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Phy2Climate

NEWSLETTER PHY2CLIMATE PROJECT

# Clean biofuel production and phytoremediation solutions from contaminated lands worldwide

We are pleased to share the second issue of Phy2Climate newsletter, keeping you up to date with all the latest news and developments from the project. Phy2Climate is a project funded by Horizon 2020 EU's Research and Innovation programme. The overall objective of the Phy2Climate project is to build the bridge between the phytoremediation of contaminated sites with the production of clean drop-in biofuels and bio-coke.

CONTENTS



## Phytoremediation pilots

### Pot experiments

The Pilot Sites have carried out preliminary pot tests with the aim of optimizing the phytoremediation strategy to be applied in situ, according to each specific condition (clime, contamination source/s, and soil).

First, a harmonized pot tests experimental plan, defining a common framework in which pot tests had to be performed was agreed upon. It provides sampling (including type and frequency of sampling and storing procedure for both soil and energy crops) and monitoring (including soil and energy crop characterisation) procedures and defines the main parameters of the experimental design such as controls, number of replicates and the duration of the experiments. Visual inspections have also been agreed as important indicators of the plants' response to the hostile conditions.

Particularly, the common parameters for soil characterisation included physical parameters (water content, texture), chemical parameters (pH, electrical conductivity, N, C, S, Total C (CT), Total N (NT), organic matter, Mg, Ca, B, Fe, Mn, Na, K, Cd, Cr, Cu, Pb, As, P (available), K (available), P (total), K (total), TPH, PAH), and biological parameters (microbial biomass). Whereas common

parameters for energy crop characterisation included yield of production of biomass that is an important factor to estimate the future available feedstock for biofuel/biodiesel production. However, each Pilot Site has its own characteristics, where the different contamination sources and soil conditions play a major role.
Specific targets have been pursued, depending on the site-specific characteristics such as:

- Optimization of the soil – plant species – amendments – fertilizers – biostimulants matrix.
- Biomass production including seeds, for its valorisation as feedstock for biofuels/biodiesel production.
- Seed germination under hostile conditions (contaminated soils) for later transplantation to the pilot parcels.
- Assessment of the phytoremediation mechanism (rhizosphere effect or translocation to roots/stems/leaves/seeds) to, additionally, determine the possible environmental impact of the loss of the contaminated aboveground biomass.

To meet the main and specific objectives, each Pilot Site has defined its own experimental plan to perform pot trials, based on the agreed common framework in which to conduct phytoremediation actions.
Accordingly, each Pilot Site Leader, after characterising the contaminated site and defining the main contamination sources and contamination level, has defined the set of vegetative species and amendments/fertilizers/biostimulants to be investigated as well as the specific experimental conditions (experimental design and experimental set-up).
Specific parameters for soil and energy crop characterisation have been measured together with the common ones, with a minimum frequency of 1 sampling event for season. Translocation and bioaccumulation factors have varied among pilot sites since they are strictly connected to the specific contaminants.

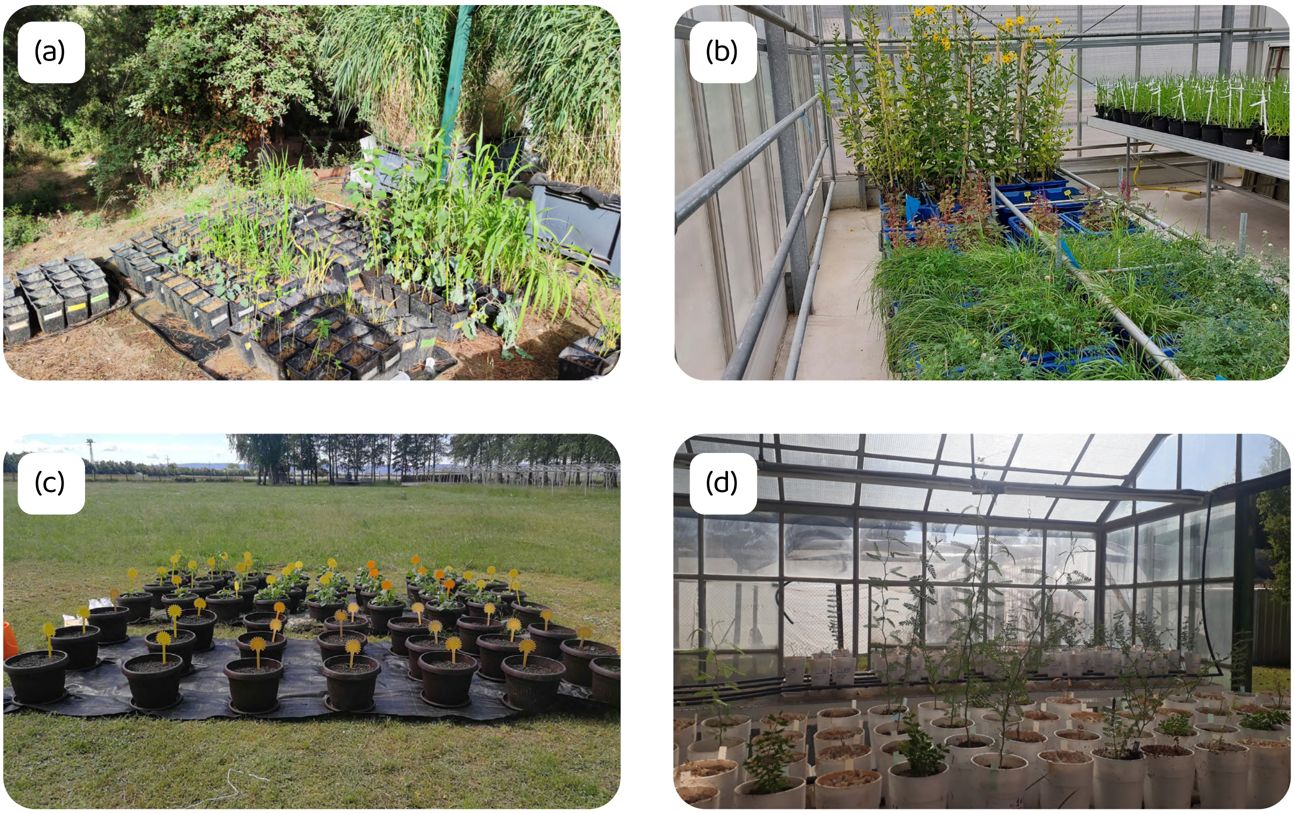



Figure 1 Pot tests performed at a) LEITAT facilities-Spain Pilot Site, Credit: LEITAT; at b) BVA facilities-Lithuania Pilot Site, Credit: BVA; at c) IFVCNS facilities-Serbia Pilot Site, Credit: IFVCNS, and at d) INTA facilities-Argentina Pilot Site, Credit: INTA.

