Biodiversity of microbial communities in the rhizospheres of Alnus glutinosa contaminated by HCH isomers.

Aday Amirbekov Aday.amirbekov@tul.cz , Pavel Hrabák Pavel.hrabak@tul.cz, Alena Ševců Alena.sevcu@tul.cz

In this exterior study, we planted 36 Alnus glutinosa seedlings in pots and treated 27 of them with HCH isomers, leaving 9 for control. The experiment was installed on the roof of the building, and all the pots were watered for 3 months. Soil, rhizosphere and sections of seedlings (roots, trunks, branches, leaves) where analysed by chemical and molecular biology methods.

Key words: Bioacumulation, biodegradation, Alder seedlings, PCR, NGS,

Introduction

The harmful effect of an hexachlorocyclohexane isomers on the environment and organisms is well known, and although field application was banned in 2010, contamination of soil and water is alarming. Chemical reduction and degradation of HCH isomers are well studied (Dominguez et al., 2016; Homolková et al., 2015; Wacławek et al., 2019). Detailed studies on the anaerobic microbial degradation of HCH isomers started to appear in the 1960s, and aerobic bacteria capable of degrading HCH isomers have also been discovered. Different levels of anaerobic degradation of HCH isomers by variable microbial strains and consortium has been observed. (Boyle et al., 1999; van Doesburg et al., 2005).

The aim of this study was to determine phytoremediation potential of α-, β-, and δ-HCH isomers by Alnus glutinosa, furthermore describe biodiversity of microbial communities in the rhizosphere

Material and methods

Each HCH isomer alone was mixed with soil to reach a final concentration of cca 5 mg/kg in one pot. Control was prepared with the same soil without HCH. All variants were set in triplicate and had instant access to water for three months. HCH was analysed in soil and sections of seedlings (roots, trunks, branches, leaves) on GC/MS assembles RSH/Trace 1310/TSQ8000 (PAL, Switzerland; ThermoFisher Scientific, USA) used the DB-5ms column for semivolatile HCH transformation products. The extraction of DNA from all samples in duplicate was carried out by using a DNeasy power Soil KIT (Qiagen, Netherlands). DNA yield and quality were analysed with the Qubit fluorometer (Thermo Fisher, USA) and by agarose gel electrophoresis. Amplicon 16S rRNA sequencing was applied to study the microbial community in rhizosphere samples by using the Ion PGM Hi-Q Sequencing Kit with the Ion 314 Chip on Ion Torrent PGM (Thermo Fisher Scientific). V4 region of bacterial 16S rDNA gene was amplified with primers 530F (5'–TGCCAGCMGCNGCGG–3') and 802R (5' TACNVGGGTATCTAATCC–3') in a final volume of 50 µL(Claesson et al., 2010; Dowd et al., 2008).

Fig. 1: Each triplicate was set on one tray. Control 1, control 2, control 3, alpha 1, alpha 2, alpha 3, beta 1, beta 2, beta 3, delta 1, delta 2, delta 3.

Results and Discussion

The highest concentrations of HCH isomers were detected in the roots with a decreasing trend towards the branches and the leaves. The δ-HCH isomer was taken up in highest quantities (14.7 µg/g in roots, 7.2 µg/g in trunks, 1.53 µg/g in branches and 1.88 µg/g in leaves) while α-HCH and β-HCH were found in much lower concentrations. Most interestingly, in the β-HCH treatment, we detected high concentrations of α-HCH as well.

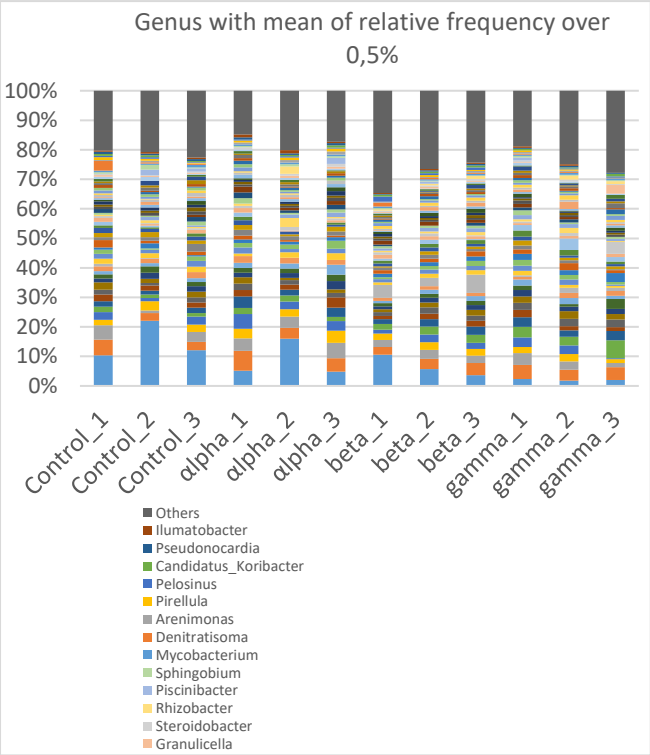

Fig. 2: Relative representation of microorganisms at family level (abundance > 0,5 %) in the rhizosphere samples.

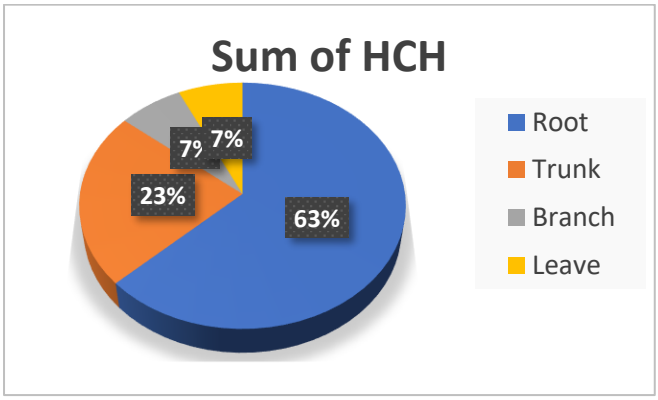

Fig. 3: Sum of HCH distributed by parts of A. glutinosa.

The abundance of rhizosphere populations was similar in all HCH isomer samples with some exceptions. For example, *Pseudomonas sp*. significantly decreased in all HCH-amended samples, the lowest abundance was found in the δ-HCH – the isomer that was detected in highest quantities. In contrast to *Pseudomonas*, the abundance of an anaerobic *Opitutus sp.* was the highest. In samples treated with β- and δ-HCH, *Rhizobacter sp.* appeared, while not present in control samples.

Conclusion

To conclude, the A. glutinosa seedlings were able to accumulate all HCH isomers, thereby proving that it has high phytoremediation potential. The reason why was the δ-HCH most easily bioaccumulated will be subjected to further study.

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