

**Phytoremediation combined with biorefinery on the example of two agricultural crops  
grown on Ni soil and degraded by *P. chrysosporium***

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**Abstract**

During the last few decades, phytoremediation process has attracted much attention because of the growing concerns about the deteriorating quality of soil caused by anthropogenic activities. Here, a tandem phytoremediation/biorefinery process was proposed as a way to turn phytoremediation into a viable commercial method by producing valuable chemicals in addition to cleaned soil. Two agricultural plants (*Sinapis alba* and *Helianthus annuus*) were grown in

moderately contaminated soil with *ca.* 100 ppm of Ni and further degraded by a fungal lignin degrader - *Phanerochaete chrysosporium*. Several parameters have been studied: the viability of plants, biomass yield and their accumulating and remediating potentials. Further down-stream processing showed that up to 80% of Ni can be easily extracted from contaminated biomass by aqueous extraction at mild conditions. Finally, it was demonstrated that the grown onto contaminated soil plants can be degraded by *Phanerochaete chrysosporium* and the effect of nickel and biomass pre-treatment on the solid state fermentation was studied. The proposed and studied in this work methodology can pave the way to successful commercialization of the phytoremediation process in the near future.

#### Keywords

phytoremediation, metal accumulating plants, nickel, biorefinery, lignocellulose degradation, *Phanerochaete chrysosporium*

## **1. Introduction**

Phytoremediation -- a process of cleaning land from toxic metals using hyperaccumulating plants -- has been coined in 1994 (Raskin et al. 1994). Despite a significant effort in studying the mechanism of phytoextraction and metals deposition, in improving metals uptake by using chelating agents (Sun et al. 2009) or even genetic modification of the known species or searching for native hyperaccumulators, some significant hurdles still hamper successful commercialization of the phytoremediation process. The main intrinsic obstacles are: 1) high variety of the plant species involved in extraction of various metals and high inconsistency in rates of uptake even within one family of the hyperaccumulating plants (Adamidis et al. 2014); 2) highly specific growth conditions required for hyperaccumulators to grow; 3) low biomass yields; 4) very slow remediating process (up to several decades even for high annual uptake) (Shelmerdine et al. 2009); 5) high monetary and energy costs associated with downstream processing and metals recovery (the most common method to recover metals is biomass combustion followed by extraction of the metals from the ashes with strong acids); 6) very low revenue due to low demand and price for recovered metals.

Taking the above into consideration, a new concept of the combination of phytoremediation with biorefinery is being proposed (Fig. 1). Biorefinery, in this case processing of waste agricultural biomass for the production of valuable platform chemicals, has been intensively scrutinized during the last decade as the most sustainable and CO<sub>2</sub> negative way for the production of various commodities and fuels (Werpy & Petersen 2004). Phytoremediation by plants (agricultural and energy crops, hyperaccumulators, perennial grasses) can be followed by

lignocellulosic transformation. The combined process will bring additional benefits, such as valuable crops and cleaned land at first stage (phytoremediation) and various chemicals and recovered metals at the following stage (biorefinery). Moreover, some surplus revenue can be generated via pyrolysis of the final lignocellulosic residue (Koppolu & Clements 2003). The solid residue after gasification, pyrolysis or carbonization of biomass can be further used as fertilizer (Carter et al. 2013; George et al. 2012) to enhance growth of plants during phytoremediation, thus accomplishing a closed loop of the entire process. Overall, the process itself is a demonstration of the sustainable development paradigm where environmental problems are being resolved while generating useful commercially viable products.

In this work, the feasibility of the proposed combined phytoremediation/biorefinery process has been studied using agriculturally relevant plants, such as *Hileanthus annuus* (sunflower) and *Sinapis alba* (white mustard) and for remediating soil moderately contaminated with Ni.

Sunflowers are annually grown crop plants and characterized by ease of cultivation, tolerance to various soil types, low demand for fertilizers, high biomass yield and finally by good acceptance by farmers and widespread established application across the UK and Europe (Martínez-Force et al. 2015; Cook 2008; Cook et al. 2003). Currently, rapeseed crop production outnumbers sunflowers in the UK, however it is predicted that up to 79% of the UK arable area will be suitable to grow sunflowers by 2050 due to climate change (Cook 2009). Also, yielding up to 70-90 tones of biomass per hectare of cultivated land, sunflowers have attracted much attention as promising energy crops where both seeds and biomass residues can be used (Ion et al. 2014; Venturi & Venturi 2003). *Sinapis alba* also proposes some benefits in comparison to other crop

plants, such as low-demand growth conditions (cooler weathers, any type of soil), fast growth (Rosenfeld & Rayns 2011), relatively high yield due to which white mustard is used as a green manure plant (Overthrow & Brookes 2007).

The drive to utilize non-specific plants rather than well known hyperaccumulating plants is based on several reasons: 1) most of hyperaccumulating plants, (although offering high metal uptake), originate from Mediterranean or subtropical climates with warm weather and therefore are not suitable to be grown in higher latitudes with cooler and wet climates, like in the UK; 2) hyperaccumulators are characterized by low biomass yield; 3) lowering of the metal content in biomass will reduce health and safety risks and therefore enhance and ease the processing of the biomass; 4) easy adaptation of the combined phytoremediation-biorefinery process using the commonly grown plants rather than different hyperaccumulators for cleaning soil from contaminant metals.

Indeed, the best known and studied nickel hyperaccumulating plants are of genus *Alyssum* (Brassicaceae) (most reported *Alyssum murale* and *Alyssum bertolonii*) and *Berkheya coddii* which could provide nickel uptake as high as 10,000 ppm (Robinson, Chiarucci, et al. 1997; Tappero et al. 2007; Robinson, Brooks, et al. 1997). However, only *Berkheya coddii* yielded as high as 22 t ha<sup>-1</sup> of dried biomass, which can be suitable for effective phytoextraction. In reality though, it is difficult to achieve any significant growth of the hyperaccumulating species, as has been demonstrated in field trials (Robinson et al. 2015).

The concept of designing cropping systems for metal contaminated sites has been assessed by Tang *et al.* (Tang et al. 2012) along with other remediation techniques (Tang et al. 2016).

Depending on the initial concentration of metal in soil, different strategies have been proposed: growing low-accumulating edible plants on low and moderately contaminated soils and non-edible or energy crops on highly contaminated sites. One of the options proposed is co-cropping of low accumulating and hyperaccumulating plants which further needs to be investigated and developed. Low-accumulating plants have a capacity to accumulate same total amount of contaminant metals due to their high biomass yield in comparison to hyperaccumulating plants which are usually characterized by low biomass yields.

Another aspect, which cannot be ignored but is difficult to assess when developing a phytoremediation process using hyperaccumulating plants, is health and safety risks associated with transporting and handling plant material contaminated with high metal content (up to 10,000 ppm). When only biomass is dried on the field and ready to be transported, special precautions must be taken to avoid exposure of workers and drivers to dust easily generated by dried biomass. Therefore, a significant reduction in metal content in lignocellulosic material will lessen the operational requirements and therefore reduce the costs of the process. Cumulative exposure effect can not be ignored though.