## SPAIN PILOT SITE

- **Main contaminants**: **TPH, PAH**
- **Objective: Assess the most effective species and amendments for the phytoremediation of TPH and PAH in the Spain Pilot Site, but also considering the potential for biofuel production.**
- **Experimental design**: **24 phytoremediation treatments** (5 replicates) using **4 Vegetative species (** Sorghum sp., Brassica napus, Panicum virgatum, Helianthus annuus) combined with **5 amendments** (compost, biochar, PGPR, common fertilizer, mix of compost/biochar/PGPR) + controls. Pot test performed under natural conditions and lasted about 5 months.

**MAIN RESULTS**

- **Phytoremediation of TPH/PAH seems to be mainly due to the degradation operated by microorganisms in the soil** (lixiviation effect will be further studied)
- **The highest biomass production has been detected for *Sorghum sp.* combined with compost and with the mix of amendments**
- ***Sorghum sp.*, and the mix of amendments (compost/biochar/PGPR) will be tested in field** but in rotation with *Brassica napus* to help limiting runoff and leaching of NPK and to ensure that biomass production needs will be met (40 kg DM/season)

## LITHUANIA PILOT SITE

- **Main contaminants**: **TPH, PAH, PCB**
- **Objective: Assess the potential to degrade petroleum hydrocarbons and other organics substances in the Serbia Pilot Site using specially for this purpose designed combination of plants and amendments**
- **Experimental design**: **As vegetative species Amaranth, Jerusalem artichoke and 3 mixes of herbaceous plants were tested** and were combined **with biological additives and nutrients** (vermicast and common fertilizer). Pot tests were performed under controlled conditions in a greenhouse and lasted from 3 to 5 months, depending on the species.

**MAIN RESULTS**

- **The best phytoremediation potential of TPH was achieved using herbaceous**
- **Lighter petroleum hydrocarbon fractions were degraded easier than heavier fractions (diesel, oil, residual).** However, the decrease in concentration of all fractions was observed in all cases.
- Based on the estimation of the biomass production, **all chosen plant species have shown to successfully grown on the contaminated soil** at the given contamination levels, according to the chosen cultivation strategy.
- It has been found that **plants cultivated on contaminated soil with appropriate strategy can produce equal and if not higher biomass output**.

Figure 2
Main characteristics and results of the pot tests performed at the LEITAT facilities-Spain Pilot Site (on the left) and at BVA facilities-Lithuania Pilot Site (on the right).
Credit: LEITAT

## SERBIA PILOT SITE

- **Main contaminants**: **high level of Cu, Cr, Pb, Zn** and low level of As, Cd, Ni, OCPs, PCBs, PAHs and TPH.
- **Objective: Assess if rapeseed as energy crop, has potential to be used for phytoextraction of heavy metals in Serbia Pilot Site.**
- **Experimental design: rapeseed potential to uptake heavy metals was compared to other 3 energy crops** such as sunflower, hemp and white mustard; **3 PGPR commercial products** were tested in promoting rapeseed growth. Pot test performed under natural conditions and lasted about 10 weeks.

**MAIN RESULTS**

- **Very low bioavailable fraction of the metal(oid),** most of the metal content was distributed in the oxidable ad residual fractions (non-bioavailable fraction)
- The **contaminated sediments didn't pose stress to the plant growth and obtained biomass**
- **Rapeseed had, in general, best performance regarding the BAF,** moreover **addition of PGPR Trifinder increased its biomass and BAF for Cr**
- **TF was <1** which indicates that the **main mechanism of the metal(oid) removal is phytostabilisation** and not phytoextraction

## ARGENTINA PILOT SITE

- **Main contaminants**: **As, Cu, Zn, Cd**
- **Objective: Assess the acute and chronic toxicity of a soil contaminated with mining waste on native plant species as well as their metal(loid) bioaccumulation capacity with and without amendment application**
- **Experimental design: 4 vegetative species (**Plectrocarpa tetracantha, Bulnesia retama, Larrea cuneifolia and Prosopis flexuosa) **and 2 amendments** (dolomite and compost) **were tested.** Chronic toxicity was assessed by exposing 3 months plants to different mixtures of contaminated and reference soil in greenhouse conditions for 90 days.

**MAIN RESULTS**

- **Chronic exposure to contaminated soil causes mortality of the plants at concentrations higher than 10%.**
- **The negative effect was reverted using dolomite and compost.**
- **Bioavailability of metal(loid)s was reduced due to the increase of the pH value, which reduce the inhibition in the plant growth.**

Figure 3
Main characteristics and results of the pot tests performed at IFVCNS facilities-Serbia Pilot Site (on the left) and at INTA facilities-Argentina Pilot Site (on the right).
Credit: LEITAT

Very interesting and promising results have been obtained by each Pilot Site showing a large potential for a successful clean-up of the contaminated sites in few years.

