Using *in vivo* nickel to direct the pyrolysis of hyperaccumulator plant biomass

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Experimental

The impregnation of nickel in the Control samples.

Ground leaf material from the control samples were impregnated with Ni, using 0.1 M $NiCl_2 \cdot 6H_2O$ aqueous solution to achieve final nickel concentrations equivalent to the those in the hyperaccumulator species - 0.93 and 0.35 wt% for *Stackhousia* and *Alyssum* respectively. The water was removed on a rotary evaporator at 40°C and 50 mbar. The samples were placed on a glass filter and carefully washed with distilled water (figure 1S) several times until the negative reaction on the chloride anions with $AgNO_3$ solution (figure 2S). The possible leaching of the metal during washing step was checked by NaHS solution. Precipitation of NiS was not observed (figure 3S).

Fig. 1S. Washing on a glass filter from the excess of chloride anions

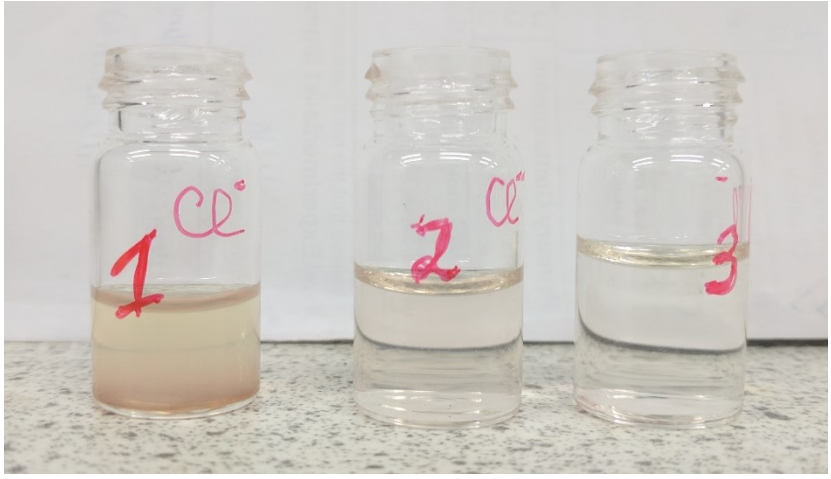

Fig. 2S. Analysis for the presence of chloride anions by Ag^+. The partial occurrence of the red colour happened because of the partial oxidation of the silver

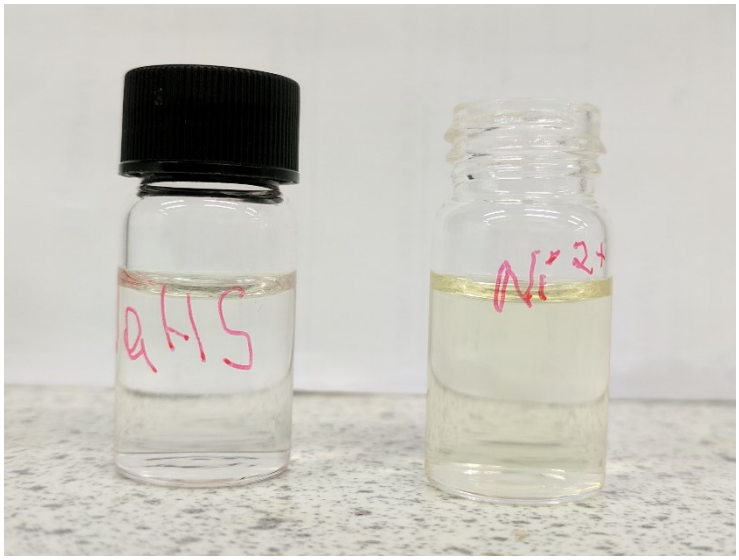

Fig. 3S. The test for the leaching of nickel with NaHS solution

MW-assisted pyrolysis

Microwave assisted pyrolysis was conducted using dynamic power mode, on a CEM Discover, equipped with 10 ml closed vial. The target temperature was 280 °C (figure 4S). The extraction of bio-oil was carried out using acetone; the extracts were centrifuged, decanted, and finally filtrated on a glass filter.

