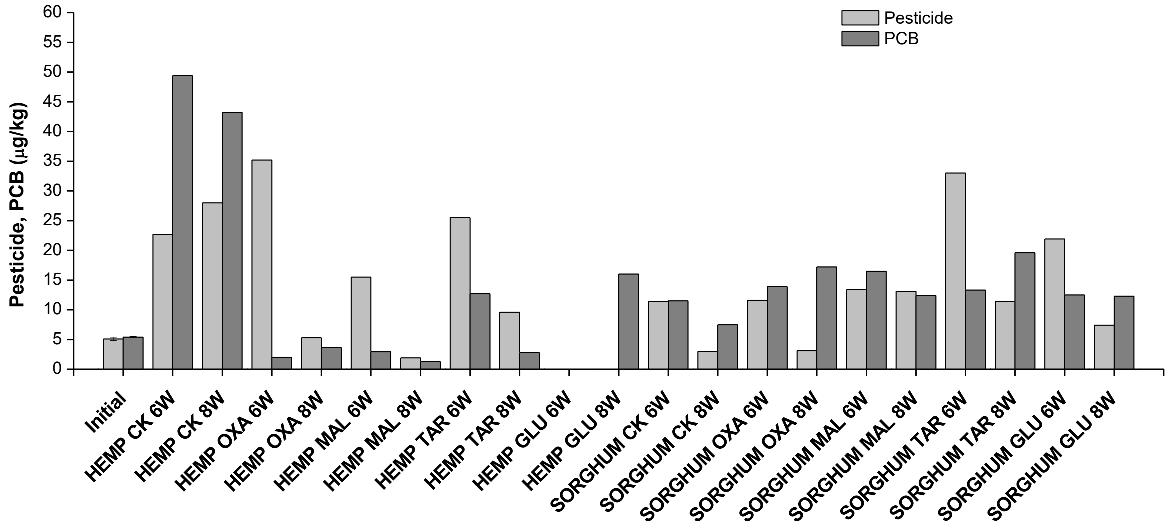

Figure 9.7-2. Total and bioavailable fraction of OCP, PCB, PAHs and TPH during different treatments in 2023 growing season

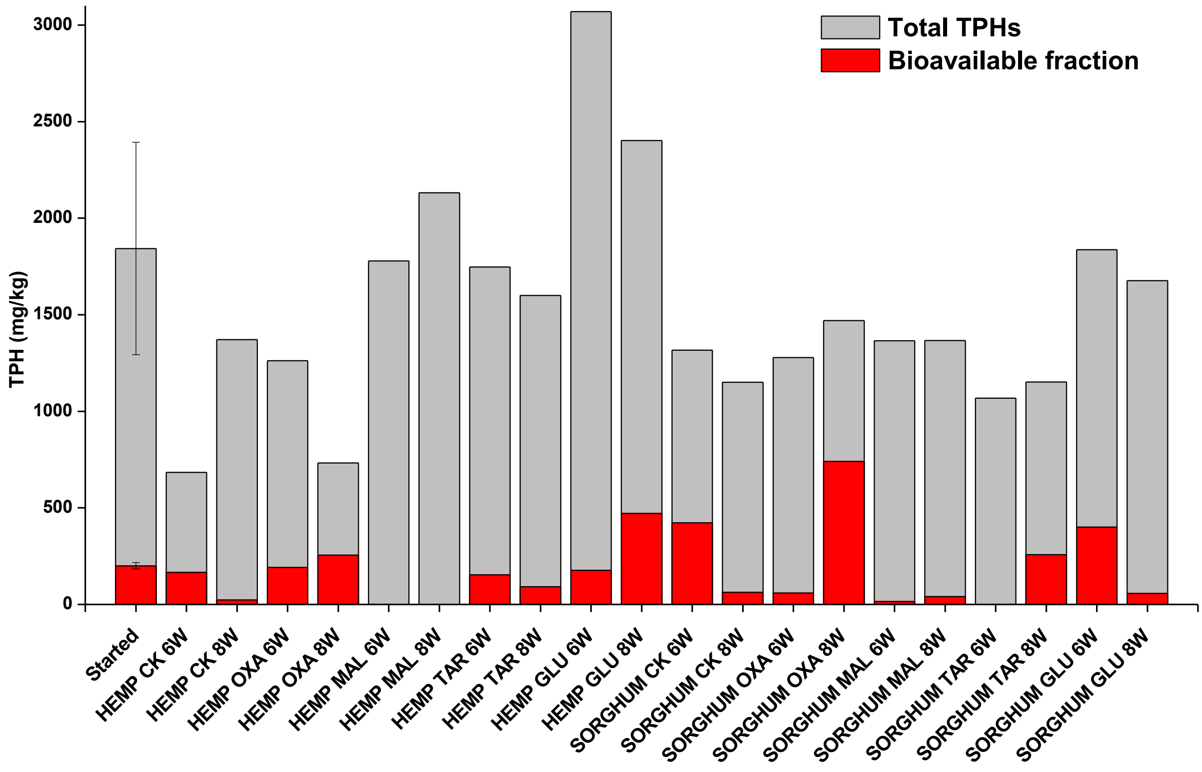

Figure 9.7-3. Total and bioavailable fraction of OCP, PCB, PAHs and TPH during different treatments in 2023 growing season