## First results from the pilot sites

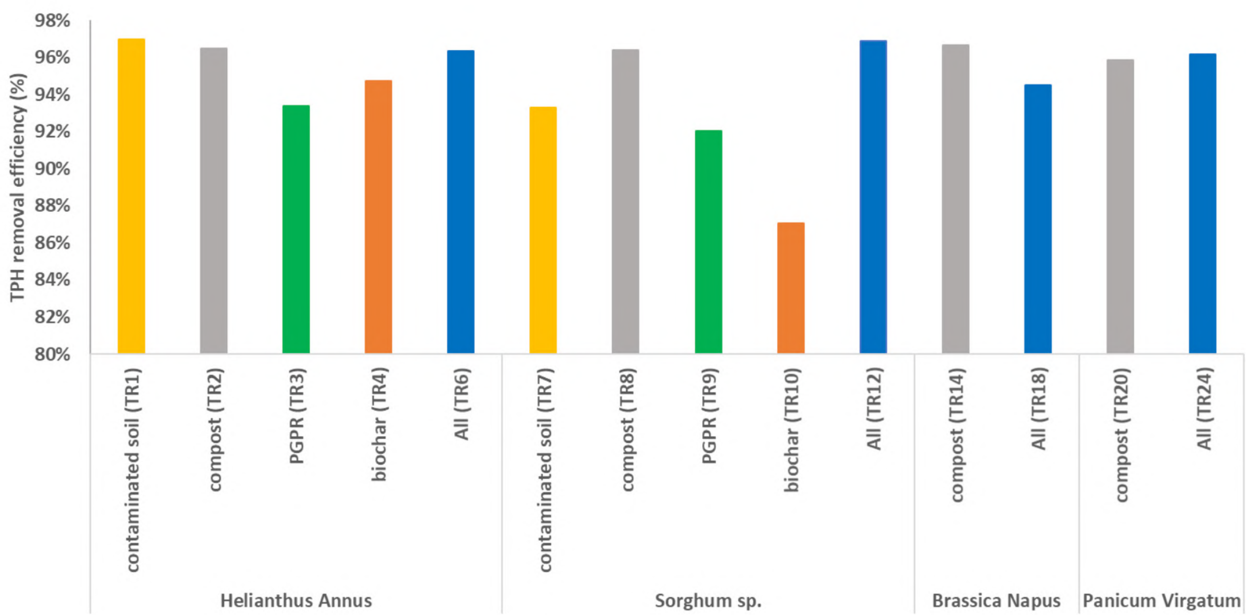
**Spain Pilot Site**: The preliminary characterisaWtion of the Spain Pilot Site has highlighted Total Petroleum Hydrocarbons (TPH) and Polycyclic Aromatic Hydrocarbons (PAH) as main contaminants. Hence, pot tests have been performed with the aim of i) determining the most effective species and amendments for the phytoremediation of TPH and PAH, but also considering the potential for biofuel production, in line with the main goal of valorisation of the produced biomass.

For this purpose, an experimental design consisting of 24 different phytoremediation treatments has been performed. Four vegetative species have been selected namely Sorghum sp., Brassica napus, Panicum virgatum, Helianthus annuus and have been tested alone and in combination with 5 amendments: 1) compost 2) biochar 3) PGPR 4) common fertilizer and 5) mix of compost/biochar/PGPR. Pot tests were performed in an outdoor experimental area at LEITAT facilities and have lasted about 4 months (from sowing to harvesting).

The pot tests have stressed that all the investigated treatments (including unplanted controls) shown almost the same and very high decrease of TPH/PAH concentrations. Hence neither the plants nor their combination with the amendments seem to have a significant effect on the improvement of the TPH/PAH phytoremediation. Concluding, phytoremediation of TPH/PAH seems to be mainly due to the degradation operated by microorganisms in the soil and rhizosphere (lixiviation effect will be further studied). Therefore, the selection of the vegetative species to be applied in field won't be performed according to the TPH removal efficiency but on biomass production. The highest biomass production has been recorded in the case of the phytoremediation treatments using Sorghum sp. combined with compost and with the mix of amendments (compost/biochar/PGPR=All).

Based on these results it has been concluded that Sorghum sp., and the mix of amendments (compost/biochar/PGPR) will be tested in field but in rotation with Brassica napus to help limiting runoff and leaching of NPK and to ensure that biomass production needs will be met (40 kg dry matter(DM)/season).

(a)



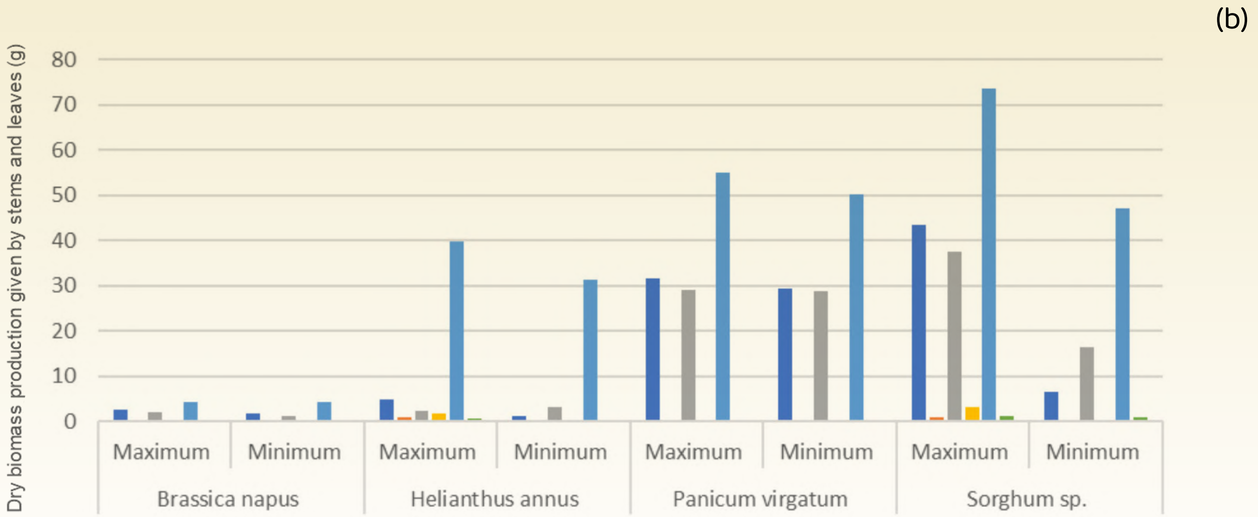



Figure 4 a) TPH removal efficiency (%) and b) minimum and maximum value of wet and dry biomass given by stems and leaves treated as a bulk sample, of the investigated phytoremediation treatments carried out at LEITAT facilities.
Credit: LEITAT

**Lithuania Pilot Site**: Based on the preliminary characterisation of the Lithuania Pilot Site PAH, TPH, PCB resulted to be the main sources of soil contamination. The main objective of the pot trials was to evaluate the potential to degrade petroleum hydrocarbons and other organics substances in the Serbia Pilot Site using specially for this purpose designed combination of plants, biological additives, and nutrients.