As a result, phytoremediation process based on *non-specific low-accumulating plants* offers some advantages in comparison to hyperaccumulating plants and can be widely adopted as a very promising way to clean soil moderately contaminated with metals. Indeed, as it can be seen from Fig. 2, most of the sites across the UK recorded in the British Geological Survey surface soil dataset are contaminated with 100-200 ppm of Ni both in profile and surface soil horizons (Ander et al. 2013). This concentration of Ni is within the range of the soil guideline values for

Ni (130 and 230 ppm for residential land use and allotments accordingly) and therefore can be regarded as of low hazardous level (Martin et al. 2009). However, the guidelines have been revised recently and new allowable level of Ni for allotment has been significantly reduced to 53 ppm (Nathanail et al. 2015). It is worth noting that the guideline value for Ni for commercial land use has been also reduced from 1,800 in 2009 to 980 ppm in 2015 (Martin et al. 2009; Nathanail et al. 2015). This trend reflects the raising concerns about Ni potential as a skin sensitiser (causing allergic reaction even at low concentrations) along with its developmental toxicity at prolonged contact times. Thus, Ni poses significant risks to human health due to several types of exposure: oral, dermal and through inhalation. Via inhalation, Ni has been reported to cause chronic bronchitis, emphysema, reduced vital capacity and asthma (Martin et al. 2009), as well as being potent carcinogen (Cempel & Nikel 2006; Burwell 2014). However, the current use of Ni accounts for 25 Mt worldwide and is expected to grow as Ni is widely used in making steel and alloys, batteries, electronics, catalysts and domestic goods (NI Booklet 2015a; NI Booklet 2015b). Although, most of the metal is recycled, the high use of Ni, which is expected to grow, will lead to higher health risks in the nearer future. Moreover, most of the sites in Fig. 2 with relatively low nickel content are currently used either for growing produce or as residential areas where soil ingestion, dust inhalation, skin contact and consumption of home-grown vegetables are the main long-term intake routes for Ni. Therefore, the problem of moderate nickel contamination is more likely to become more profound in the nearer future and needs to be addressed appropriately. Here, the proposed process of the combined phytoremediation and biorefinery can be adopted as a long-term strategy for cleaning soil, recovery of Ni and production of useful chemicals.

In this work, in order to verify the proposed phytoremediation-biorefinery tandem, *Hileanthus annuus* (sunflower) and *Sinapis alba* (white mustard) were grown onto soil contaminated with nickel and further subjected to lignocellulose degradation by the fungus *Phanerochate chrysosporium*. In addition, phytoremediating potential of the two agricultural crops for moderately contaminated soils was studied.

## **2. Experimental**

### *2.1 Chemicals*

NiSO<sub>4</sub>·7H<sub>2</sub>O (Analar, 99.0%), Ni ICP standard (1000 ppm), 3,5-dinitrosalicylic acid, Folin-Ciocalteu reagent, anhydrous gallic acid and glucose were sourced from Sigma-Aldrich, Merck or VWR International Ltd and used without any purification. 1% w/v solution of 3,5-dinitrosalicylic acid was prepared by the following method: 3,5-dinitrosalicylic acid (0.4 g) was dissolved in NaOH (4 mL, 5 M) and distilled water (8 mL) on a hot plate and further combined with sodium potassium tartrate solution (28 mL, 30% w/v) under vigorous mixing.

### *2.2 Greenhouse experimental design and sampling*

Pot-culture experiments of growing plants in Ni contaminated soil under controlled conditions were conducted in a Phytobiology Unit, Warwick University, Coventry. The seeds of *Hileanthus annuus* and *Sinapis alba* were procured from Wellesbourne Seed bank collection (Wellesbourne Crop Centre, Wellesbourne, UK). All the seeds were germinated in wetted cloth pads in an incubator with 8/10 hours day light/dark pattern for at least one week prior to the sowing into freshly prepared soil. The contaminated nickel soil was prepared by the following method: a

corresponding amount of  $\text{NiSO}_4 \cdot 7\text{H}_2\text{O}$  salt was weighed and dissolved in 50 ml of distilled water, the solution was diluted up to 1 L, and the final solution of  $\text{NiSO}_4$  was sprayed evenly onto a pile of fresh soil (John Innes compost N3), mixed carefully and potted into 1 L square bottom plastic pots (11×11 cm) for *Helianthus annuus* and 0.5 L pots for *Sinapis alba* (9×9 cm) so that the total volume was  $426 \pm 2$  g and  $221 \pm 2$  g, corresponding to wet weight of soil per 1 L and 0.5 L pots (average soil moisture content is 74%). One week old healthy seedlings were transplanted into fresh compost (control) or with addition of nickel sulphate, randomised and left to grow for a period of 4 weeks in a controlled environment at 16-19°C with a daily photoperiod of 16 hours and a constant irrigation regime. Three or five plants were chosen as controls for weekly monitoring of the height and appearance of the plants during the growth period. The plants were harvested by cutting leaves and stems with scissors. Leaves and stems were placed into paper bags and dried in an oven at 80°C for 24 hours. Soil samples were collected before and after growing the corresponding plants and dried in an oven at 80°C until constant weight. The dried soil and biomass samples were ground and sieved and a fraction of below 1 mm was taken for subsequent microwave digestion and ICP-MS analysis. Throughout sampling, an average of at least three representative samples was obtained for statistical analysis and a STDV was calculated and plotted as error bars.

### 2.3 Analytical techniques

200 mg of soil or biomass sample was weighted into teflon tubing and 3 ml of  $\text{HNO}_3$ <sub>conc</sub> was added, then the slurry was subjected to microwave digestion according to the following program: 1) heating to 100°C at 3°C per min, hold 2 min, 2) heating to 120°C at 1°C per min, hold 1 min,

3) heating to 160°C at 3 °C min, hold 2 min, 4) heating to 180°C at 2°C min, hold 20 min. The digested samples were diluted up to 25 ml with distilled water and finally filtered through Millipore 0.2 µm filters. ICP analysis of the samples were carried out using Agilent 7500cx ICP-MS, Warwick University, UK, (with 50ppb Er as internal standard and 4 ml/min of He as collision gas) and some of the data have been verified at Nikolaev Institute of Inorganic Chemistry, Russia, using iCap 6500 Duo ICP-OES, Thermo Scientific, USA, (with 0.5 mg L<sup>-1</sup> Sc as internal standard). The samples were analyzed in technical triplicates and blank samples were run with acids only.

The content of soluble phenolics and total reduced sugars in the plant extracts were determined with Folin-Ciocalteu (FC) (Ainsworth & Gillespie 2007) and 3,5-dinitrosalicylic (DNS) calorimetry (Chua et al. 2012) accordingly. For Folin-Ciocalteu analysis, in 96 well plate, each sample (2 µL) was mixed with distilled water (158 µL) and 10% Folin-Ciocalteu reagent (10 µL). After a period of 8-10 min, Na<sub>2</sub>CO<sub>3</sub> (30 µL, 16% w/v) solution was added, and the mixture was left for 1 h to complete the reaction. Then the absorbance of the samples was recorded at 760 nm.

For total sugars analysis, 1/3 or 1/6 dilution of the samples was carried out prior to the analysis. Then, in an Eppendorf tube, 60 µL of the sample was mixed with 180 µL of freshly prepared DNS solution and incubated in a block heater (2-3 min, 90°C) to allow the reaction to occur. Then, 150 µL of each solution was placed into 96 well plate and analysed at 540 nm. The absorbance of the prepared solutions was measured using a Tecan GENios plate reader equipped with Magellan 3 software. Calibration curves were constructed using solutions of gallic acid and

glucose with the final results expressed in equivalents of gallic acid and glucose for phenolics and sugars respectively. All assays were carried out in triplicates so that three representative samples from the same batch were analysed. In addition, all UV-Vis and ICP analysis was carried out in triplicates for same sample. One way ANOVA statistical analysis was used throughout the work.

#### *2.4 Extraction of nickel from plant biomass*

Several methods for nickel extraction from plant biomass have been studied, such as agitation, hydrothermal treatment and homogenization. The extraction process has been scaled up to 10 g of biomass. All small scale extraction experiments were carried out by mixing 200 mg of each sample (stem or leaf) with 4 ml of distilled water so that the solid/water ratio was 1/20 (optimized prior to extraction). Hydrothermal treatment was carried out by autoclaving of the slurry at 121°C for 15 min in a bench top autoclave (Prestige Medical, UK). Extraction by mixing (agitation) was carried out by mixing the slurry on a stirrer plate at 600 rpm at room temperature for a period of 70 min (this time was found to be sufficient to reach the equilibrium). Homogenization was carried out by grinding the slurry using mortar and pestle for 1-2 min. After the extraction the samples were centrifuged down at 4000 rpm, 4°C, 10 min in an Eppendorf Centrifuge 5810 R, filtered through Millipore 0.2 µm filters and finally diluted (1/5) for ICP analysis.