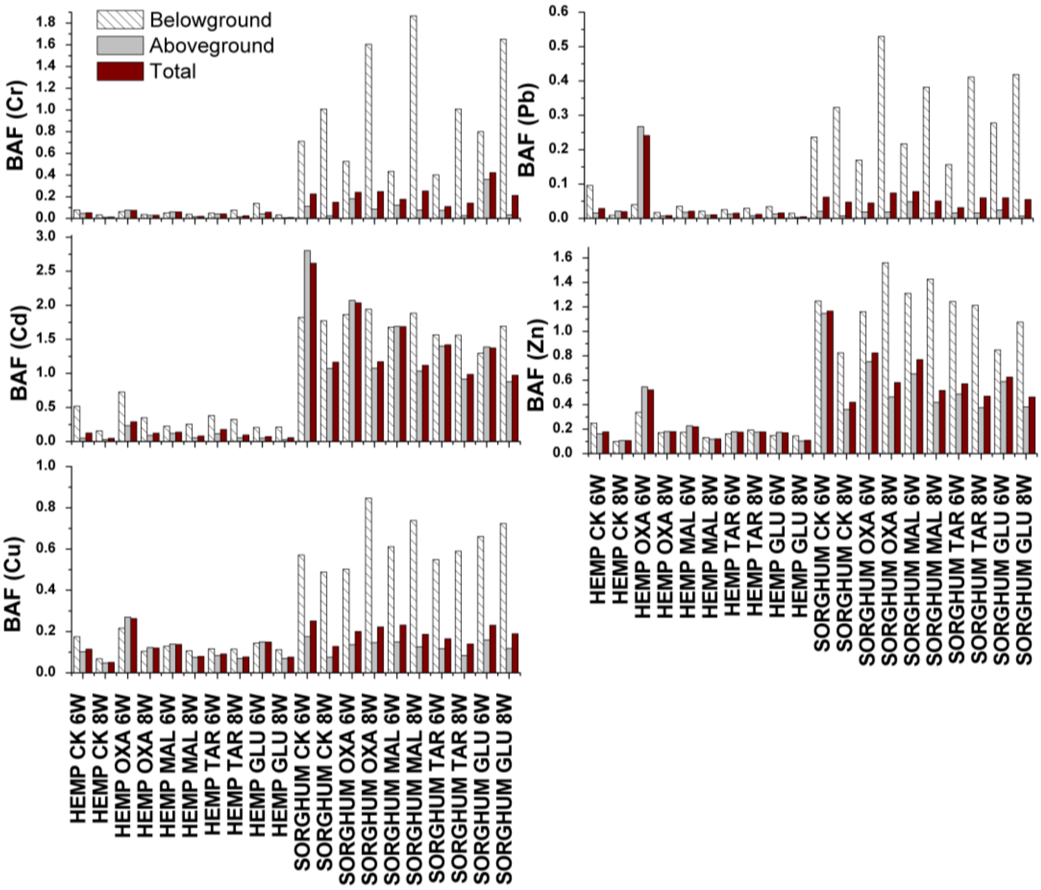

Figure 9.8. Bioaccumulation factor in the POT test

Tables from the Argentinian Pilot Site

Table 9.20 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	813±390	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	219±154	202±65	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	35±26	28±15	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	24±16	30±10	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	10±7	25±8	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	1.85E^{5}± 1.42E^{5}	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	1.22E^{5}± 9.75E^{4}	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	5.67E^{4}± 5.92E^{4}	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	1.67E^{5}± 1.26E^{5}	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	3.67E^{5}± 3.47E^{5}	ND	ND	20±13	ND	234±81	6564±5715	6662±6168

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

Table 9.21 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	21±13	51±20	11324±5133	19744±8194	ND	ND	785±306	ND
	0.2-0.4	11±11	33±11	55±3	10±2	5±1	14218±6456	15±8	68±34	13721±6356	29455±3677	ND	ND	880±343	ND
	0.4-0.6	11±2	28±3	59±4	10±2	5±1	11577±4164	10±4	62±26	11950±5419	30118±5865	ND	ND	921±351	ND
	0.6-0.8	10±3	21±9	67±8	8±3	6±0	9863±5002	11±5	76±32	11907±1660	27604±8036	ND	ND	558±273	ND
	0.8-1	9±6	25±5	66±3	6±1	6±1	9678±2275	11±6	65±27	13548±1215	34085±6395	ND	ND	743±47	ND
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	2.22E^5± 9.70E^4	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	3.92E^5± 4.64E^5	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	3.02E^5± 1.05E^5	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	2.02E^5± 1.16E^5	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	1.80E^5± 9.54E^4	ND	ND	4±1	ND	194±145	5053±3613	3878±3033