Concerning the vegetative species, Amaranth, Jerusalem artichoke and 3 mixes of herbaceous plants were tested, where a single mix is comprised of at least four different plant species. Regarding biological additives industrially prepared powdered biological material consisting of a carefully selected blend of natural micro-organisms that can degrade all main classes of compounds in oil fractions was used. Regarding nutrients, vermicast and common fertilizer were tested.

Pot tests were performed under controlled conditions in a greenhouse and lasted from 3 to 5 months, depending on the species.

Results have shown that the best phytoremediation potential of TPH was achieved using herbaceous plants above all with the mix of Tall fescue (Festuca arundinacea), Perennial ryegrass (Lolium perenne), Reed canary grass (Phalaris arundinacea) and Red clover (Trifolium pratense) It was observed that the lighter petroleum hydrocarbon fractions were degraded easier than heavier fractions (diesel, oil, residual). However, the decrease in concentration of all fractions was observed in all cases. Although, one growing season was insufficient to degrade TPH below maximum permissible values in this specific case, the obtained results show a large potential for a successful clean-up of the contaminated site in few years. An estimation of the biomass production for each of the investigated treatment was also conducted based on the pot tests results and has shown that all chosen plant species can be successfully grown on the contaminated soil at the given contamination levels, according to the chosen cultivation strategy. Furthermore, it has been found that plants cultivated on contaminated soil with appropriate strategy can produce equal and if not higher biomass output.

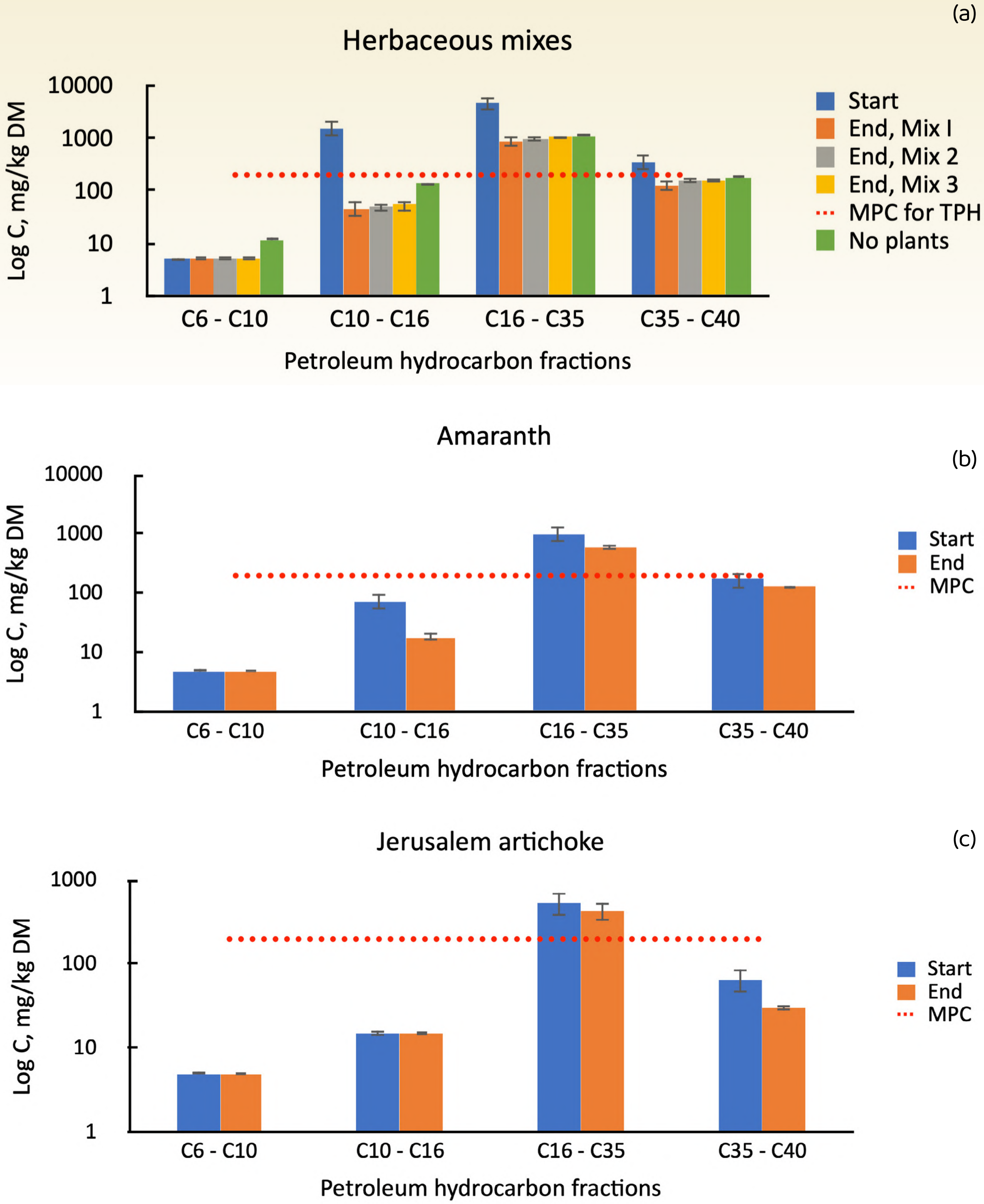



Figure 5 TPH concentrations in the contaminated soil at the start and at the end of the pot experiments performed at BVA facilities, with a) Herbaceous mixes b) Amaranth, and c) Jerusalem artichoke (n=3). Note the logarithmic scale. MPC= Maximum permissible concentration value, according to Lithuanian legislative document LAND 9-2009 (200 mg/kg).
Credit: BVA