#### *2.5 Degradation of plant biomass by *Phanerochaete chrysosporium**

The inoculum solution of *Phanerochaete chrysosporium* spores was prepared according to the following method: the fungus was grown onto Malt extract agar for 1 week and the spores

solution was collected by scraping the plates with 5 ml of sterile water. The spores solution was prepared prior to the inoculation. 1 g of each plant samples was weighed into 15 ml glass tubes and after addition of 5 ml of distilled water, the tube was autoclaved (121°C, 15 min). After that 1 ml of  $1.2 \cdot 10^6$  CFU ml<sup>-1</sup> spores solution of *Phanerochate chrysosporium* was added into each tube. The tubes were closed with polyurethane plugs to provide sufficient aeration and left for degradation in a Plus II Incubator (Gallenkamp, UK) at 37°C for 4 weeks. Every five days 1 ml of sterile water was added to each tube to compensate for water evaporation. The degradation was quenched by drying the samples to a constant weight. The degraded biomass was ground and subjected to ethanol/water (50/50) extraction via homogenization of 0.5 g of each sample with 10 ml of 50% ethanol (1/20 biomass to solvent ratio) in a mortar. The slurry was centrifuged down at 4000 rpm, 4°C for 10 min and finally filtered through Whatman 0.22 µm nylon filter. The extract was analyzed for the total amount of soluble phenolics and sugars, as well as for total extracted solids content (by drying of 0.5 ml of the extract at 60°C for 24 hours).

### **3 Results and discussion**

#### *3.1 Growing of *Hileanthus annuus* and *Sinapis alba* plants onto contaminated with nickel soil*

Firstly, the plants viability and biometric parameters for control and Ni-exposed plants were verified by conducting repetitive experiments with ca. 100 ppm of Ni in soil and one-off experiment at an extreme Ni content in soil of 2200 ppm. It can be seen from Fig. 3 that the heights and appearance of the plants grown on moderately contaminated with ca. 100 ppm of nickel soil did not differ from the control plants grown on clean compost. Similarly, no difference in biomass yields has been previously observed both for nickel hyper-accumulator

*Berkheya coddii* Rossler and the non-accumulator *Cicer arietinum* grown in moderately contaminated soil with 65-125 ppm of Ni (Conesa et al. 2009).

Nickel at a concentration as high as 2200 ppm significantly affected the growth of both the studied plants, as their heights were lowered in comparison to the controls and necrosis (discoloring) of the leaves was observed. However, it is noticeable that *S. alba* demonstrated higher tolerance towards high nickel in comparison to *H. annuus*.

Table 1 summarizes the results obtained for the repetitive experiments on growing *H. annuus* and *S. alba* in the compost contaminated with ca. 100 ppm of nickel. The biomass yield for both plants was not affected by ca. 100 ppm of nickel in compost, whereas lower biomass yield observed in the first experiment with *H. annuus* is due to seasonal variations in the plant. The biomass yield for *S. alba* is somewhat lower in comparison to *H. annuus*.

The background level of nickel in clean soil was 2.3-4.1 ppm, whereas concentration of the metal in spiked soil varied between 73-108 ppm despite the standardized procedure for soil contamination. The reduction of nickel in soil after the harvesting of the plants reached as high as 32-38% which points out at significant leaching of the metal salt with water during the course of the experiments (therefore no further increase in nickel loading was studied).

The content of nickel in the control plant samples varied between 0.4-2.9 ppm of nickel whereas at 100 ppm of nickel in soil accumulation ability of the both plants differs. Thus, *H. annuus* absorbed 7.1 ppm in leaves and up to 16.8-18.0 ppm of nickel in stems, whereas *Sinapis a.* accumulated 8.6-8.9 and 15.9-22.3 ppm of nickel in stems and leaves accordingly. It is worth pointing out that various parameters, such as season, temperature gradient and watering regime,

length of day/night periods and viability of seedlings will affect the total uptake of nickel, and some variation in the final amount of a metal extracted is common even within the same family of plants. For example, intra-specific differences in tolerance and accumulation of nickel have been observed for nine outcrops of *Alyssum bertolonii* grown under hydroponic conditions (Galardi et al. 2007).

Comparing both studied in this work plants, one can conclude that *S. alba* showed higher absorption capacity and translocation ability for nickel, whereas *H. annuus* accumulated and stored most of the nickel through its transport system in stems. Accumulation factors are estimated to be within 0.13-0.18 for both plants. These low accumulation factors result in a relatively low extraction potential of the plants in the studied experimental set up. Thus, only 1.9-5.0% of nickel per pot was extracted by *H. annuus* and *S. alba* in one run. Nevertheless, it can be concluded that both *H. annuus* and *S. alba* can be used as remediating crops, grown on moderately contaminated with nickel soil, which are able to accumulate up to 11.9-15.1 ppm of nickel per g of total dry weight of the plant. *H. annuus* is characterized by high biomass yield and allocation of the most of the absorbed nickel in stems. Whereas, *S. alba*. showed some potential to translocate Ni into leaves and higher tolerance towards higher nickel content in soil, while being also a short-rotation fast growing crop. Previously, a strong correlation between metal tolerance and accumulation was observed for some plants, for example *Alyssum lesbiacum* (Adamidis, Aloupi et al. 2014). Therefore, one might suggest that *S. alba* can demonstrate good potential to accumulate nickel at higher metal content in the soil. In order to prove this, however, further studies are required.

### 3.2 Extraction of nickel from contaminated biomass by various methods

In the downstream processing of the contaminated biomass, the metals recovery stage can be carried out either before or after biomass degradation. From a health and safety point of view, the extraction of metals prior to any degradation is favorable, however, the degradation can facilitate the release of the metals from the lignocellulose thus enhancing the recovery stage. The two options need to be studied in order to provide a better solution for the whole biorefinery process.

In this work several extraction procedures have been tested for the extraction of nickel from the contaminated biomass: agitation (mixing), hydrothermal treatment and homogenization. Here, thermally (hydrothermal treatment) and mechanically (homogenization) assisted aqueous extraction has been verified as a way to improve the efficiency rather than conducting multiple extraction which was initially regarded as cost ineffective and therefore not studied in this work. As it can be seen from Fig. 4, all the extraction methods (except for *H. annuus* stems) provided high extraction efficiency up to 64-82% of nickel extracted from both plants in one step. Here, some variations in the extraction efficiency were observed, such as 1) somewhat better extraction of nickel from both leaves and stems of *S. alba* in comparison to *H. annuus*, 2) poor extraction of nickel from *H. annuus* stems (8-28%) due to probably a highly rigid structure of the stems tissue, 3) homogenization is characterized by somewhat lower extraction capacity. In addition, hydrothermal treatment yielded much better extraction of nickel from *H. annuus* stems in comparison to agitation and homogenization techniques pointing out at beneficial effect of elevated temperatures on extraction process.

The most common method for metal extraction from biomass is aqueous extraction under agitation/stirring. This could be facilitated by high stirring speed, higher temperature or addition of acids, chelating agents, oxidizers or organic solvents like ethanol, methanol, ethyl acetate. For example, only several consecutive washings at high temperature of 90°C and with at least 0.5 M acidic solution of H<sub>2</sub>SO<sub>4</sub> allowed efficient extraction of nickel from *Alyssum murale* (11,400 ppm of Ni), whereas pure water and high biomass/solvent ratios (more than 1/10) reduced the extraction efficiency (Barbaroux et al. 2009). In order to improve extraction of nickel, some other, rather complicated, techniques (selective precipitation, electroplating, flocculation) were developed and tested, however solvent extraction was found to be the most effective method (Barbaroux et al. 2011). In the process proposed in this work, high agitation speed and/or temperature will increase energy demand, whereas the addition of organic solvents is undesirable because the residual organic solvent can be toxic for the bio-catalyst in the following biomass degradation stage. Surprisingly, under the moderate conditions studied in this work (low temperature, short times, single washing), pure water appeared to be sufficiently effective in the extraction of nickel due to perhaps highly soluble forms of nickel being present in the plants (more likely as free ion and salts of low molecular weight organic acids). Indeed, citric acid was found to be the main chelating agent for nickel in a study across nine nickel hyperaccumulating plant species collected in New Caledonia (Callahan et al. 2012). In another study of hyperaccumulator *Alyssum serpyllifolium* ssp. *Lusitanicum* (3,000 ppm dw Ni), 30% of nickel in the xylem sap was coordinated with citric acid and some other acids such as oxalic, malic, malonic, aspartic acids, whereas the most of nickel was found to be in the free ion form (Alves et al. 2011). The authors concluded that the plant used simple and “cheap” ways of transporting

nickel, because only 5% of nickel was bound to amino acids and no histidine was detected, although another study claimed that histidine is used by *Alyssum lesbiacum* to facilitate the phytoextraction of nickel (Singer et al. 2007).

