

GC-MS identification of Lindane metabolites in bacterial cultures

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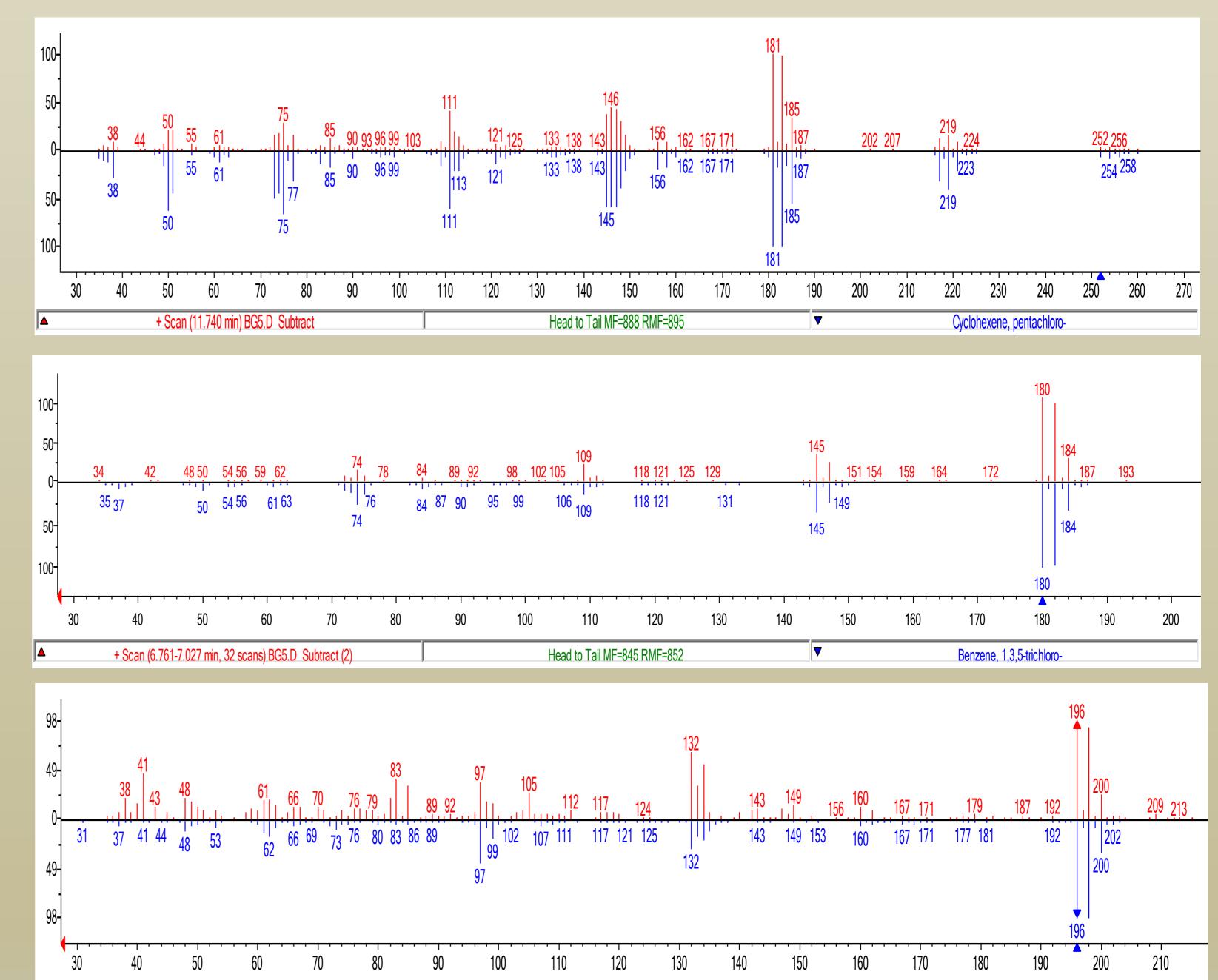
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INTRODUCTION

GC-MS is widely used as a tool for the analysis of small, volatile, non-polar compounds. In our work this method has been chosen for the analysis of chloroorganic pesticides metabolites suspected to be present in bacterial cultures, where the only source of carbon. Unique bacterial strains collected near the pesticides burial sites were used for experiments. It was expected that some bacterial strains or microbial consortiums could be very effective in degradation of very toxic for the environment substances like DDT or Lindane.