**Serbia Pilot Site**: The preliminary characterisation of the Serbia Pilot Site soil has highlighted high level of: Cu, Cr, Pb, Zn and low level of: As, Cd, Ni, OCPs, PCBs, PAHs and TPH. Hence, the objective of pot trials was to assess if rapeseed (Brassica napus) as energy crop, has potential to be used for phytoextraction of heavy metals from soil from Serbian pilot site. The effect of different PGPR commercial products in promoting rapeseed growth was also validated and rapeseed potential to uptake heavy metals was also compared to other energy crops such as sunflower, hemp and white mustard. Specifically, commercial products based on 1) Trichoderma strains; 2) on rhizosphere bacteria; and 3) on auxins and gibberellins producing bacteria to be applied foliar, were tested. Pot experiments were performed on open air under natural weather conditions and lasted 10 weeks (from sowing to harvesting). The pot tests results have highlighted that i) the conditions for plant grooving regarding the nutrient and water content was optimal; ii) the content of the metal in the contaminated sediment did not change significantly during the experiment; iii) bioavailable fraction of the metal(oid)s was very low, and most of the metal content was distributed in the oxidable ad residual fractions (non-bioavailable fraction); iv) The contaminated sediments didn't pose stress to the plant growth and obtained biomass; v) Rapeseed had, in general, best performance regarding the BAF, moreover addition of PGPR Trifinder increased its biomass and BAF for Cr vi) Hemp showed similar performance for the relevant metal(oid)s (As, Cr and Cu), and due to the higher biomass, it can accumulate higher absolute amount of these metals; vii) the TF was <1 which indicate that the main mechanism of the metal(oid) removal is phytostabilisation and not phytoextraction.

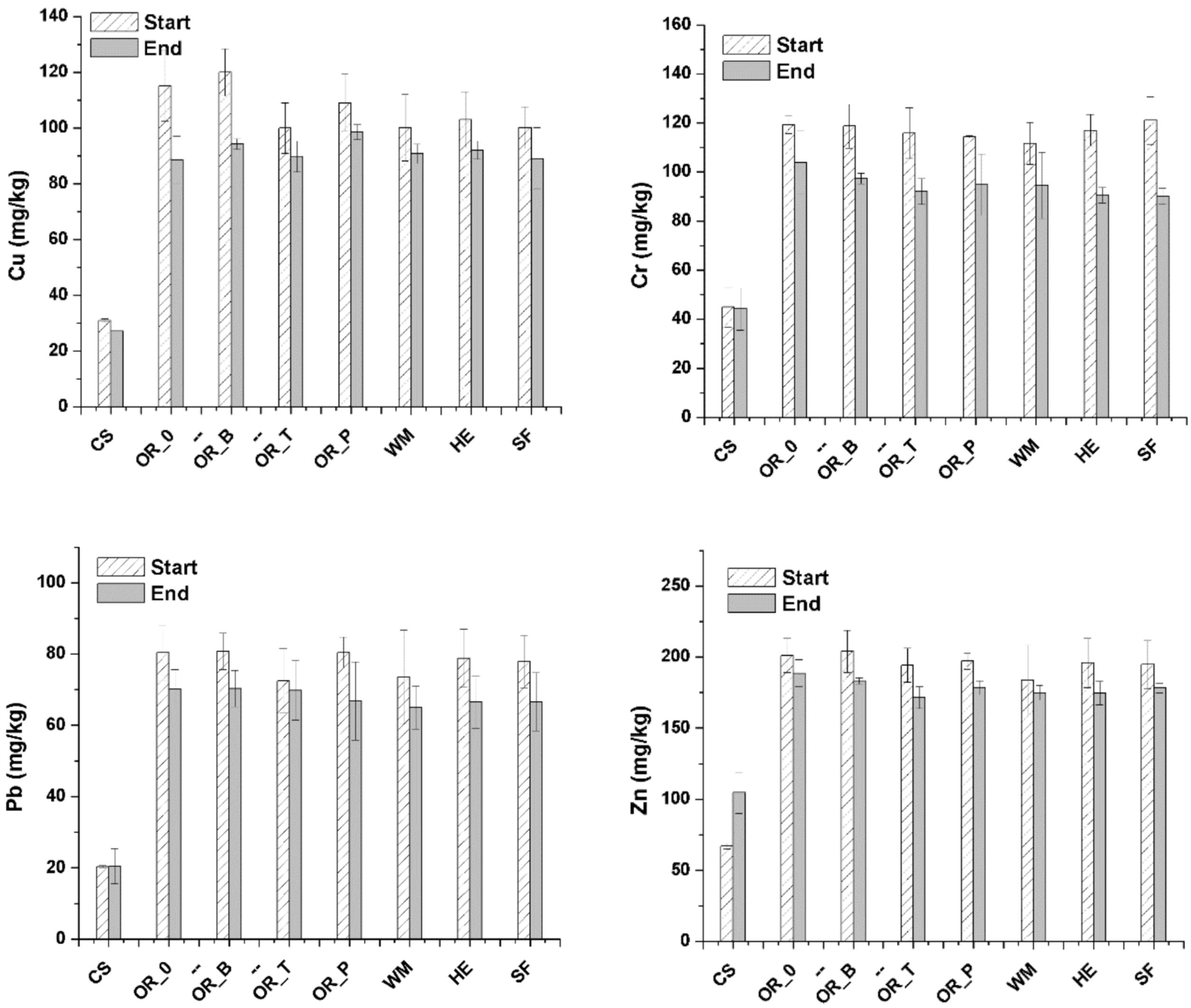



Figure 6 Concentration of Cu, Cr, Pb, Zn (the metals detected in the highest concentrations) at the beginning and at the end of the pot tests for the different investigated conditions (CS: contaminated soil, OR_0: rapeseed and no PGPR treatment, OR_B: rapeseed and treatment with Bio Eho PGPR; OR_T: rapeseed and treatment with Trifender Pro PGPR, OR_P: rapeseed and treatment with Panorama Bio Plus PGPR, WM: White Mustard; HE: Hemp; SF:Sunflower).
Credit: IFVCNS

**Argentina Pilot Site**: The preliminary characterisation of the Argentina Pilot Site has highlighted As, Cu, Zn, Cd as main contaminants.

The aim has been i) to evaluate the acute and chronic toxicity of a soil contaminated with mining waste on Plectrocarpa tetracantha, Bulnesia retama, Larrea cuneifolia and Prosopis flexuosa; ii) to evaluate the metal(loid) bioaccumulation capacity of Plectrocarpa tetracantha, Bulnesia retama, Larrea cuneifolia and Prosopis flexuosa growing on a soil contaminated with and without amendment application (dolomite and compost).