Fig. 4S. The microwave reactor – CEM Discover

GC-MS and GC-FID procedures

GC-MS and GC-FID analysis was performed using a premier TOF mass spectrometer coupled to an Agilent Technologies 6890A gas chromatograph equipped with a 30 m x 0.25 x 0.25 ZB-5HT inferno column. The MS acquisition mass range was between m/z 50 and 750, electron energy 70 eV, trap current 200 uA. The GC method used was isothermal for 2min at 50 °C, then increased to 280 °C at 10 °C/min, held for 15 mins at maximum temperature, with a total runtime of 40 mins. The GC-FID analysis was performed on a Hewlett Packard HP 6890 with a 30 m x 0.25 x 0.25 Rxi-5HT column. The GC method held a starting temperature of 50 °C and then an increase to 300 °C at 30 °C/min, held for 5 mins, with a total runtime of 13.33 mins. The GC-MS spectra for all the samples are portrayed in figures 5S and 6S.

ATR-FTIR

The analysis was carried out on the Perkin Elmer FTIR/FTNIR Spectrum 400 in the range of 600-4000 cm^{-1}. The amount of scans were 32 with 4 cm^{-1} recording step.
The spectra for the *Alyssum bertolonii* hyperaccumulator and its control are displayed on the figure 7S. The Control sample revealed increased amount of lignin in comparison to the hyperaccumulator. The similar absorbance of the carbonyl bands (figure 7S), which can be attributed to the acid constituents of the hemicellulose, makes it possible to assume that amount of hemicellulose is similar for the samples.

Thermal analysis

The thermal analysis was conducted on the Netzsch STA 409 using linear heating with ramping rate 10°$Cmin^{-1}$. The experiment was performed in inert atmosphere of N_2 with a gas-flow of 100 ml/min. The processing of the data (including the deconvolution procedure) was done in OriginPro 2018 (figure 8S). The integrated signal of the lignin for the *Alyssum* Control was larger than that of the *Alyssum bertolonii*, in line with the ATR-FTIR analysis.

