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

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In this exterior study, we aimed to determine the bioaccumulation potential of α, β, and δ isomers of HCH by Alnus glutinosa (Alder tree) seedlings. 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.

Keywords: Phytoremediation, Alnus Glutinosa, HCH, bioaccumulation, PCR.

Introduction

Pesticides were invented by people to control pests for widespread use in agriculture. Trying to solve one problem, we spawned several new ones, sometimes the value of which we often underestimate. The widespread production and use of lindane, as well as a large number of other HCH isomers and chlorobenzenes (CB), has caused pollution in soil, water and atmospheric systems around the world. [1], [2]. The rhizosphere and plant endosphere host plant growth-promoting bacteria and microbes with the capacity to degrade organic contaminants and/or modify their bioavailability[3]. Inoculating the tolerant plant Withania somnifera with the lindane-degrading rhizobacteria Staphylococcus cohnii subsp. urealyticus can enhance dissipation of this organochlorine and improve plant growth.

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). Amplicon 16S rRNA and ITS region sequencing was applied to study the microbial and fungal community in rhizosphere samples.

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. 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.1). 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). 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.

		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. 1: 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 highest quantity; NA – not detected, or below the detection limit.

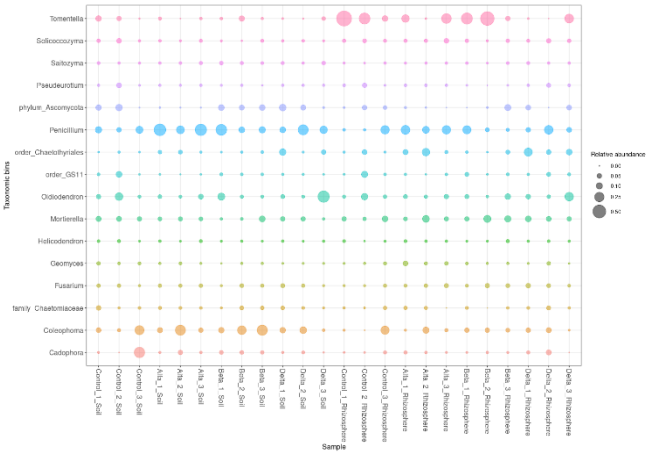

Fig. 2: Abundance of fungi taxa's in rhizosphere and soil samples. (mean 0.01 revised)

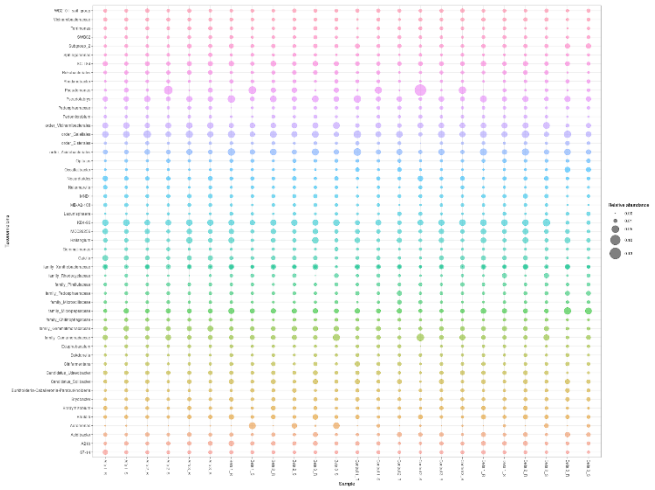

Fig. 3: Abundance of bacterial taxa's in rhizosphere and soil samples. (mean 0.05 revised)

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.

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Reference

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