Regarding chronic toxicity, 3 months plants were exposed to different mixtures of contaminated and reference soil in greenhouse conditions for 90 days. Specifically, 0%, 1%, 10%, 50%, and 100% contaminated soil percentages were investigated. Concerning the amendments three concentrations of dolomite (10%, 20% and 40%) were tested while the compost was fixed to 5% with the aim of reaching a pH value of 6.5.

The series of conducted experiments allowed to conclude that the chronic exposure to contaminated soil causes mortality of all tested species at concentrations higher than 10%. This effect was reverted using dolomite and compost. Also, the bioavailability of metal(loid)s was reduced due to the increase of the pH value, which reduce the inhibition in the plant growth.

Results in terms of stem elongation are shown in the following figure where it can be observed the inhibition at 10% of contaminated soil after 75 d of exposure.

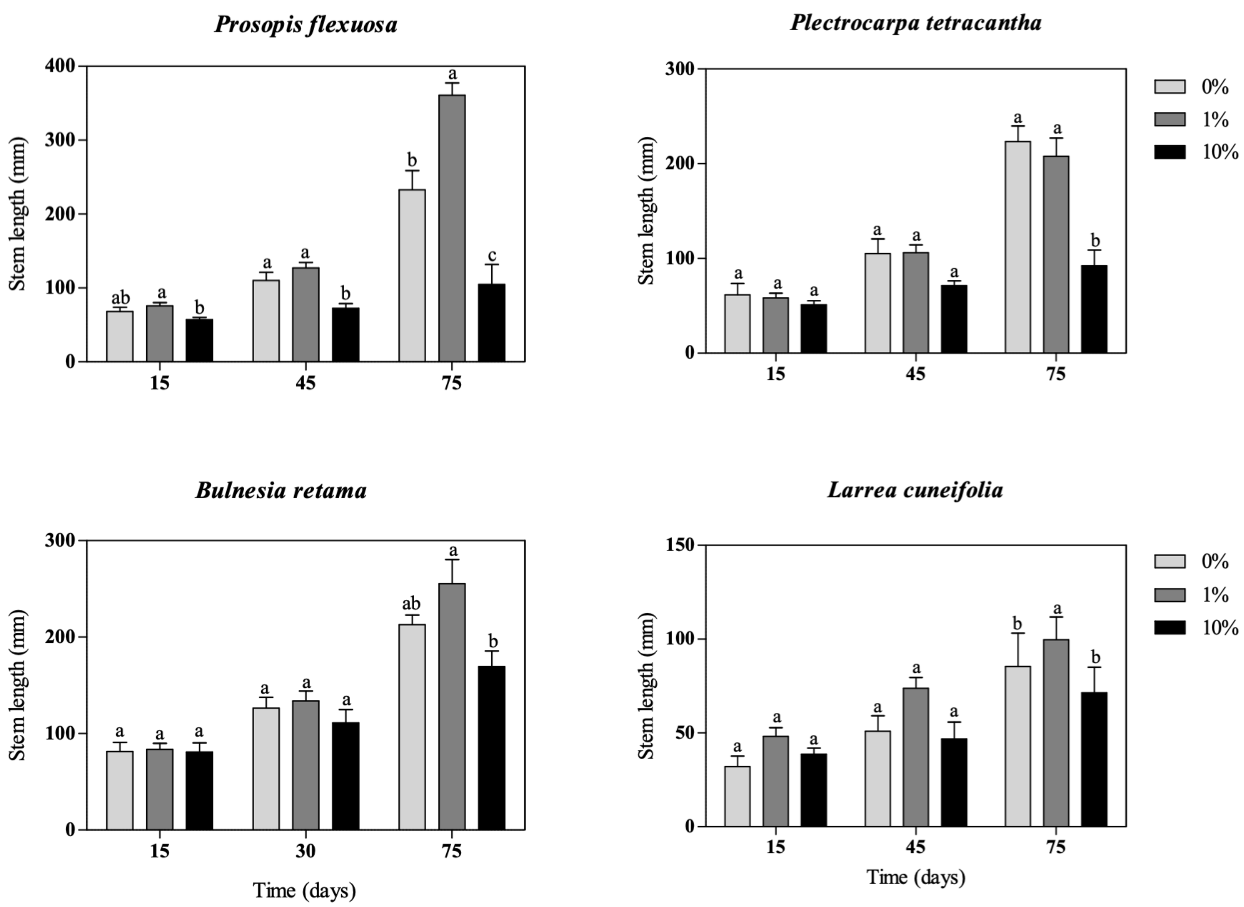



Figure 7 Mean (± SE) stem length obtained for all tested species in different exposure times (15, 30, and 75 days) and for different mixtures of contaminated and reference soil (0%, 1% and 10% of contaminated soil. Letters indicate significant differences ($p<0.05$) between treatments for each exposure time.
Credit: INTA

