Microbial and Fungal abundance in rhizosphere of Alnus Glutinosa contaminated by HCH isomers

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OBJECTIVES

- To compare removal of α, β, and δ isomers of HCH by Alnus glutinosa (Alder tree) seedlings.
- - To study development of microbial and fungal communities in tested setups and to monitor genes encoding enzymes involved in biodegradation of HCH isomers.

Design of experiment

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.

Material and methods

The soil and rhizosphere samples were collected for chemical analysis, DNA extraction and analysis by real-time quantitative PCR (qPCR) to determine total bacterial biomass, and presence of functional genes (LinA, LinB, LinB-RT, LinD). Based on the qPCR results, water and sediment samples were chosen to be analyzed by next-generation sequencing (NGS) to estimate the bacterial diversity.

Results

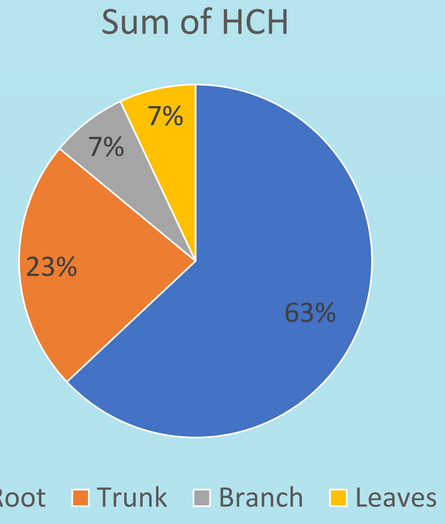

Fig. 3: Sum of HCH distributed by parts of A. glutinosa.

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.

		Total bacterial biomass	Lindane-degrading bacteria			
		16S rDNA	dehydrochlorinase	haloalkane dehalogenase	haloalkane dehalogenase	reductive dechlorinase
		U16SRT	linA	linB	linB-RT	linD
Rhizosphere	Control 1	+++	+-	+-	+-	+-
	Control 2	+	NA	+-	+-	+-
	Control3	+	+-	+-	+-	+-
	alpha 1	++	+-	+-	+-	+-
	alpha 2	++	+-	+-	+-	+-
	alpha 3	++	NA	+-	NA	+-
	beta 1	+++	NA	+-	+-	+-
	beta 2	+++	NA	+-	+-	+-
	beta 3	+++	+-	+-	+-	+-
	delta 1	++	NA	+-	+-	+-
	delta 2	+++	+-	+-	+-	+-
	delta 3	+	NA	+-	+-	+-
Soil	Control 1	+	+-	+++	NA	NA
	Control 2	+	+-	+-	NA	NA
	Control3	++	+-	+++	+-	+-
	alpha 1	++	+-	++	NA	+-
	alpha 2	++	+-	++	NA	NA
	alpha 3	+++	+-	+++	NA	NA
	beta 1	++	+-	++	+++	NA
	beta 2	++	+-	+++	+-	NA
	beta 3	+++	+-	+++	+++	+-
	delta 1	++	+-	+	NA	NA
	delta 2	+++	+-	+++	+-	NA
	delta 3	+++	+-	+	+-	NA

Fig. 4: Presence of genes for dehydrochlorinase (linA), haloalkane dehalogenase (linB, linB-RT), re-ductive dechlorinase (linD) in control (C, different depths) and biochar sediments. The color scale indicates the relative quantity of a given marker, and the lowest numbers have the high-est quantity; X – not detected, or below the detection limit.

The highest quantity of total bacterial biomass marker was discovered in rhizosphere samples treated with beta isomer, on the other hand soil samples treated with beta isomer has less quantity of this marker (Fig.4). In soil samples treated with delta has highest quantity of 16 s gene. LinA gene was less amont or not detected at all in rhizosphere samples comparing to soil samples. Interestingly gene LinB was highest in soil samples comparing to rhizosphere smaples. Haloalkane dehalogenase is enzyme encoded by LinB gene and its involved in the pathway gamma HCH degradation and it also catalyzes conversion of (1,4-TCDN) to (2,5-DDOL) via the intermediate (2,4,5-DNOL).

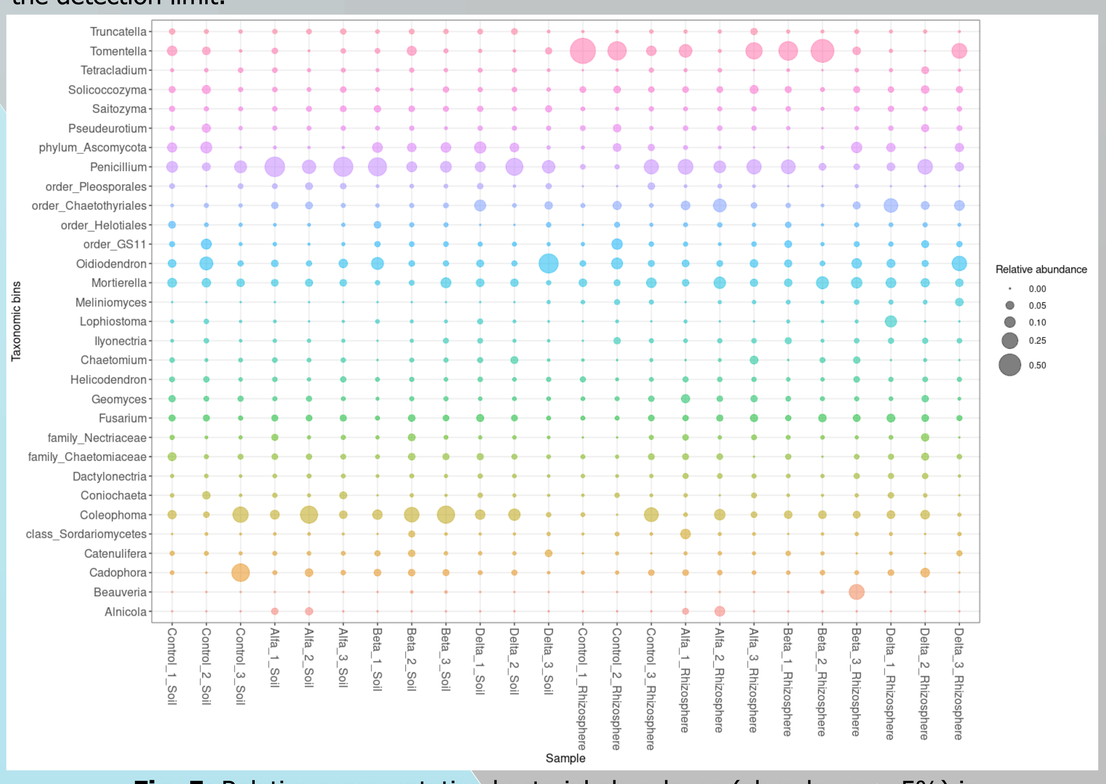

Fig. 5: Relative representation bacterial abundance (abundance > 5%) in soil and rhizosphere samples.

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 (Fig.2). Rhizosphere samples were rich for Tomentella. On the other hand Coleophoma was dominant taxa in soil samples.

Conclusion

To conclude, the A. glutinosa seedlings were able to accumulate all HCH isomers, mostly in root. Overall presence of lin genes was confirmed almost in all samples. LinA and LinB genes were present in high quantity in soil samples comparing to rhizosphere samples. Analysis of the metabolic pathways related to microbial abundance and detailed phytoremediation experiment using selected HCH isomers will be studiyed in future.

Acknowledgements

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