It is worth pointing out that hydrothermal treatment is a facilitating extraction carried out at high temperature (121°C) and under pressure (2 atm), however mass transfer limitations due to the absence of any agitation are expected to accompany this extraction procedure. Hydrothermal treatment may be considered as the most suitable method for the subsequent biomass degradation which includes sterilization of the biomass prior to the fermentation. Thus, both extraction and sterilization could be carried out at one stage by using hot water vapor with the subsequent drainage of the spent water (which will contain the metals) and the remaining sterilized biomass ready to be fermented. Because no agitation is required, the energy demand and capital investment can be significantly reduced.

### *3.3 Biomass degradation by *Phanerochaete chrysosporium**

Solid state fermentation offers some advantages in comparison to aqueous fermentation, such as no requirement for mixing and therefore lower energy demand, lower consumption of additional chemicals and supplements, such as salts, vitamins, simpler control (only temperature, humidity and aeration need to be maintained) and a greater flexibility and simplicity in downstream processing, where the final products can be separated and isolated through several consecutive extraction stages. White rot fungi *Phanerochaete chrysosporium* has been extensively studied during the last few years and has proved to be the most promising lignin-degrading white rot fungus due to its high competitiveness and moderate requirements for fermentation process

(Chang et al. 2012; Taccari et al. 2009; Kumar et al. 2006). *Phanerochaete chrysosporium* has been studied for the degradation of agricultural waste, such as wheat straw and corn stalks (Bhatnagar et al. 2008).

Here, the application of this fungus has been extended towards degradation of other types of biomass such as *H. annuus* and *S. alba* and also biomass contaminated with metals, in this case with nickel. Also, two strategies have been verified: post extraction fungal degradation and bio-degradation of the biomass contaminated with nickel.

Unlike in microbial aqueous fermentations, the fungal growth in solid state fermentation is difficult to monitor. However, the change in total biomass weight can provide some valuable information on the growth of fungi. Thus, as it can be seen from Fig. 5, a significant difference in the reduction of weight due to the degradation of biomass along with water loss can be observed for degraded samples in comparison to non-degraded. Also, the solid state fermentation of biomass by fungi is accompanied by significant loss of water, which can be overcome by addition of water during the process. No degradation was observed for control samples (no fungi) as the final weight reduction was within 1-2%.

It was found, that contaminated with 7-20 ppm of nickel lignocellulose and the biomass subjected to hydrothermal treatment or aqueous extraction can be easily degraded by *Phanerochaete chrysosporium* (Fig. 6). The fungus fully colonized the biomass during the first week of the fermentation and by the end of the experiment the structure of the biomass was fully distorted as it can be seen in Fig. 5. However, it can be noticed, that the contamination with Ni affected the degradation of *H. annuus*, but not *S. alba*, whereas quite oppositely, the pretreatment

stage enhanced the degradation of *S. alba*, but not *H. annuus*. Thus, 46-48% of *H. annuus* grown in clean soil was degraded by *P. chrysosporium* in comparison to 36% for *H. annuus* grown on Ni soil and therefore containing up to 12.6 ppm of nickel. The extraction of nickel from *H. annuus* prior to the fungal fermentation stage did not improve the subsequent degradation process. The observed effect of inhibition by Ni is due to, perhaps, high stem/leaf ratio and poor extraction of nickel from stems of *H. annuus*, as was previously discussed.

Surprisingly, unlike for *H. annuus*, contamination of *S. alba* with 15.1 ppm of nickel did not affect the fungal degradation of biomass, having even somewhat beneficial effect and increasing the degradation yield by 3% (Fig. 6). More likely, the forms of nickel in the two plants are different depending on the mechanism of accumulation and chelating of the metal. The solid state fermentation experiment showed that the fungal growth was inhibited in contaminated *H. annuus* but not in *S. alba*. This suggests that the inhibitory effect of Ni onto the fungus is more likely associated with the chelating agents and forms of nickel in the plants rather than with the metal itself.

An obvious beneficial effect of aqueous extraction on the degradation was seen for *S. alba*, for which the pre-treatment of biomass prior to fungal fermentation improved the degradation yield by 14-15% in comparison to non-treated biomass. Thus, due to the lack of easily available substrates after the extraction, *P. chrysosporium* attacks rigid lignocellulosic polymers. This leads to the release of simple mobile chemicals and eventually to an increase in total extractable solids, as can be seen in Fig. 6 for both plants which underwent pre-treatment and fungal degradation. The change in the amount of soluble compounds which can be extracted into 50%

of ethanol from the contaminated Ni plants *without any pre-treatment* of biomass was found to be negligible. Thus, the final content of extractable solids before and after the fermentation did not change. This points at a more complex mechanism of lignocellulose fungal degradation when one type of soluble chemicals available initially in the plant is replaced by fungal metabolites released during the fermentation.

Apparently, some inhibitory to the fungus substances, present in *S. alba*, were extracted into water and therefore aqueous treatment of biomass improved the growth of *P. chrysosporium*. It was also noticed that the degradation of *S. alba* was somewhat faster in comparison to *H. annuus* degradation which might be due to either less rigid structure of *S. alba* in comparison to *H. annuus*. Also, the effect of contamination of *S. alba* with nickel on degradation is negligible. Overall, the fermentation of *S. alba* was characterized by higher degradation yields of 43-60% in comparison to 36-48% for *H. annuus*. Thus, one may conclude that the correlation between contamination with nickel or the biomass pre-treatment and the degradation of lignocellulose by *P. chrysosporium* is not straightforward and the response of the fungus to inhibitors in various plants is highly specific and needs to be investigated.

Apart from the degradation yield and final extracted solids, the total amount of sugars and phenols in the extracts were analyzed as the main products of the fermentation. Sugars could be further used for the production of alcohols, acids or bio-gas, whereas phenolic compounds could include valuable derivatives of p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) lignols. For example, the key compounds of phenols have been previously identified as vanillin, coumaric acid, 5-methoxy proto-catehuic acid, syringyl alcohol and ferulic acid (Koncsag et al. 2012).

Simple phenols are known to possess antioxidant and antimicrobial properties (Guil-Guerrero et al. 2016; Shahidi & Ambigaipalan 2015).

The amount of the extracted total phenols and sugars in 50% ethanol extracts of the plants are presented in Fig. 7. Firstly, some effects of the aqueous extraction can be observed for both plants. Thus, the pretreatment of biomass significantly reduced the soluble sugars content in both *H. annuus* and *S. alba* from 56-106 to 18-24 mg g<sub>dw</sub> which was increased to 55-59 mg g<sub>dw</sub> for *S. alba* and 48 mg g<sub>dw</sub> for *H. annuus* (with no nickel) as a result of fungal degradation and release of sugars during this process. Surprisingly, despite lower degradation yields for *H. annuus*, the final concentration of total phenols extracted after the fermentation process was found to be higher for *H. annuus* (4.9-13.1 mg g<sub>dw</sub>) in comparison to *S. alba* (4.5-6.6 mg g<sub>dw</sub>). Thus, the effect of the fungal degradation of lignocellulose on the release of free phenols was more profound for *H. annuus* (increase in phenolics by 49-55%) in comparison to *S. alba* (increase in phenolics by 24-30%). The effect of contamination of biomass with nickel is less profound in comparison to the effect of the pretreatment stage. As no influence of nickel on the final degradation yield of *S. alba* was found, the difference in content of soluble sugars and phenols in contaminated biomass is also negligible. Somewhat lower final sugars and phenolic content in contaminated *H. annuus* in comparison to non-contaminated biomass is more likely due to overall lower degradation yield in the presence of the metal.