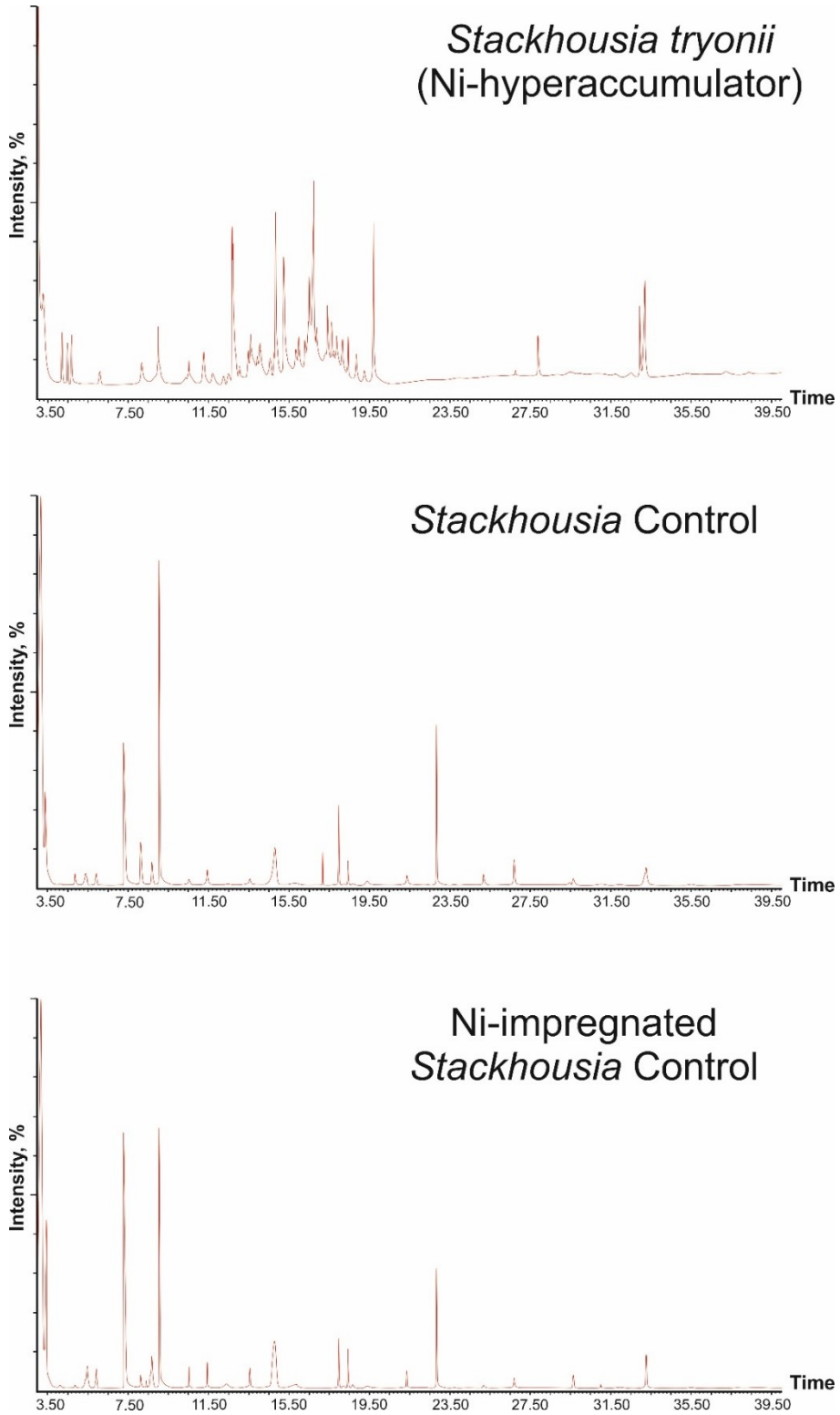

Fig. 5S. GC-MS of the *Stackhousia* related samples

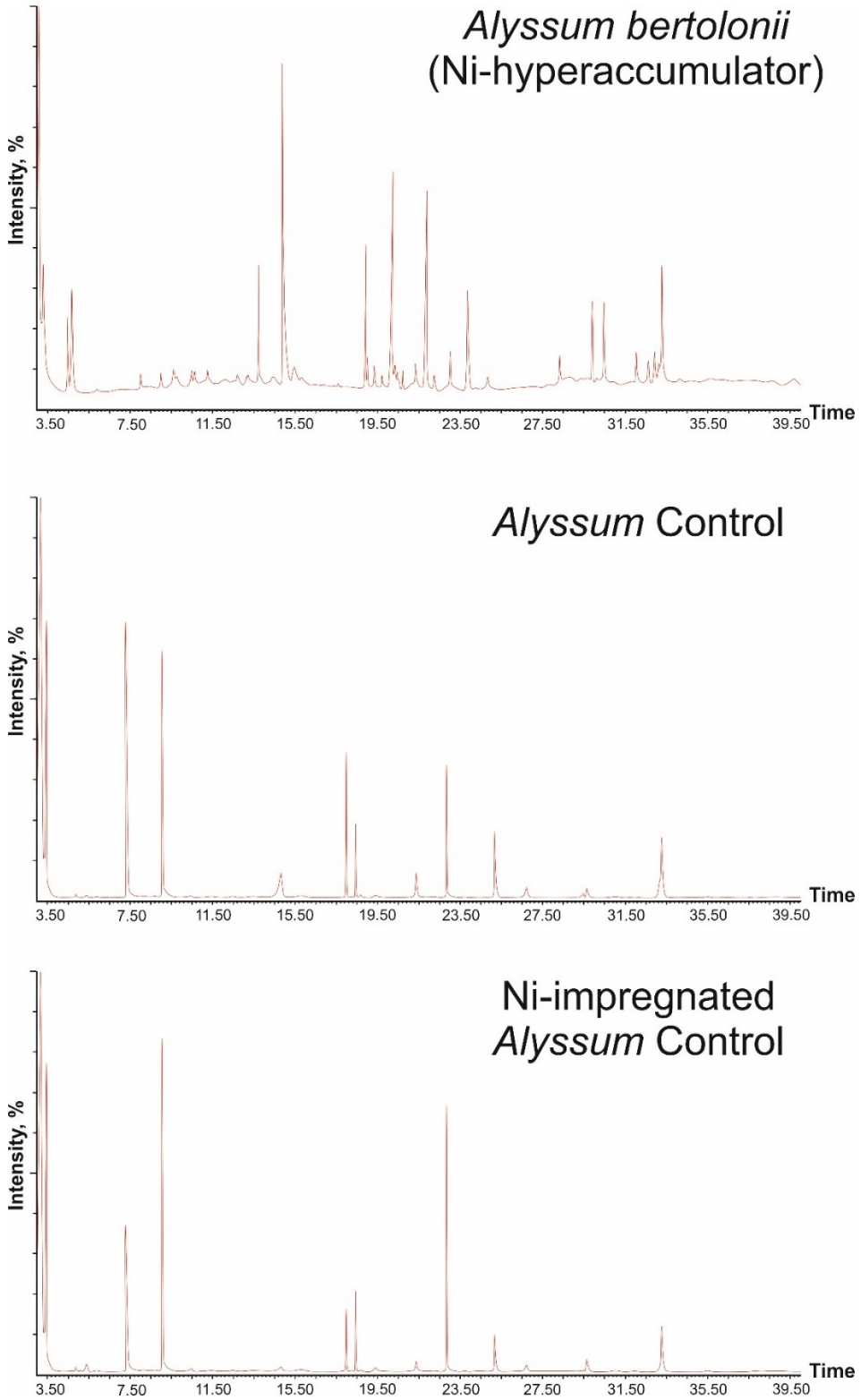

Fig. 6S. GC-MS of the *Alyssum* related samples

