BIODEGRADATION OF SOIL POLLUTED WITH POPS (HCH AND DDT) – RESULTS OF LABORATORY TRIALS

Diana Dobre, Cristiana Cosma, Elisabeta Pena-Leonte, Sorin Ion Florescu, Mihai Stefanescu, Costel Bumbac, Daniela Niculescu

National Research and Development Institute of Industrial Ecology
90-92 Panduri St., Bucharest, Romania

ABSTRACT

This work shows the results up to date of the biodegradation experiments started at ECOIND in April on soil samples collected from Turda and Oltchim sites, within Eurostars E!5164 – POPELIM project. The project partners are: Dekonta, ECOIND and DFR System SRL. Initial analyses showed contamination of Turda soil with HCH isomers (particulary β-HCH) of hundreds of µg/kg d.m. and with DDT of tens of µg/kg d.w. Initial analyses of Oltchim samples showed the pollution of soil with tens of mg/kg d.w. of HCH isomers and tens of µg/kg d.w. of DDX.
The purpose of the biodegradation experiments was to demonstrate the performance of stimulation of indigenous soil bacteria to degrade pesticides (HCH and DDT) and to isolate bacterial strains with such degrading capacities.

INTRODUCTION

Organochlorinated pesticides, such as hexachlorocyclohexanes (HCHs) and dichlorodiphenyltrichloroethanes (DDTs) are well known as persistent organic pollutants (POPs) [2], [3]. Organochlorine pesticides are toxic and carcinogenic and may cause serious environmental and health problems on animals, including humans [4]. They have become a major issue of environmental research in order to investigate the level of their occurrence in environmental compartments [5]
DDT and HCH isomers constitute a large part of obsolete pesticides stockpiled in many countries of Africa, Asia and the former communist block in Europe [6]. It is estimated that during the 1970s and 1980s more than 20 000 t of obsolete pesticides were stored in nearly 300 hazardous waste repositories, called ''tombs". Additionally, about 80 000 t of pesticide waste were deposited at industrial landfills. Nowadays, leakages from these places spread contamination to surrounding soil, groundwater, and water courses, in some cases endangering drinking water intakes.
During remedial actions wastes are removed and incinerated; however, such procedures cannot be applied to large areas of contaminated soil and groundwater. This raises the issue of effective soil bioremediation methods [1].

EXPERIMENTAL SETUP

Soil samples

Soil samples taken from Turda and Oltchim sites at different depths were homogenized, resulting three soil samples (1 Turda and 2 Oltchim) which were air dried and sieved through 4 mm mesh, then analyzed for physical chemical parameters. These three samples were analyzed initially for HCH and DDX and the results are presented in the table below:

	pH	Σ HCH (µg/kg)	Σ DDX(µg/kg)	Hexachlorbenzene (HCB) (µg/kg)
Turda soil sample	7-8	284.3	7.7	1.7
Oltchim soil sample I	7-8	46,023	48.8	45.6
Oltchim soil sample II	7-8	5440.5	6.3	5.5

Table 1. Chemical analysis of initial soil samples

Preparation of soil samples

500 g of dried soil from the three samples was transferred in 2.5 L brown bottles cap sealed, after water was added to 50% humidity, resulting in soil slurry. For Turda soil, a sample bottle was made with nutrient addition and a blank bottle with no nutrient addition. Due to insufficient quantity of sample, no blank was possible for Oltchim soil. Soil samples were treated with 1% Daramend product - Oltchim and Turda samples, 1% molasses - Turda soil, 5% acetate – Oltchim soil, 5% Fe(0) - Oltchim soil and *Phanerochaete chrysosporium* - Oltchim soil.
The biodegradation variants are presented below:

	1 bottle Turda sample for:	1 bottle Oltchim sample for:
Variant I – Daramend product 1%	x	x
Variant II – molasses 1%	x	-
Variant III – acetate 5%	x	x
Variant IV – Fe(0) 5%	-	x
Variant V – inoculum	-	x

Table 2. Experimental variants of bioremediation

One biodegradation cycle consisted in 10 days anaerobic and 4 days aerobic (with good shaking, manual) for all variants, at room temperature (20-24^{0}C). In case of variants II and IV humidity 50% was assured by means of groundwater from the two locations.

