GC-MS identification of DDT metabolites in bacterial cultures

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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 pesticides were 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 H_2O/acetonitrile solution 3:7 and for GC-MS the liquid-liquid extraction with DCM was done.

sample	soil	DDT	sod acet
Az		+	
AzAc		+	+
BG5	+		
BG5Ac	+		+
BG5Az	+	+	
BG5AzAc	+	+	+

In the „experiment 1" one set of samples with the DDT as the only source of carbon for growing bacteria was prepared.
The microorganisms were isolated from the soil sample that has been chosen for the experiments and were cultivated in the medium.

The experiment 2 was prepared in three sets of samples where DDT was added in acetone solution directly into the soil. The control samples were prepared by pure acetone addition and by the sterilization of the samples with the pesticide added. The samples were analyzed at start and end of the experiment after 4 weeks.

sample type	sample	soil	acetone	DDT	sterilization
control sample	A1/A2/A3	+	+	+	+
test sample	AD1/AD2/AD3	+	+	+	+
control sample	ADS1/ADS2/ADS3	+	+	+	+

For the GC-MS analyses samples from experiment 1 were extracted directly into the dichloromethane and ethyl acetate. The samples from experiment 2 were prepared like the soil samples (described above).

RESULTS

The DDT, Lindane and Methoxychlor where the pesticides that were mainly found in the BG5 sample chosen for the analysis (Fig. 2). The possible metabolites or degradation products of DDT like DDE, DDD, DDMU or dichlorobenzophenone (Fig. 1) where found in the soil samples.

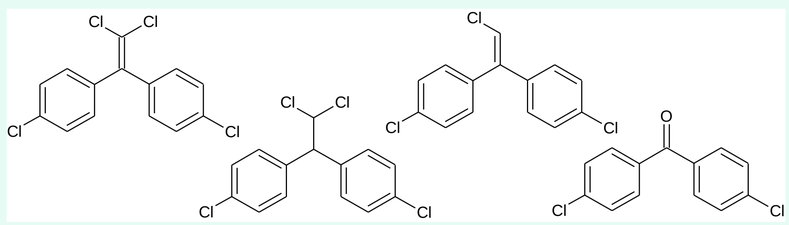


Fig. 1. The main metabolites/degradation products found in the samples.