#### **4. Conclusions**

In this work, a tandem phytoremediation/biorefinery process with two agricultural crops (*Hileanthus annuus* and *Sinapis alba*) grown on soil with *ca.* 100 ppm of nickel and degraded by

the fungus *P. chrysosporium* was verified as a suitable strategy to add value to phytoremediation which is known to be an environmentally friendly method of cleaning contaminated soil. It has been found, that *S. alba* shows higher tolerance towards higher nickel content in soil, whereas both plants grow well on moderately contaminated compost with *ca.* 100 ppm of nickel. The two plants accumulated 11.9-15.1 ppm of nickel, and *S. alba* showed a somewhat better metal translocation ability in comparison to *H. annuus*, which accumulates nickel mainly within the stems. It was shown that up to 80% of nickel can be easily extracted by water via a simple procedure, although the release of nickel from *H. annuus* stems proved to be inefficient. It was suggested that hydrothermal treatment could be considered as the most suitable method for combined extraction and sterilization as the most energy effective way to carry out the tandem phytoremediation/biorefinery process.

The contaminated nickel biomass was subjected to solid state fermentation by *P. chrysosporium* for 4 weeks at 37°C under static condition with regular additional of water. The contamination of biomass with nickel worsened the degradation of *H. annuus* by *ca.* 10% but not of *S. alba*. The pre-treatment of biomass (aqueous extraction) prior to the fermentation increased the degradation yield by 14-15% for *S. alba*. Extraction was also found to significantly reduce the amount of soluble sugars in both plants from 56-106 to 18-24 mg g<sub>dw</sub>. This eventually should have led to the deficiency of the available sugars and phenols and to enhancement of the growth of the degrading fungus, which was indeed observed for *S. alba* but not for *H. annuus*. The degradation of lignocellulose which underwent pre-treatment led to higher final amount of sugars (*ca.* 50 mg g<sub>dw</sub>) and phenols (5-6 mg g<sub>dw</sub>) in extracts for both plants. Higher increase in available soluble phenols after the fermentation stage was observed for *H. annuus*. Overall, one may conclude that

the both plants -- *H. annuus* and *S. alba* -- are suitable candidates for the combined phytoremediation/biorefinery process which can be used to clean land from moderate contamination with nickel and to recover the metal and produce valuable phenolic compounds and sugars.

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## 6. References

- Adamidis, G.C. et al., 2014. Chemosphere intra-specific variation in Ni tolerance , accumulation and translocation patterns in the Ni-hyperaccumulator *Alyssum lesbiacum*. *Chemosphere*, 95, pp.496–502. Available at: <http://dx.doi.org/10.1016/j.chemosphere.2013.09.106>.
- Ainsworth, E.A. & Gillespie, K.M., 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin – Ciocalteu reagent. *Protocol*, 2(179337), pp.875–877.
- Alves, S. et al., 2011. Nickel speciation in the xylem sap of the hyperaccumulator *Alyssum serpyllifolium* ssp . *lusitanicum* growing on serpentine soils of northeast Portugal. *Journal of Plant Physiology*, 168, pp.1715–1722.
- Ander, E.L. et al., 2013. Science of the total environment methodology for the determination of normal background concentrations of contaminants in English soil. *Science of the Total Environment*, 454-455, pp.604–618. Available at: <http://dx.doi.org/10.1016/j.scitotenv.2013.03.005>.
- Barbaroux, R. et al., 2011. A new method for obtaining nickel metal from the hyperaccumulator plant *Alyssum murale*. *Separation and Purification Technology*, 83, pp.57–65.
- Barbaroux, R. et al., 2009. Hydrometallurgy Chemical leaching of nickel from the seeds of the metal hyperaccumulator plant *Alyssum murale*. *Hydrometallurgy*, 100(1-2), pp.10–14. Available at: <http://dx.doi.org/10.1016/j.hydromet.2009.09.005>.

- Bhatnagar, A., Kumar, S. & Gomes, J., 2008. Operating conditions of a 200 l staged vertical reactor for bioconversion of wheat straw by *Phanerochaete chrysosporium*. *Bioresource Technology*, 99, pp.6917–6927.
- Burwell, S., 2014. Nickel compounds and metallic nickel. *Report on carcinogens*, pp.1–4. Available at: <http://ntp.niehs.nih.gov/ntp/roc/content/profiles/nickel.pdf>.
- Callahan, D.L. et al., 2012. Phytochemistry elemental and metabolite profiling of nickel hyperaccumulators from New Caledonia. *Phytochemistry*, 81, pp.80–89. Available at: <http://dx.doi.org/10.1016/j.phytochem.2012.06.010>.
- Carter, S. et al., 2013. The impact of biochar application on soil properties and plant growth of pot grown lettuce (*Lactuca sativa*) and cabbage (*Brassica chinensis*). *Agronomy*, 3, pp.404–418.
- Cempel, M. & Nikel, G., 2006. Nickel: a review of its sources and environmental toxicology. *Polish Journal of Environmental Studies*, 15(3), pp.375–382.
- Chang, A.J., Fan, J. & Wen, X., 2012. Screening of fungi capable of highly selective degradation of lignin in rice straw. *International Biodeterioration & Biodegradation*, 72, pp.26–30. Available at: <http://dx.doi.org/10.1016/j.ibiod.2012.04.013>.
- Chua, M. et al., 2012. Methodologies for the extraction and analysis of konjac glucomannan from corms of *Amorphophallus konjac* K. Koch. *Carbohydrate Polymers*, 87(3), pp.2202–2210. Available at: <http://dx.doi.org/10.1016/j.carbpol.2011.10.053>.

- Conesa, M. et al., 2009. Root responses to soil Ni heterogeneity in a hyperaccumulator and a non-accumulator species. *Environmental Pollution*, 157, pp.2189–2196.
- Cook, S., 2008. Sunflowers - a growers guide. *Defra Innovation Network Press*, (January), pp.1–12. Available at:  
[https://cereals.ahdb.org.uk/media/184967/sf6\\_sunflower\\_growers\\_guide.pdf](https://cereals.ahdb.org.uk/media/184967/sf6_sunflower_growers_guide.pdf).
- Cook, S., 2009. Sunflowers and climate change. *Defra Innovation Network Press*, pp.1–16.  
 Available at: [http://stage.hgca.com/media/185127/sf5\\_sunflowers\\_and\\_climate\\_change.pdf](http://stage.hgca.com/media/185127/sf5_sunflowers_and_climate_change.pdf).
- Cook, S. et al., 2003. The role and position of sunflower in UK agriculture. *Defra Innovation Network Press*, pp.1–6. Available at:  
[http://isasunflower.org/fileadmin/documents/aaProceedings/15thISCToulouse2000/PosterWorkshopI-O/N\\_UK1.pdf](http://isasunflower.org/fileadmin/documents/aaProceedings/15thISCToulouse2000/PosterWorkshopI-O/N_UK1.pdf).
- Galardi, F. et al., 2007. Intra-specific differences in nickel tolerance and accumulation in the Ni-hyperaccumulator *Alyssum bertolonii*. *Environmental and Experimental Botany*, 60, pp.377–384.
- George, C. et al., 2012. Divergent consequences of hydrochar in the plant – soil system: arbuscular mycorrhiza , nodulation , plant growth and soil aggregation effects. *Applied Soil Ecology*, 59, pp.68–72. Available at: <http://dx.doi.org/10.1016/j.apsoil.2012.02.021>.