After 10 days of anaerobic treatment, the slurry was analyzed for anaerobic bacteria developed on anaerobe agar medium at 37^{0}C.

After each cycle of 14 days, all soil samples were analyzed for pH, HCH isomers, DDX, HCB, Σ DCB and Σ TCB.

For the isolation of bacterial strains from polluted soil, 1 mL of soil suspension was transferred on agar medium containing mineral salt medium (Winogradsky) and pesticides – lindane (10 mg/l) and DDT (10 mg/l), and incubated for 48 h at 22^{0}C, in aerobic conditions.

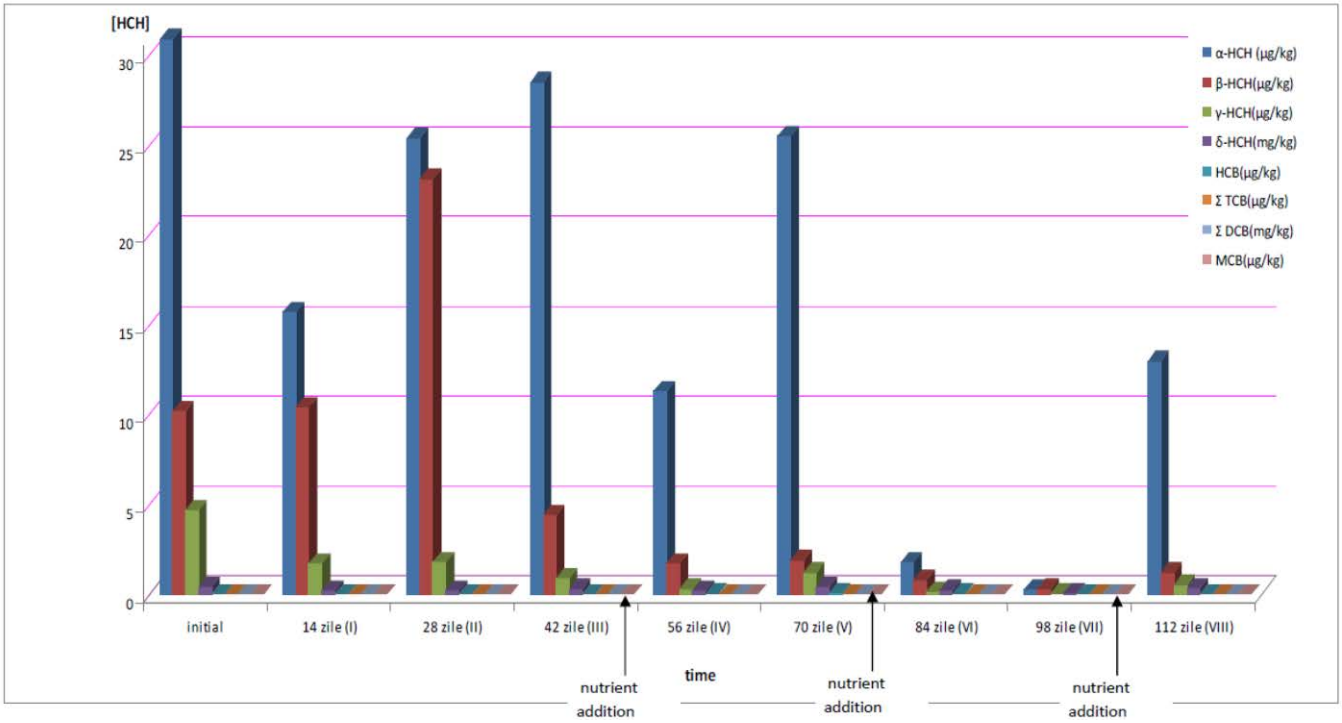
Since no colonies developed, we made the presumption that bacteria isolated from these soil samples degrade pesticides through cometabolism, therefore the MSM agar was supplemented with peptone. The colonies developed in 48h were counted. Some of these colonies representing pure bacterial strains were transferred on slants with LB medium and kept at 4^{0}C. They were tested for their potential of HCH degradation by 14 day incubation at 30^{0}C on liquid MSM – peptone medium supplemented with 5 mg/l technical lindane. The lindane contained 98-99% γ-HCH and 1-2% α- HCH. The strains were compared with *R. jostii* and *Ph. chrysosporium*.