Table 9.22. Third physicochemical characterisation of Site 1, Plot 2 (P2) quinoa (Mean ± SD) carried out after the second 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	NMY	NMY	NMY	NMY	7.36±0.10	6348±286.52	NMY	NMY	NMY	NMY	ND	ND	NMY	NMY
	0.2-0.4	NMY	NMY	NMY	NMY	7.23±0.08	3970±131.91	NMY	NMY	NMY	NMY	ND	ND	NMY	NMY
	0.4-0.6	NMY	NMY	NMY	NMY	7.32±0.05	3975±71.41	NMY	NMY	NMY	NMY	ND	ND	NMY	NMY
	0.6-0.8	NMY	NMY	NMY	NMY	7.43±0.04	3855±31.09	NMY	NMY	NMY	NMY	ND	ND	NMY	NMY
	0.8-1	NMY	NMY	NMY	NMY	7.38±0.02	3668±169.98	NMY	NMY	NMY	NMY	ND	ND	NMY	NMY
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	NMY	NMY	NMY	NMY	NMY	NMY	NMY	ND	ND	NMY	NMY	NMY	NMY	NMY
	0.2-0.4	NMY	NMY	NMY	NMY	NMY	NMY	NMY	ND	ND	NMY	NMY	NMY	NMY	NMY
	0.4-0.6	NMY	NMY	NMY	NMY	NMY	NMY	NMY	ND	ND	NMY	NMY	NMY	NMY	NMY
	0.6-0.8	NMY	NMY	NMY	NMY	NMY	NMY	NMY	ND	ND	NMY	NMY	NMY	NMY	NMY
	0.8-1	NMY	NMY	NMY	NMY	NMY	NMY	NMY	ND	ND	NMY	NMY	NMY	NMY	NMY

n.d.: not detected; ND: no data; NMY: no measured yet.

Table 9.23. Third physicochemical characterisation of Site 1, Plot 2 (P2) - control without quinoa (Mean ± SD) carried out after the second 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	NMY	NMY	NMY	NMY	7.27±0.08	4708± 57.95	NMY	NMY	NMY	NMY	ND	ND	NMY	NMY
	0.2-0.4	NMY	NMY	NMY	NMY	7.25±0.10	3620±57.15	NMY	NMY	NMY	NMY	ND	ND	NMY	NMY
	0.4-0.6	NMY	NMY	NMY	NMY	7.23±0.07	3535±91.74	NMY	NMY	NMY	NMY	ND	ND	NMY	NMY
	0.6-0.8	NMY	NMY	NMY	NMY	7.39±0.04	3423±43.49	NMY	NMY	NMY	NMY	ND	ND	NMY	NMY
	0.8-1	NMY	NMY	NMY	NMY	7.24±0.20	3588±231.86	NMY	NMY	NMY	NMY	ND	ND	NMY	NMY

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	NMY	NMY	NMY	NMY	NMY	NMY	NMY	ND	ND	NMY	NMY	NMY	NMY	NMY
	0.2-0.4	NMY	NMY	NMY	NMY	NMY	NMY	NMY	ND	ND	NMY	NMY	NMY	NMY	NMY
	0.4-0.6	NMY	NMY	NMY	NMY	NMY	NMY	NMY	ND	ND	NMY	NMY	NMY	NMY	NMY
	0.6-0.8	NMY	NMY	NMY	NMY	NMY	NMY	NMY	ND	ND	NMY	NMY	NMY	NMY	NMY
	0.8-1	NMY	NMY	NMY	NMY	NMY	NMY	NMY	ND	ND	NMY	NMY	NMY	NMY	NMY

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

Table 9.24. Bioaccumulation and Translocation Factors determined in shrubs and trees after chronic exposure test in soil amended with compost and dolomite

Species	Metal(loid)	BAF aerial biomass	BAF root	TF
Prosopis flexuosa	Cu	0.60	0.10	6.00
	Zn	0.30	0.20	3.50
	As	0.20	0.03	5.40
	Cd	0.30	0.01	14.90
Plectrocarpa tetracantha	Cu	0.50	0.20	3.00
	Zn	0.40	0.10	3.50
	As	0.10	0.01	8.60
	Cd	0.20	0.01	30.40
Bulnesia retama	Cu	0.10	nd	14.00
	Zn	0.10	0.20	0.60
	As	0.01	nd	20.70
	Cd	0.01	nd	3.20
Larrea cuneifolia	Cu	0.04	0.40	0.10
	Zn	0.10	0.30	0.70
	As	0.01	0.04	0.20
	Cd	0.02	nd	3.40

BAF: bioaccumulation factor, TF: translocation factor