- Guil-Guerrero, J.L. et al., 2016. Antimicrobial activity of plant-food by-products: a review focusing on the tropics. *Livestock Science*, 189, pp.32–49. Available at: <http://dx.doi.org/10.1016/j.livsci.2016.04.021>.
- Ion, V. et al., 2014. Results regarding biomass yield at sunflower under different planting patterns and growing conditions. *Series A. Agronomy*, LVII, pp.205–210.
- Koncsag, C.I. et al., 2012. Resources , conservation and recycling extracting valuable compounds from straw degraded by *Pleurotus ostreatus*. *Resources, Conservation & Recycling*, 59, pp.14–22. Available at: <http://dx.doi.org/10.1016/j.resconrec.2011.04.007>.
- Koppolu, L. & Clements, L.D., 2003. Pyrolysis as a technique for separating heavy metals from hyperaccumulators. Part I: Preparation of synthetic hyperaccumulator biomass. *Biomass and Bioenergy*, 24, pp.69–79.
- Kumar, A.G., Sekaran, G. & Krishnamoorthy, S., 2006. Solid state fermentation of *Achras zapota* lignocellulose by *Phanerochaete chrysosporium*. *Bioresource Technology*, 97, pp.1521–1528.
- Martin, I. et al., 2009. Soil guideline values for nickel in soil. *Environment Agency Press*, pp.1–10.
- Martínez-Force, E., Dunford, N.T. & Salas, J.J. eds., 2015. *Sunflower: chemistry, production, processing and utilization*, Elsevier Inc.

Nathanail, C.P. et al., 2015. The LQM/CIEH S4ULs for human health risk assessment. *Land quality Press*, pp.1–12. Available at: <http://www.lqm.co.uk/publications/s4ul/>.

NI Booklet, 2015a. Ni: an abundant resource. *Nickel Institute Press*, pp.1–2. Available at: [www.nickelinstitute.org](http://www.nickelinstitute.org).

NI Booklet, 2015b. Nickel compounds: the inside story... *Nickel Institute Press*, pp.1–16. Available at: [www.nickelinstitute.org](http://www.nickelinstitute.org).

Overthrow, R. & Brookes, P., 2007. Influence of green manuring in wheat rotations under different cultivation systems. *HGCA project report*, (414), pp.1–51.

Raskin, I. et al., 1994. Bioconcentration of heavy metals by plants. *Current Opinion in Biotechnology*, 5(3), pp.285–290.

Robinson, B.H., Chiarucci, A., et al., 1997. The nickel hyperaccumulator plant *Alyssum bertolonii* as a potential agent for phytoremediation and phytomining of nickel. *Journal of Geochemical Exploration*, 59, pp.75–86.

Robinson, B.H., Brooks, R.R., et al., 1997. The potential of the high-biomass nickel hyperaccumulator *Berkheya coddii* for phytoremediation and phytomining. *Journal of Geochemical Exploration*, 60(1997), pp.115–126.

Robinson, B.H., Anderson, C.W.N. & Dickinson, N.M., 2015. Phytoextraction : Where 's the action ? *Journal of Geochemical Exploration*, 151, pp.34–40. Available at: <http://dx.doi.org/10.1016/j.gexplo.2015.01.001>.

Rosenfeld, A. & Rayns, F., 2011. Sort out your soil. A practical guide to green manures.

*Costwold Grass Seeds booklet*, pp.1–39.

Shahidi, F. & Ambigaipalan, P., 2015. Phenolics and polyphenolics in foods , beverages and spices: Antioxidant activity and health effects – a review. *Journal of Functional Foods*, 18, pp.820–897. Available at: <http://dx.doi.org/10.1016/j.jff.2015.06.018>.

Shelmerdine, P.A. et al., 2009. Modelling phytoremediation by the hyperaccumulating fern , *Pteris vittata* , of soils historically contaminated with arsenic. *Environmental Pollution*, 157(5), pp.1589–1596. Available at: <http://dx.doi.org/10.1016/j.envpol.2008.12.029>.

Singer, A.C. et al., 2007. Phytoremediation of mixed-contaminated soil using the hyperaccumulator plant *Alyssum lesbiacum*: Evidence of histidine as a measure of phytoextractable nickel. *Environmental Pollution*, 147, pp.74–82.

Sun, Y. et al., 2009. Chelator-enhanced phytoextraction of heavy metals from contaminated soil irrigated by industrial wastewater with the hyperaccumulator plant (*Sedum alfredii* Hance ). *Geoderma*, 150(1-2), pp.106–112. Available at: <http://dx.doi.org/10.1016/j.geoderma.2009.01.016>.

Taccari, M. et al., 2009. Effect of *Phanerochaete chrysosporium* inoculation during maturation of co-composted agricultural wastes mixed with olive mill wastewater. *Waste Management*, 29(5), pp.1615–1621. Available at: <http://dx.doi.org/10.1016/j.wasman.2008.12.014>.

- Tang, X. et al., 2016. Review of remediation practices regarding cadmium-enriched farmland soil with particular reference to China. *Journal of Environmental Management*, 181, pp.646–662.
- Tang, Y. et al., 2012. Designing cropping systems for metal-contaminated Sites: a review. *Pedosphere: an international journal*, 22(4), pp.470–488. Available at: [http://dx.doi.org/10.1016/S1002-0160\(12\)60032-0](http://dx.doi.org/10.1016/S1002-0160(12)60032-0).
- Tappero, R. et al., 2007. Hyperaccumulator *Alyssum murale* relies on a different metal storage mechanism for cobalt than for nickel. *New Phytologist*, 175(4), pp.641–654.
- Venturi, P. & Venturi, G., 2003. Analysis of energy comparison for crops in European agricultural systems. *Biomass and Bioenergy*, 25(3), pp.235–255.
- Werpy, T. & Petersen, G., 2004. Top value added chemicals from biomass: results of screening for potential candidates from sugars and synthesis gas. *National Renewable Energy Laboratory report*, pp.1–66. Available at: [www.osti.gov/bridge](http://www.osti.gov/bridge).

**Table 1.** Summarized data on growing *H. annuus* and *S. alba* in spiked with Ni soil.

| Plant                                                | <i>Hileanthus annuus</i> |                   | <i>Sinapis alba</i>   |                       |
|------------------------------------------------------|--------------------------|-------------------|-----------------------|-----------------------|
| Experiment number                                    | 1                        | 2                 | 1                     | 2                     |
| Background Ni content in soil, ppm dw                | 2.3 ± 0.4                | 2.3 ± 0.5         | 2.3 ± 0.4             | 4.1 ± 0.1             |
| Ni content in spiked soil, ppm dw                    | 73 ± 3                   | 96 ± 3            | 108 ± 1               | 79 ± 4                |
| Ni content in soil post-harvest, ppm dw              | 45 ± 0.3                 | 61 ± 9            | 73 ± 4                | 67 ± 4                |
| Reduction in Ni content post-harvest, %              | 38                       | 36                | 32                    | 15                    |
| Control total biomass (leaves) yield, g per pot      | 15.5 (4.1 ± 0.3)         | 27.3 (11.3 ± 0.6) | 19.8 (8.8 ± 0.6)      | 22.5 (8.0 ± 1.1)      |
| Contaminated total biomass (leaves) yield, g per pot | 11.5 (5.0 ± 0.2)         | 25.5 (11.0 ± 0.7) | 19.4 (9.2 ± 0.3)      | 18.9 (8.2 ± 0.6)      |
| Ni content in control leaves (stems), ppm            | 2.9 ± 1.4 (2.1 ± 0.3)    | 0.4 ± 0.3 (-)     | 2.8 ± 0.5 (1.5 ± 0.2) | 2.0 ± 0.6 (1.4 ± 0.8) |
| Ni content in contaminated                           | 7.1 ± 0.6                | 7.1 ± 1.9 (16.8   | 22.3 ± 2.1            | 15.9 ± 2.3 (8.9       |

|                                                                                     |                  |           |                 |           |
|-------------------------------------------------------------------------------------|------------------|-----------|-----------------|-----------|
| leaves (stems), ppm                                                                 | $(18.0 \pm 1.0)$ | $\pm 3.0$ | $(8.6 \pm 1.0)$ | $\pm 0.5$ |
| Ni average in whole plant, ppm                                                      | 13.3             | 12.6      | 15.1            | 11.9      |
| Translocation factor,<br>$C(\text{Ni})_{\text{leaves}}/C(\text{Ni})_{\text{stems}}$ | 0.4              | 0.4       | 2.6             | 1.8       |
| Accumulation factor,<br>$C(\text{Ni})_{\text{plant}}/C(\text{Ni})_{\text{soil}}$    | 0.18             | 0.13      | 0.14            | 0.15      |
| % Ni extracted per pot                                                              | 1.9              | 3.0       | 4.7             | 5.0       |