From the lyophilized strains of bacteria and fungi we recovered *Rhodococcus jostii* RHA1 and *Phanerochaete chrysosporium* in 1 ml saline solution, followed by dilution of cell suspension and transfer on LB agar (*Rhodococcus jostii*) and Sabouraud medium (*Phanerochaete chrysosporium*). The cell suspension of *R. jostii* was incubated at 22^{0}C for 72h and *P. chrysosporium* at 37^{0}C for 7 days. The colonies developed were then transferred on slants and kept at 4^{0}C.

RESULTS AND DISCUSSIONS

Figures 1 to 6 show the evolution of pesticide concentrations - HCH isomers and DDX – as well as the number of aerobic and anaerobic bacteria for each experimental variant, during the 112 days of experiment. Variants treated with acetate, Fe(0) and inoculum were initiated after the start up of Daramend variant.

A.



B.

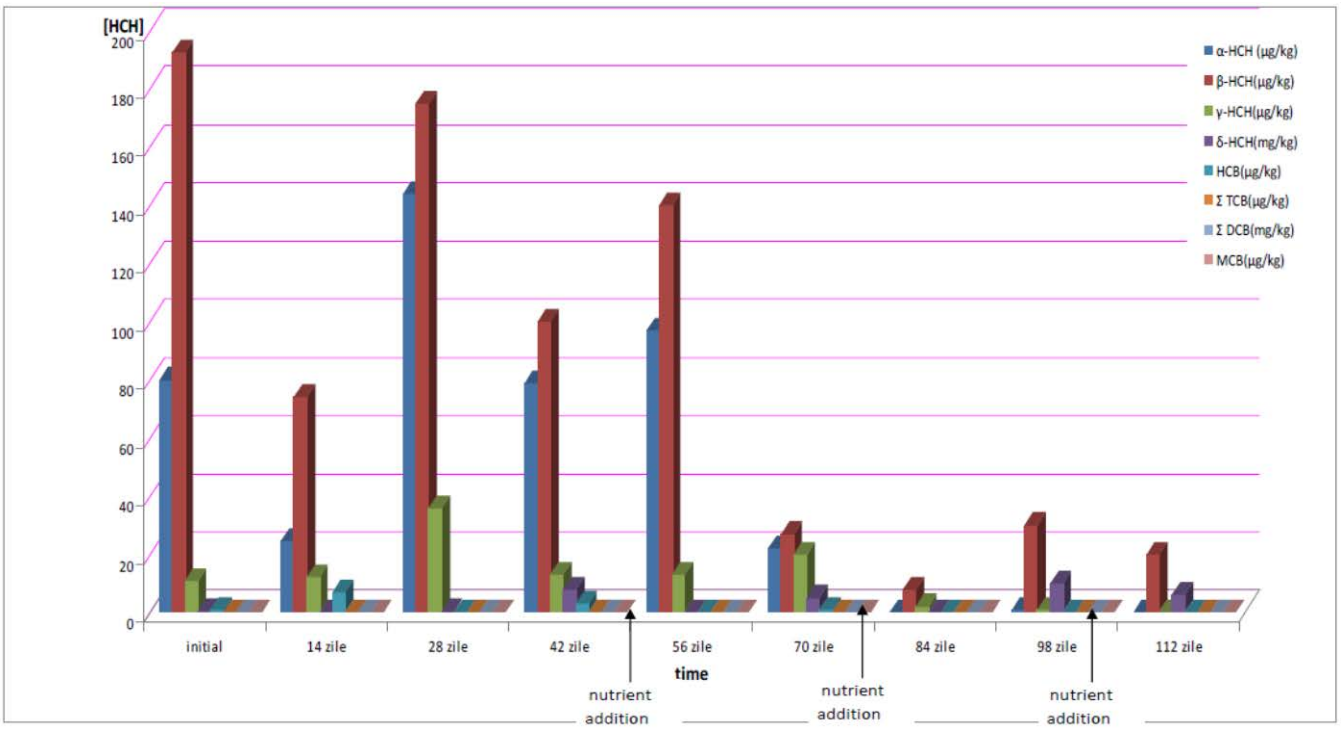


Figure 1. Variant 1 – Daramend: A) Turda soil; B) Oltchim soil

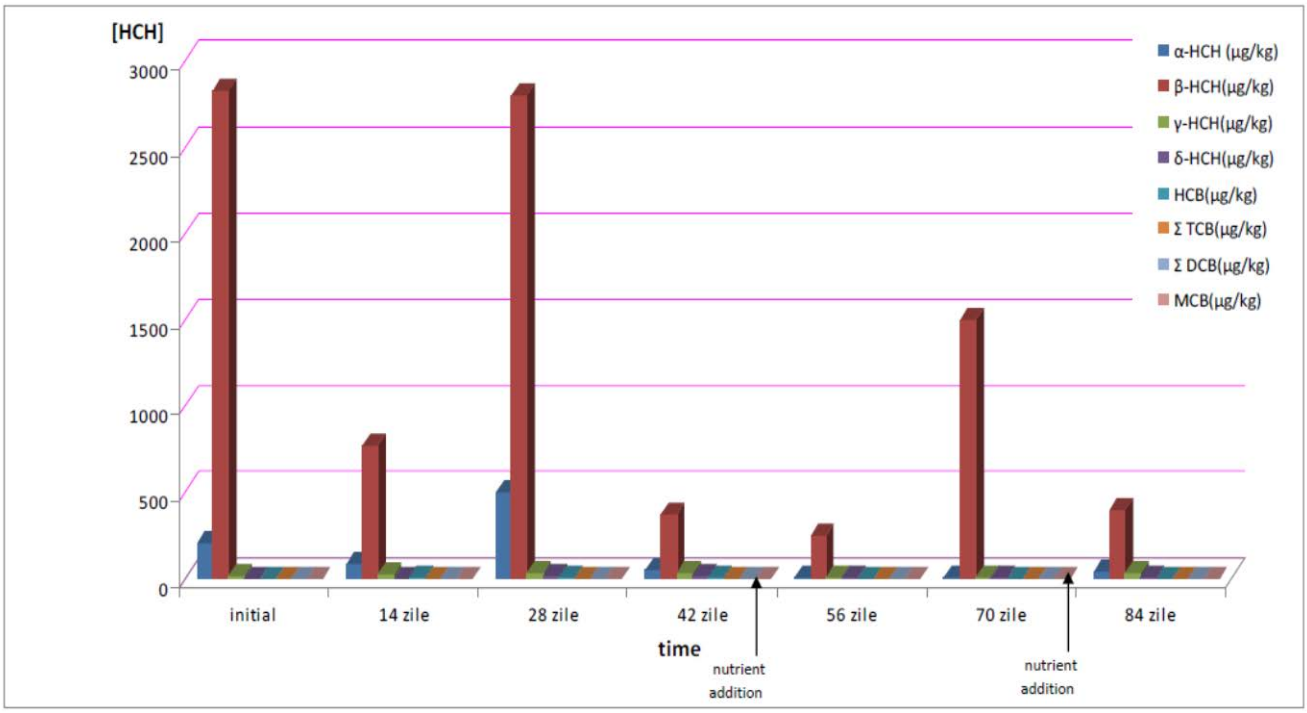


Figure 2. Variant 2 – Turda soil and groundwater treated with molasses

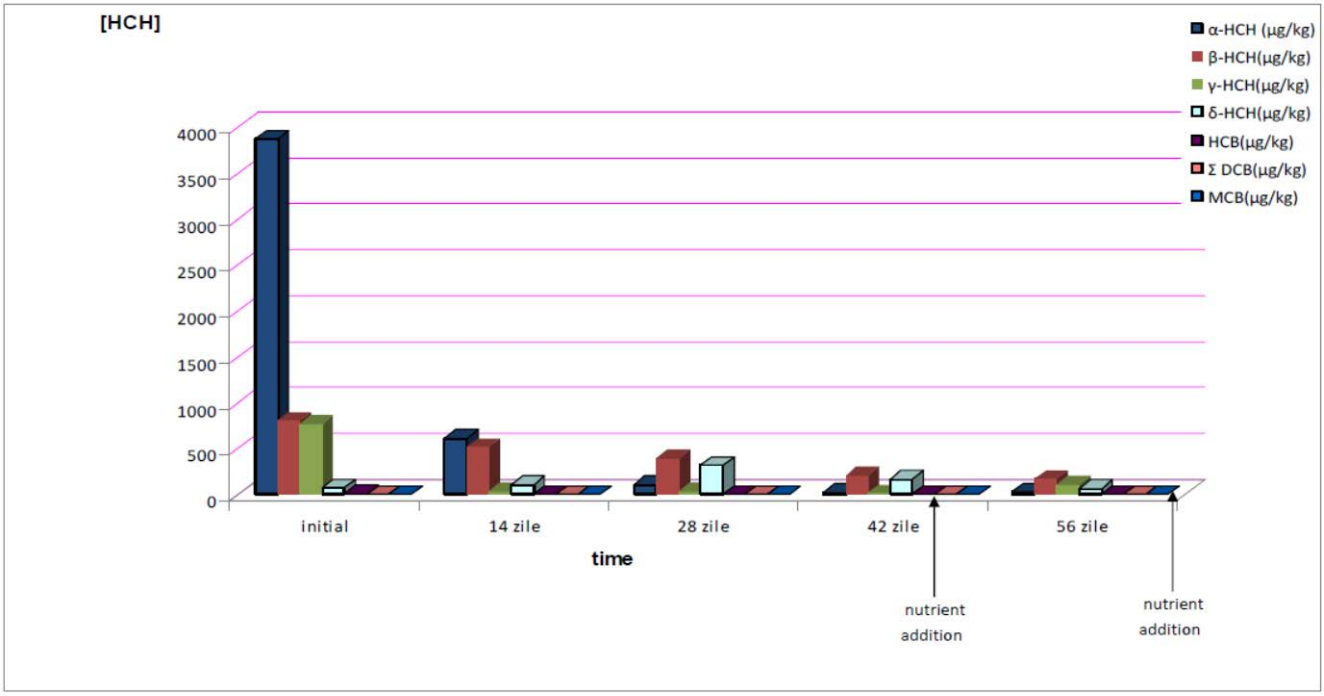


Figure 3. Variant 3 – Oltchim soil and groundwater treated with acetate