METHODS

The soil samples were pre-analyzed for the presence of pesticides with HPLC-MS and GC-MS techniques. The pesticides were extracted for the analyses using H₂O/acetonitrile solution 3:7 and for GC-MS the liquid-liquid extraction with DCM was done. In the biological experiment the isolated bacterial strains were cultivated in the two chosen mediums with the addition of Lindane as the carbon source. After one month the extraction to DCM was done and GC-MS analyses were performed



RESULTS x10 ⁵ + TIC Scan BG5.D Methoxychlor p,p-DDT * 15.579 Lindane × 18.673 × 19.187 19,702 Counts vs. Acquisition Time (min)

Fig. 1. The GC-MS chromatogram of the sample BG-5.

The Lindane was the pesticide that were found among the others in the BG5 sample chosen for the analysis (Fig. 1). The compounds of dechlorination of Lindane were found in samples and were considered to be the metabolites –

pentachlorocyclohexene, 2,4,5 – trichlorophenol and trichlorobenzene (Fig. 2). The identification was done by the comparison with the library spectra (Fig. 4).

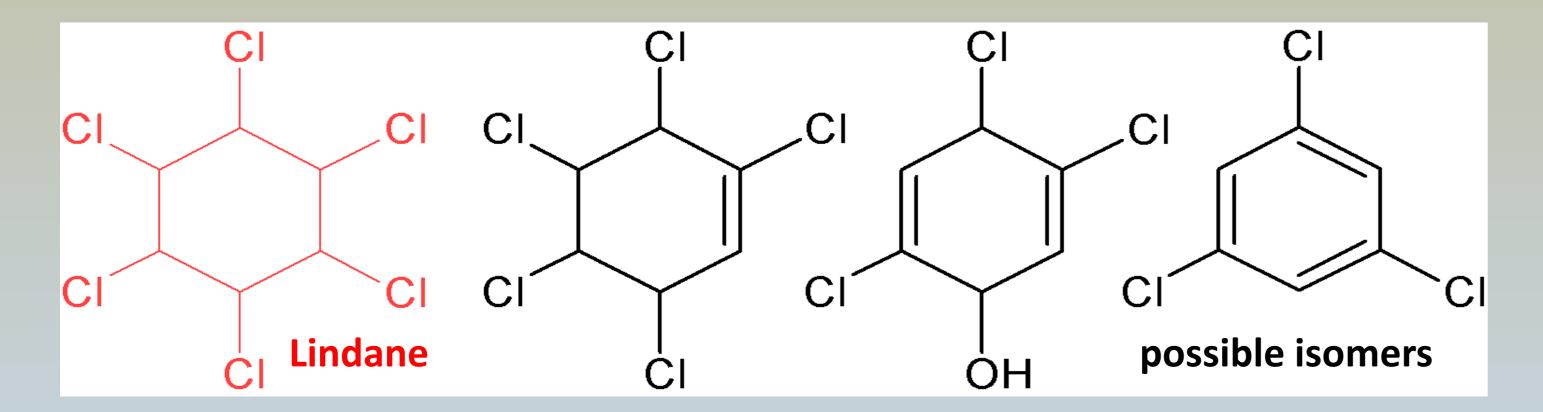
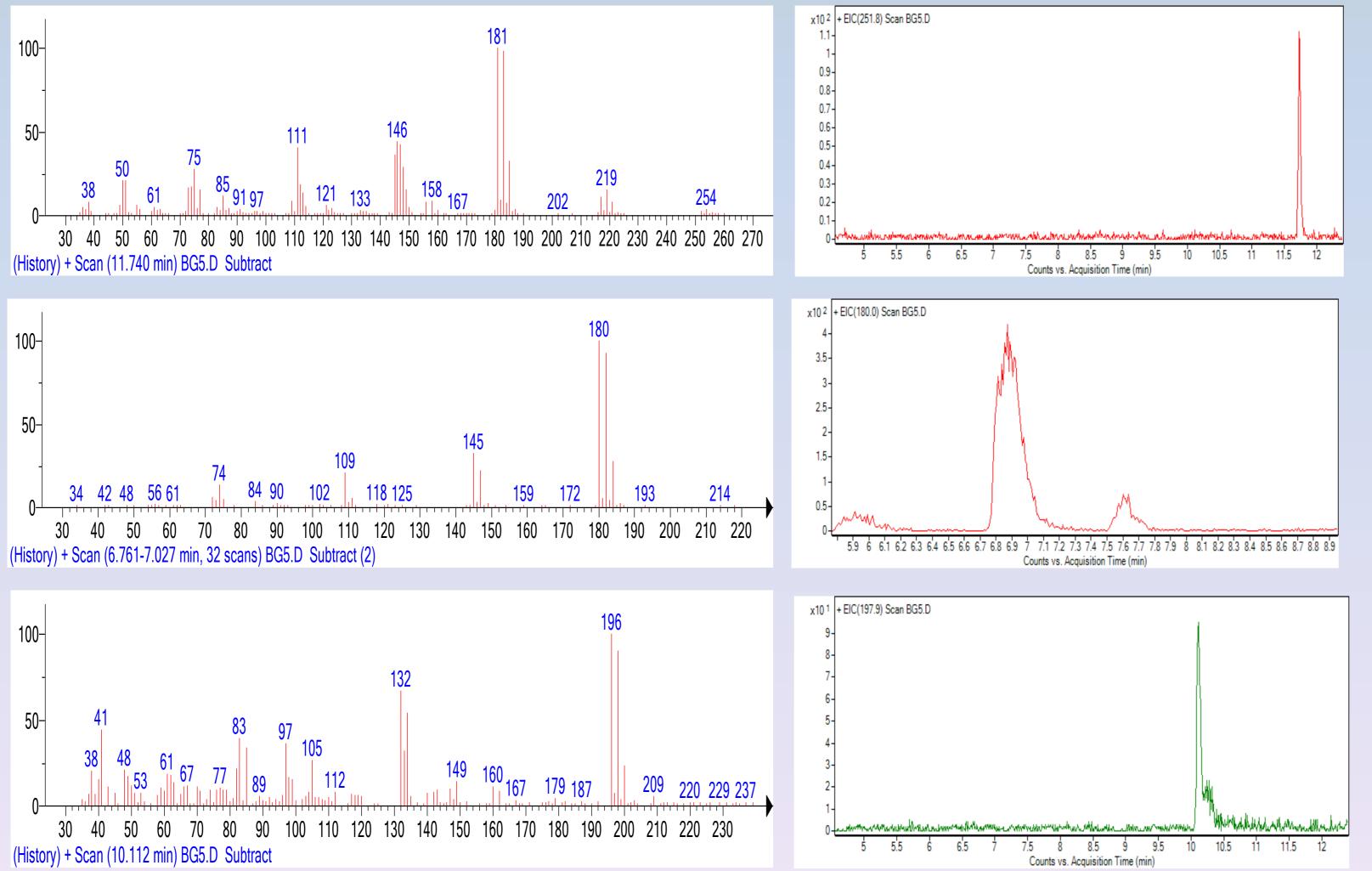