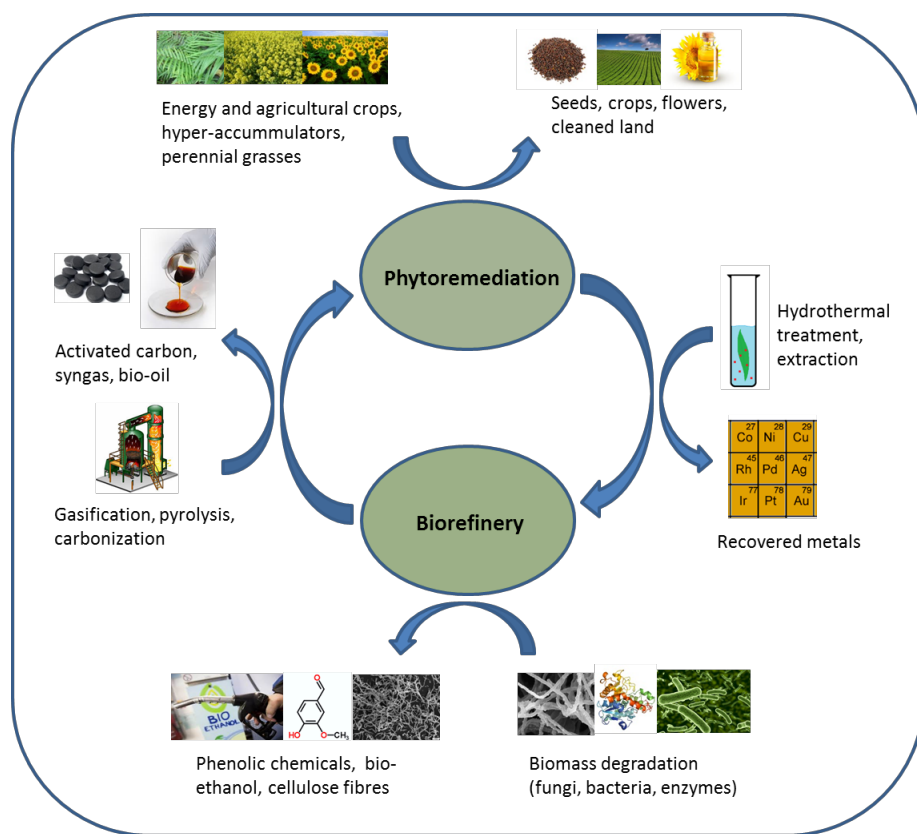


**Fig. 1.** The scheme of the tandem phytoremediation-biorefinery process.

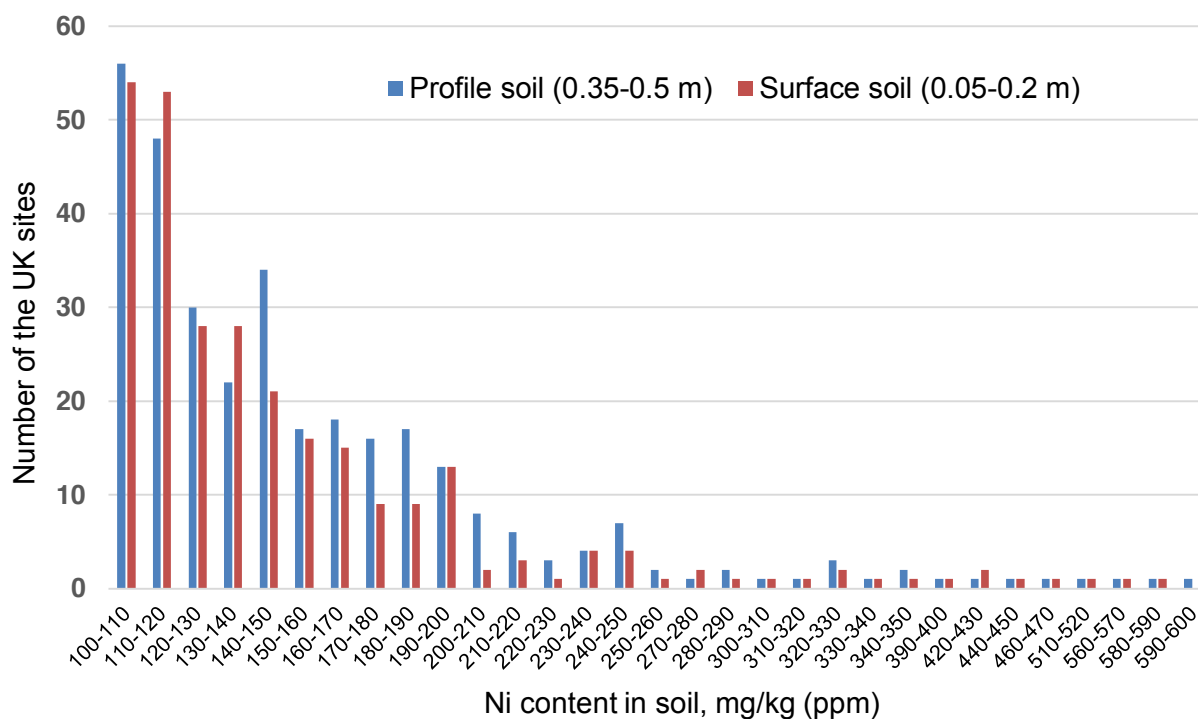


**Fig. 2.** Distribution of the number of the reported UK sites contaminated with nickel depending on the concentration of the metal in the surface and profile horizons.

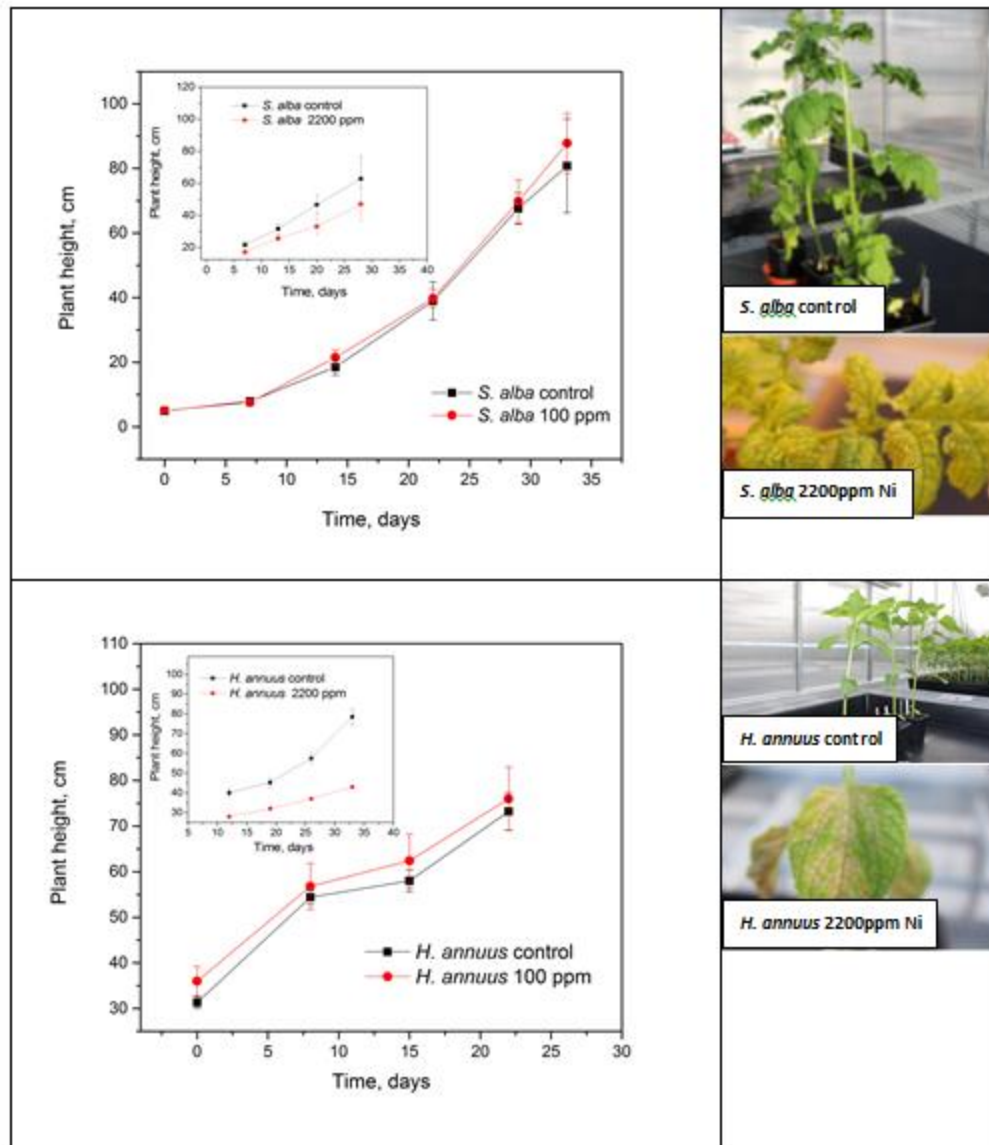


**Fig. 3.** The dependence of the plants heights and appearance on the Ni content in soil in comparison to controls grown in clean compost.