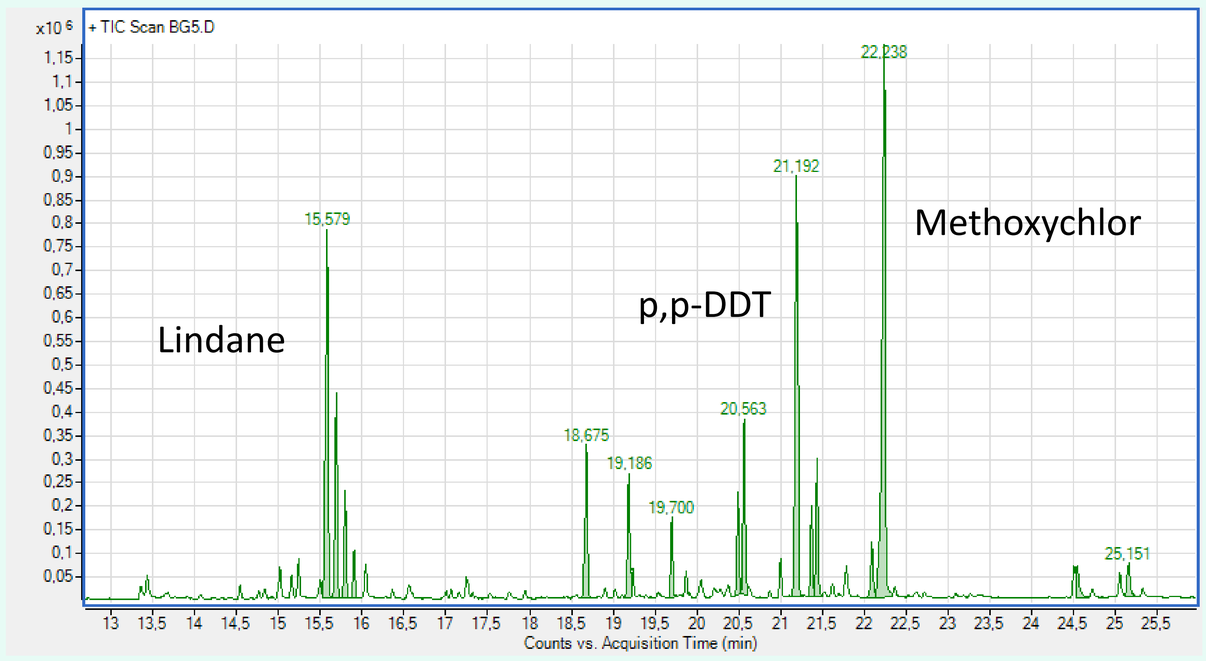
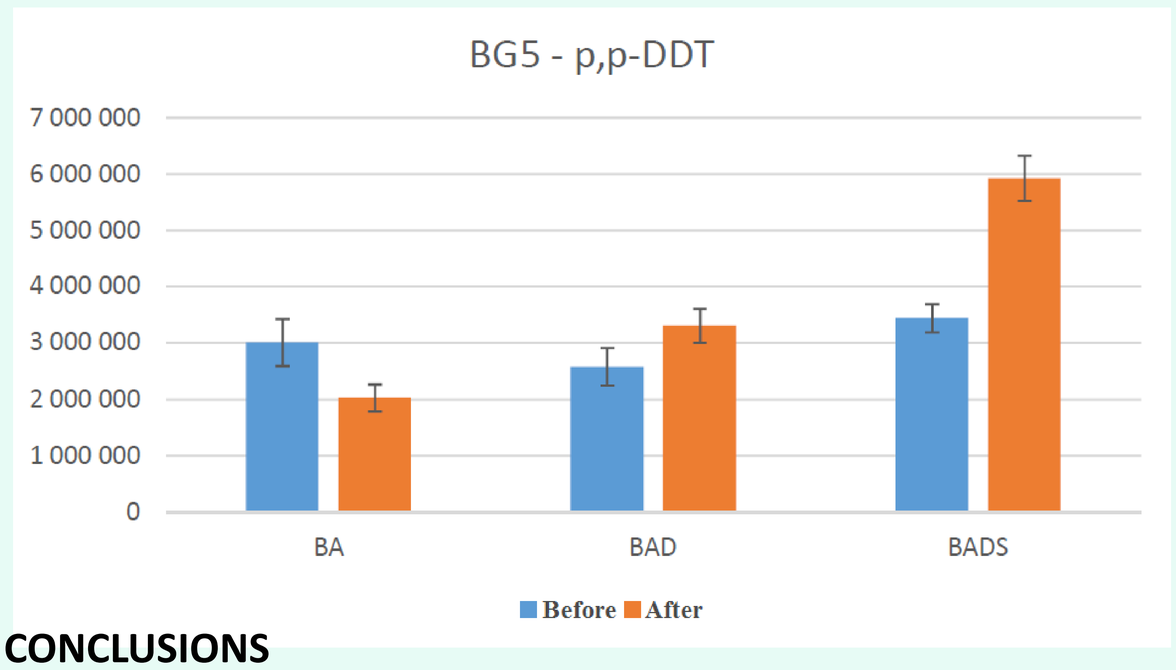


Fig. 1. The GC-MS chromatogram of the saple BG-5.

The same metabolites and degradation products as in the soil sample were found in the samples obtained in the biological experiments but no correlation in changes in their amounts where found and no new metabolites were present indicating the microbial activity in the DDT metabolism.

The amounts of investigated pesticide - p,p-DDT varied strongly without reasonable correlation with the assumed results (Fig. 3) in the experiment 2. The reason were probably the changes in the extraction conditions



CONCLUSIONS

The method used for the analysis where suitable for the DDT metabolites identification what was confirmed by finding compounds considered to be the DDT metabolites. The weak bacterial activity did not let us to find some further metabolites. The changes of the DDT level cannot be the biological activity indicator due to the unrepeatable extraction process. The experiments conditions and the extraction methods should be improved in the future.

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This study was partially supported by grant funded by the Polish National Cohesion Strategy Innovative Economy Grant, No. UDA-POIG 01.01.02-14-054/09