# Environmental and social sustainability

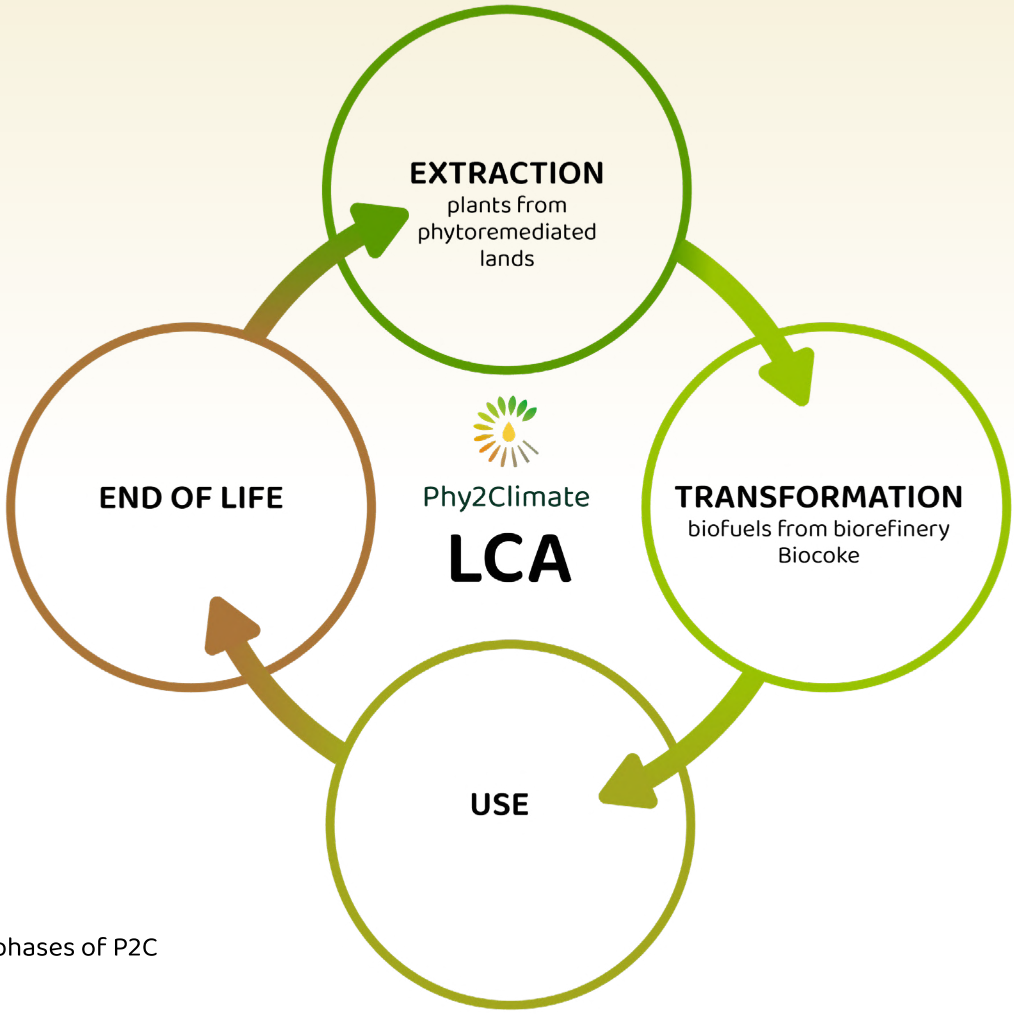



Figure 8
Life Cycle phases of P2C
Credit:SUT

## WP4 - 'Let's talk sustainable'

As mentioned in previous materials, the Phy2Climate approach aims at becoming an environmental impact role model by combining different complementing processes with a positive environmental effect. The phytoremediation of contaminated sites in 5 regions all over the world is combined with innovative biomass processing technologies to produce clean drop-in biofuels for the road and shipping transport as well as bio-coke as substitution of petroleum coke (pet-coke) in the metallurgical industry. Greenhouse Gas reduction should be achieved by substituting fossil fuels and pet-coke as well as by enhancing the organic carbon content in the soil. This overall picture of Phy2Climate approach seems very promising, but to be able to fully and independently judge the sustainability of the technology, its environmental impact has to be analyzed in terms of the whole life cycle. The Life Cycle Assessment is a standardized tool, however each technology deserves an individual approach. The analysis of a multi-effect system with variable feedstock scenarios is indeed a complex and challenging task. The Silesian University of Technology (SUT) is now preparing the methodology to be able to face that mission. The whole process has to be disassembled into factors and the whole LCA framework for this case has to be built. First steps, now undergoing, are to initially define the goal and scope, system boundaries, functional units, process and reference flows, data collection rules. LCA is an iterative process and the assumptions can be, luckily, continuously revised the more data appears. The LCA framework is presented in the figure below.

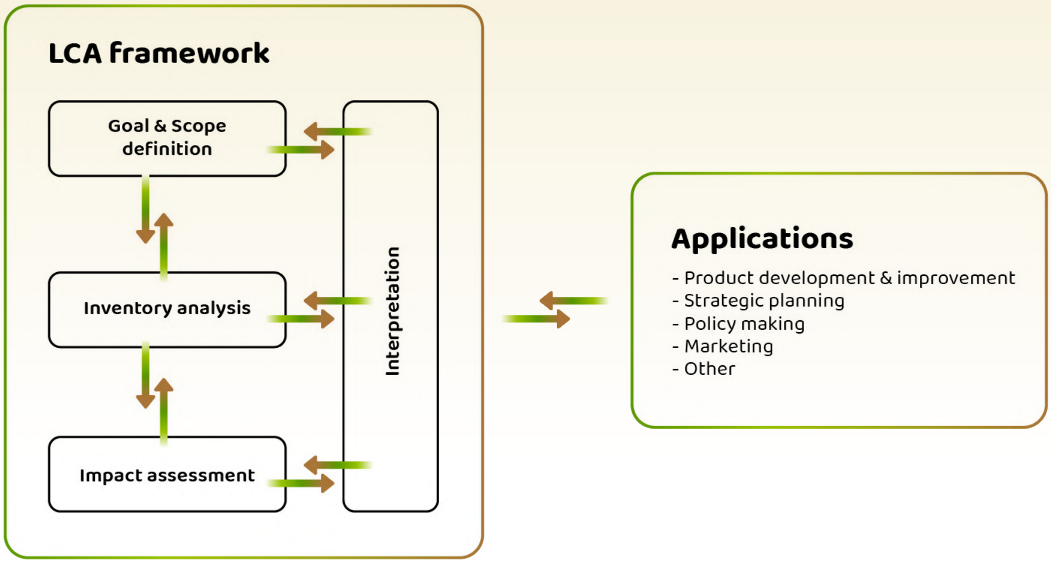



Figure 9
General LCA framework with focus on iterative actions
Credit:SUT

First attempts toward the system boundaries definition are visualized in the following figure. More approaches will be discussed with the Sustainability Board in the beginning of 2022.