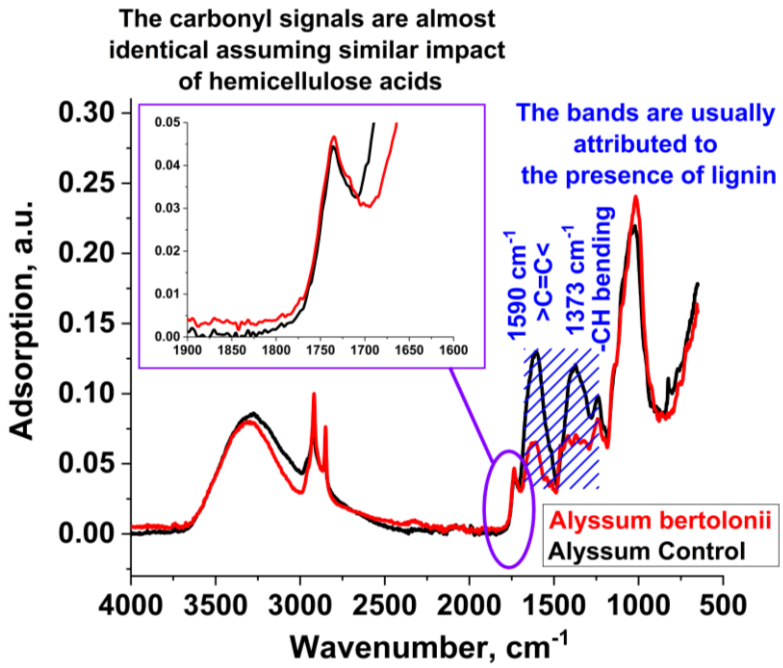

Fig. 7S. ATR-FTIR of the samples before MW-assisted pyrolysis

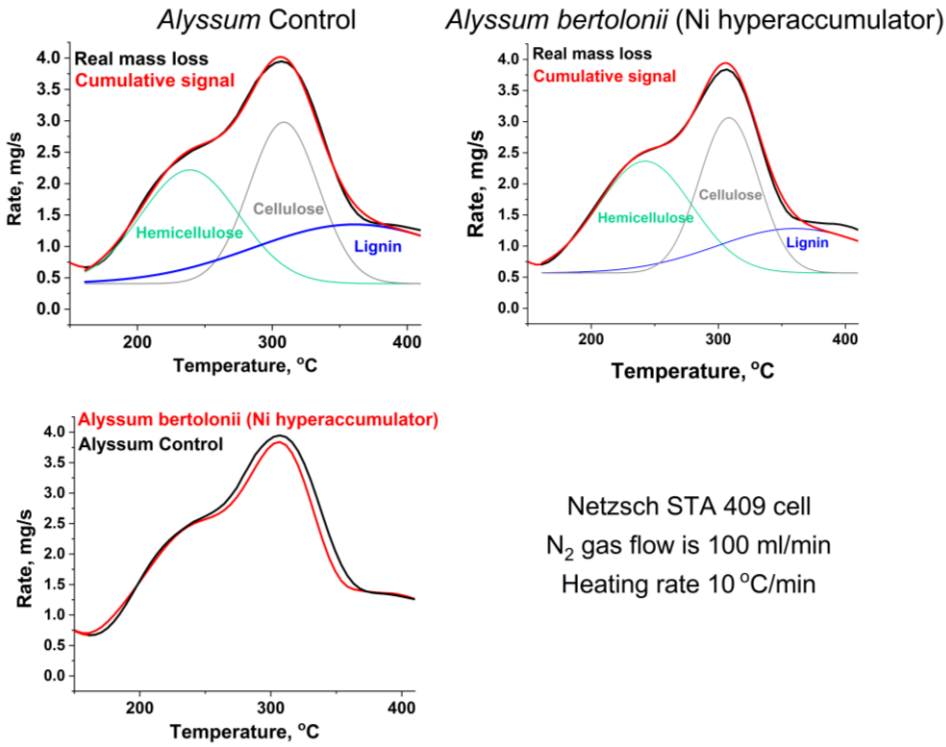

Fig. 8S. Mass-loss curves resulted from the conventional heating