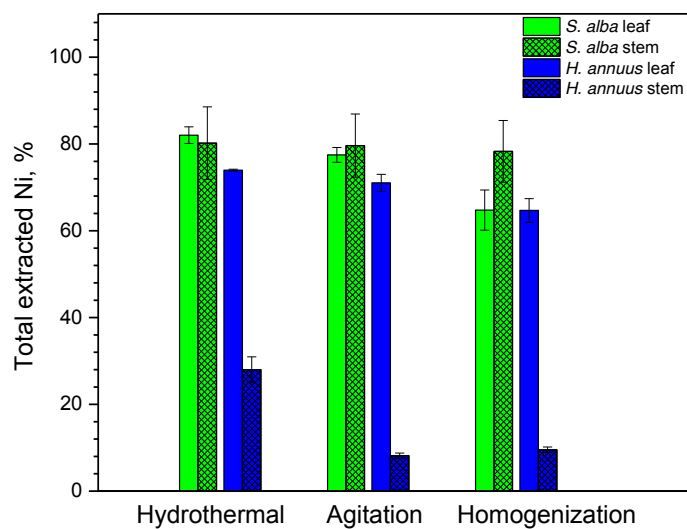


**Fig. 4.** The fraction of nickel extracted from stems and leaves of *S. alba* and *H. annuus* depending on the method of extraction.

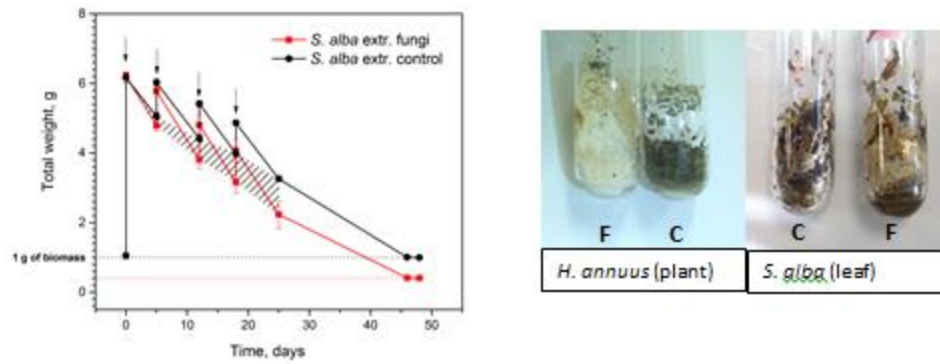


**Fig. 5.** Weight loss during the time course of the degradation of *S. alba* (addition of water is shown by arrows, dashed area demonstrates weight loss due to fungal degradation only) and the demonstration of the colonization of the fungus (F) onto *H. annuus* in comparison to control (C) and the destruction of the structure of *S. alba* leaves.

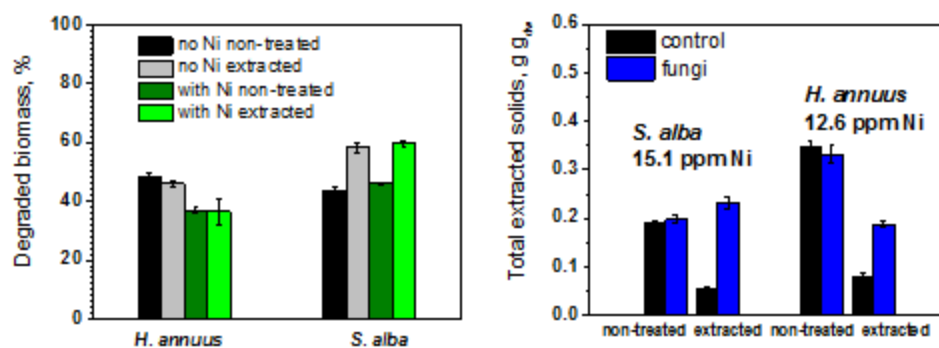


**Fig. 6.** The final degradation yields for *H. annuus* and *S. alba*, contaminated and non-contaminated with nickel, and the total extractable solids in the plants contaminated with Ni.

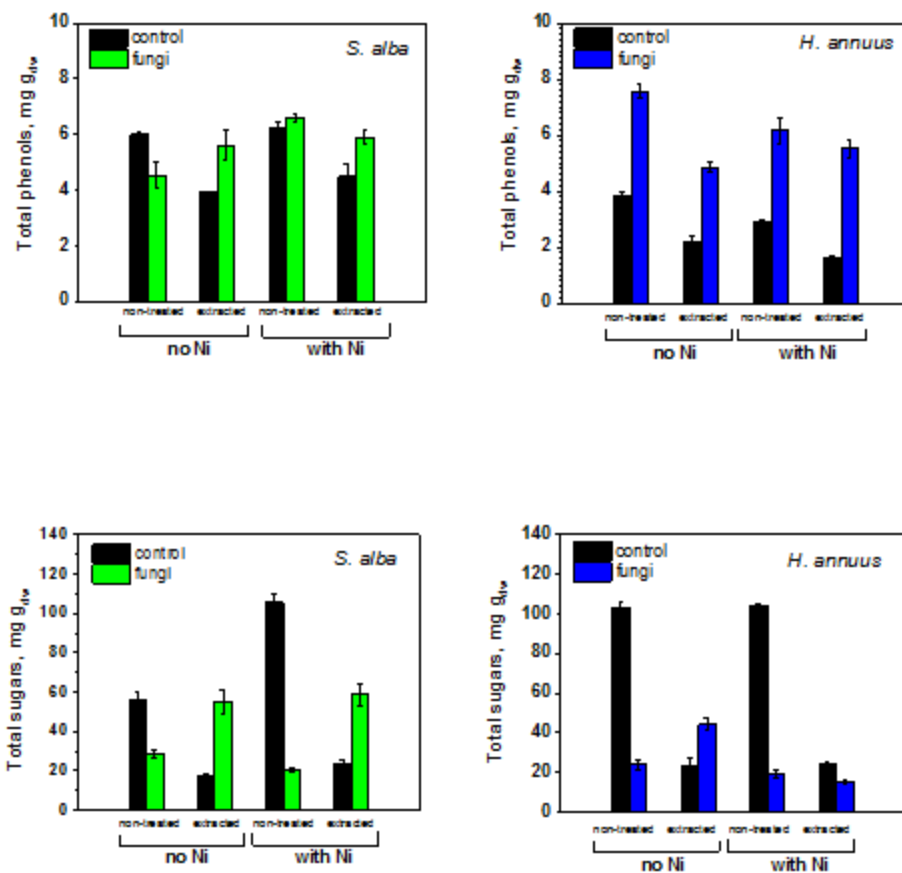


**Fig. 7.** The final concentrations of soluble sugars and phenols in 50% ethanol extracts after the degradation of *H. annuus* and *S. alba* by *P. chrysosporium*.



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**Phytoremediation combined with biorefinery on the example of two agricultural crops grown on Ni soil and degraded by *P. chrysosporium***

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