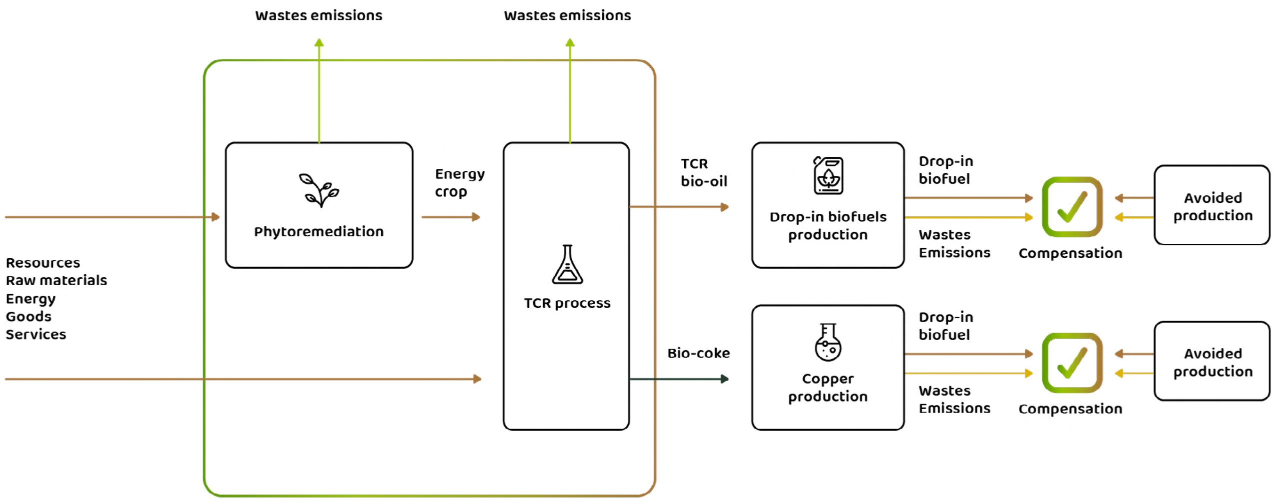



Figure 10 Solving the multifunctionality problem of the Phy2Climate technology – Beta version
Credit: SUT

## Social acceptance studies

In order to prove the technology meets the sustainability principles, it is inevitable to find a balance between environmental, economic and social aspects. It is widely acknowledged that the new energy project technology does not depend on technological advances and favorable economic conditions alone. It is important to recognize the perceptions of social acceptance of new energy technologies in order to better implement and develop of projects.

One of the aims of this project is to develop a practical toolbox for project managers to deal with societal acceptance issues in the development of a new approach in the Phy2Climate project. How does societal acceptance emerge (or does not) in new energy projects and what are the underlying mechanisms?
In this project the social acceptance of the Phy2Climate approach is discussed based on three different dimensions of social acceptance – socio-political, community, and market acceptance. All three are sometimes interdependent categories of social acceptance as indicated in Fig. 11. SUT-team is now preparing the methodology by defining the target groups and survey thematic fields.
In the socio-political dimension, opinion of general public, stakeholder, and policy makers are being considered. The aspects of public acceptance with focus on policy makers will be analyzed relying on expertise of WP6 leader, Hasselt University, while the stakeholders are recognized by the WP5 leader, ITS. The second dimension of social acceptance - community acceptance will deal with controversies at the local level. Three main factors have revealed for community acceptance, namely, procedural justice, distributional justice and community trust on information. The final aspect of social acceptance is the market acceptance that refers to the adoption of a new technology in a market or the process by which market parties adopt and support the energy innovation. The market acceptance aspects will be revealed with a close cooperation with WP5 leader, ITS.

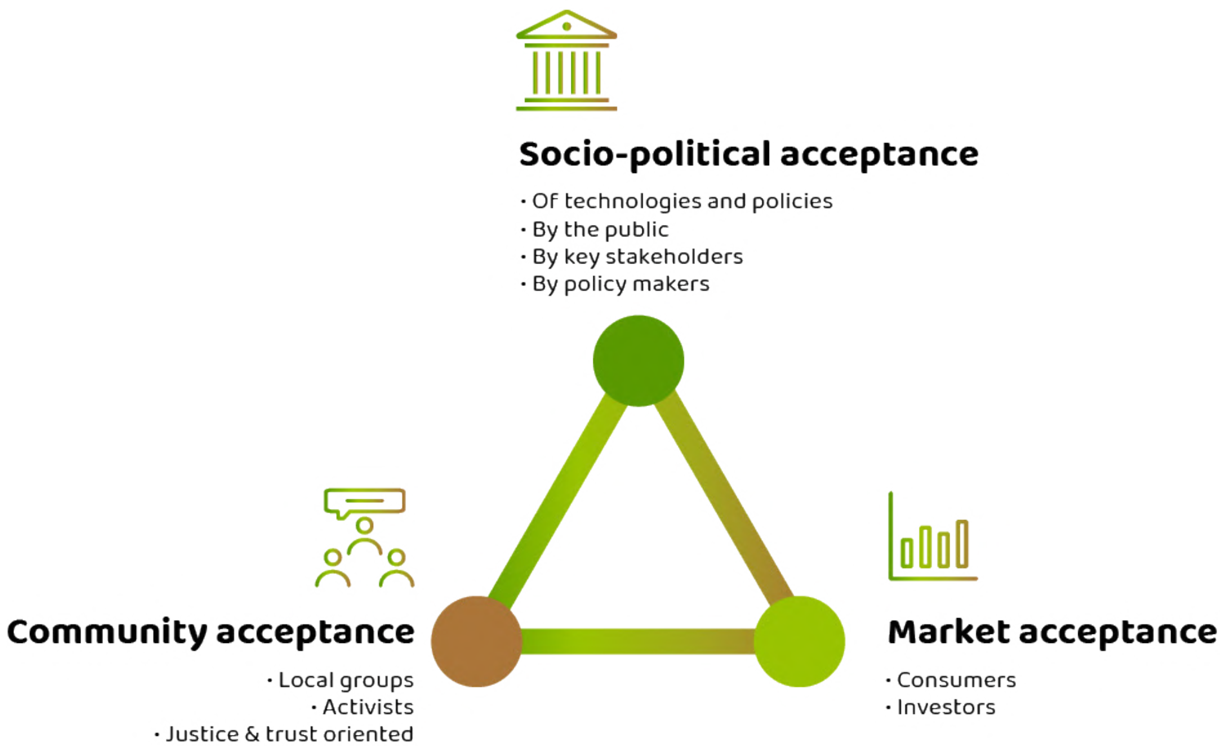



Figure 11 The triangle of social acceptance
Credit: SUT

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The project consortium has put together 17 partners from 10 countries with long-term expertise in soil remediation, phytoremediation, biofuel technologies and energy processes, environmental and social sustainability, legislative analysis, communication and dissemination as well as business development for innovative technologies.

Phy2Climate is a H2020 project with title "A global approach for recovery of arable land through improved phytoremediation coupled with advanced liquid biofuel production and climate friendly copper smelting process

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