Fig. 2. The main metabolites/degradation products found in the samples.



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. Comparioson of the mass spectra (red colour) of pentachlorocyclonexene, 2,4,5 – trichlorophenol and spectra from library (blue colour).

The extracts from bacteria cultures samples were analysed for the presence of previously found in BG5 sample metabolites. Only the traces of the pentachlorocyclohexene (table 1) where found in few samples and the compound was not found in the blank medium samples without the bacteria strain. It can be proof for the pesticides metabolism by some bacterial strains, but it cannot be confirmed because of low levels of found compound.

Table 1. The results of experiment with bacteria.

Sample (bacteria strain)	Pentachlorocyclohexane	Trichlorobenzene	2,4,5-trichlorophenol
LB E5 Lin	+	-	-
LB G10 Lin	-	_	-
LB H1A Lin	+	-	_
LB H1AB Lin	-	-	-
LB H1B Lin	+	-	-
M9 E5 Lin	-	-	-
M9 G10 Lin	-	-	_
M9 H1A Lin	-		-
M9 H1AB Lin			

Fig. 3. Extracted ion chromatograms for the m/z 251.8, 197.9, 180.0 and mass spectra from library for pentachlorocyclohexene, 2,4,5 – trichlorophenol and trichlorobenzene.



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enzymatic bioremediation and biotransformation.





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M9 H1B Lin

CONCLUSIONS

The method used for the analysis where suitable for the Lindane metabolites identification what was confirmed by finding compounds considered to be the Lindane metabolites. The traces of the pentachlorocyclohexene were found in some bacteria cultures samples, but very weak, and the experiments should be improved in future mainly by trying to increase the efficiency of the metabolism process.