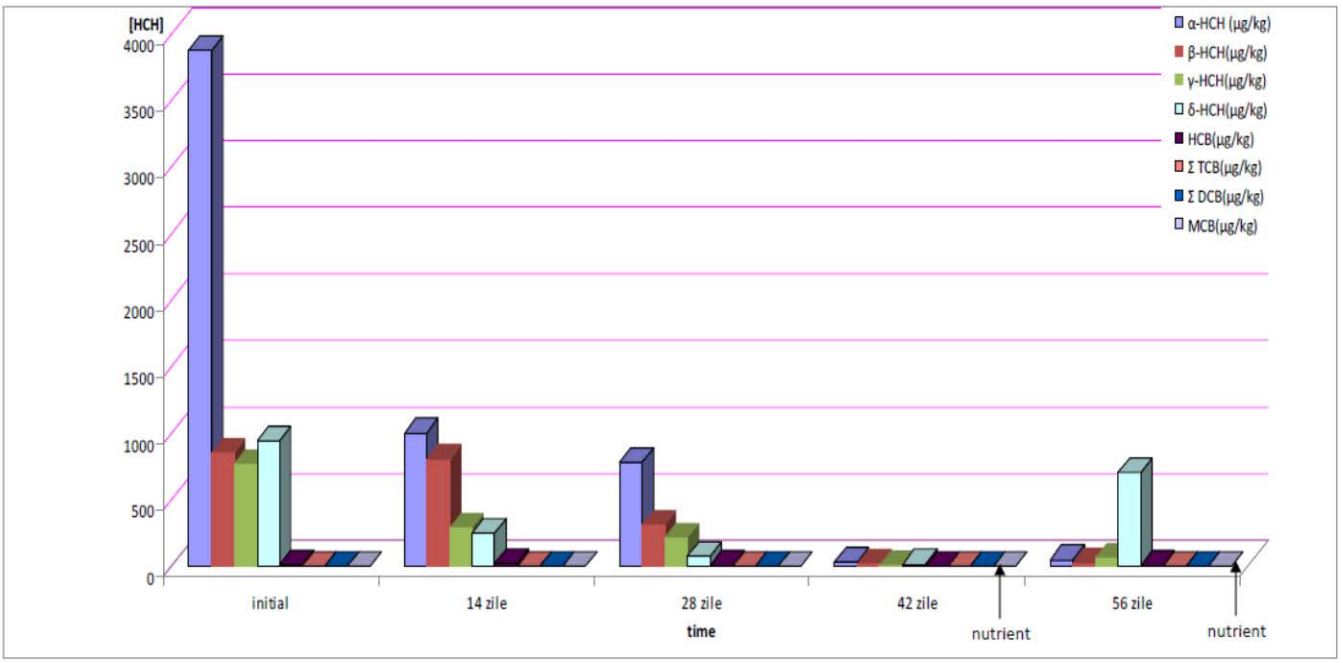


Figure 4. Variant 4 – Oltchim soil treated with Fe(0)

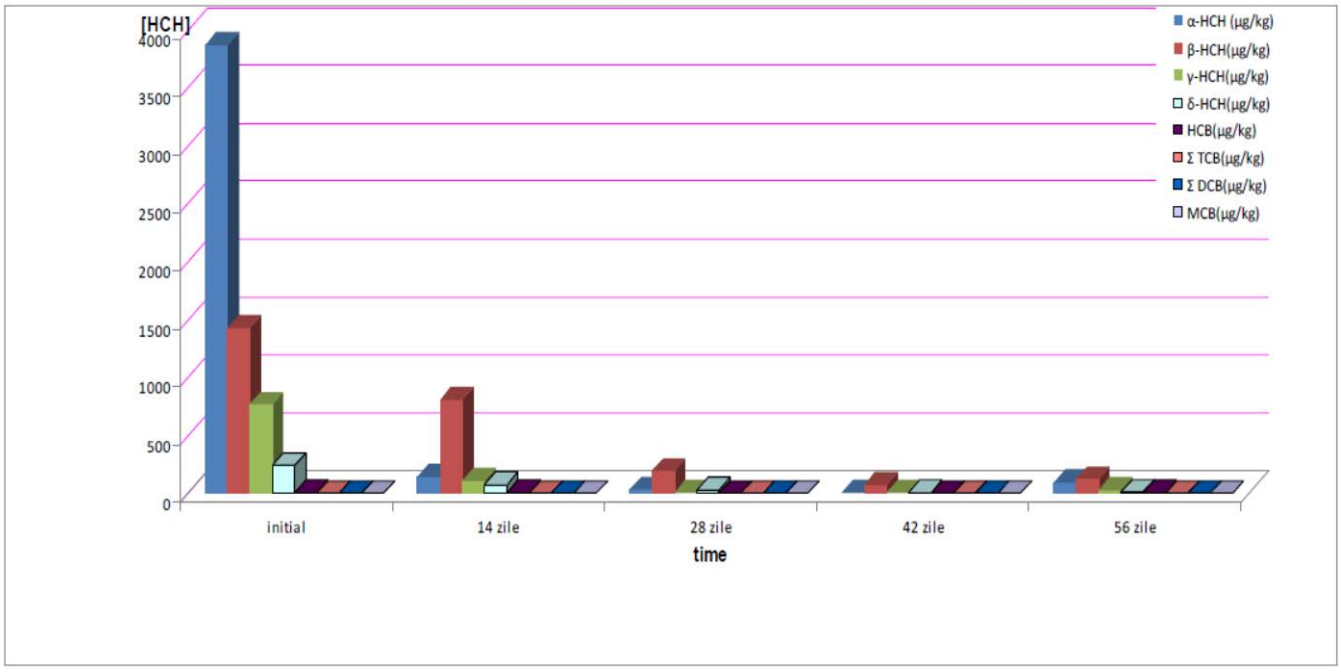


Figure 5. Variant 5 – Oltchim soil treated with *Ph. chrysosporium*, aerobic conditions

We also analyzed the composition of headspace gas produced during anaerobic phase, after 112 days. The results are shown below:

Variant	%Butane	%Propane	%Methane	%CO_2
Variant I – Daramend				
Oltchim	-	0.6	-	0.2
Turda	-	0.6	-	0.07
Variant II – molasses 1%	0.01	1	0.3	25
Variant III – acetate 5%	-	0.7	-	4
Variant IV – Fe(0) – 5%	-	0.6	-	1.2
Turda Blank	-	0.6	-	-

Table 3. Composition of headspace gas

We isolated two bacterial strains from Turda soil, one from Daramend variant (TD-5) and one from molasses (TSM-5), and one bacterial strain from Oltchim soil treated with Daramend (PO-5). They all degraded HCH in aqueous solution, as follows:

Strain	Percentage of removing γ-HCH	Percentage of removing α-HCH
TD-5	65	27
TSM-5	85	77
PO-5	30	71
R. jostii RHA1	90	62
Ph. chrysosporium	75	68

Table 4. Efficiency of isolated bacterial strains compared to reference strains

Gram staining revealed that all three bacterial strains were Gram negative.
A significant decrease of all forms of HCH in soil treated with Daramend product, Fe(0), molasses, acetate and *Ph. chrysosporium* was detected after 112 days of experiment corresponding to 8 sequential cycles. The number of aerobic bacteria was 560 CFU/10g soil in Turda samples and 530 CFU/10g soil after the first cycle. The number of anaerobic bacteria at 37^0C increased significantly in samples treated with Daramend and molasses. α and β-HCH decreased after 98 days (cycle VII) in Oltchim soil and in 84 days (cycle VI) in Turda soil, after stimulation with Daramend product. Molasses stimulated biodegradation of β-HCH from 2800 µg/kg to 400 µg/kg in Turda soil after 56 days and one supplementation of carbon source. Acetate stimulated degradation of α and δ-HCH, their concentrations decreased significantly after 42 days and no supplementation of nutrient. Fe (0) was very efficient in the degradation of all HCH isomers after 42 days. The bacterial strains isolated successfully degraded lindane in liquid nutrient medium. HCB was present as an intermediate of HCH biodegradation. No TCB, DCB and MCB were detected. Since these substances were analyzed after drying the sample at room temperature, they could have been lost through volatilization. The concentration of CO2 in headspace gas 25% indicates that the process of complete degradation of organic matter took place.

CONCLUSIONS

Two Romanian soil samples contaminated with POPs – HCH and DDX – were used in laboratory experiments of bioremediation through biostimulation of indigenous bacteria with different organic sources. The technology chosen for testing was biological sequential treatment in cycles of 14 days: 10 days anaerobic and 4 days aerobic. The results up to date performed in our laboratory show that, beside Daramend product, known as organic stimulator of pesticide bioremediation, molasses and acetate are carbon sources that can be used successfully in experiments of bioremediation of soil containing HCH isomers and DDX, at pilot scale. Molasses stimulated 86% of β-HCH removal in 56 days and acetate stimulated 99% of α-HCH and 93% of δ-HCH removal in 42 days. Neither molasses nor acetate stimulated the removal of DDE, DDD and DDT.

REFERENCES

1. Baczynski, T. P., Pleissner, D., Grotenhuis, T. "Anaerobic biodegradation of organochlorine pesticides in contaminated soil – Significance of temperature and availability." *Chemosphere*, 2010.
2. Cofield, N., Banks, M.K., Schwab, A.P. "Evaluation of hydrophobicity in PAH-contaminated soils during phytoremediation." *Environmental Pollution*, 2007.
3. Jones, K.C., de Voogt, P. "Persistent organic pollutants (POPs): state of the science." *Environmental Pollution*, 1999.
4. Safe, S. "Development, validation and limitations of toxic equivalency factors." *Chemosphere*, 1992.
5. Shi, Z., Tao, S., Pan, B., Liu, W.X., Shen, W.R. "Partitioning and source diagnostics of polycyclic aromatic hydrocarbons in rivers in Tianjin,China." *Environmental Pollution*, 2007.
6. Vijgen, J. "Obsolete pesticides: how to solve a worldwide society problem?", *IWA Publishing, London*, 2005.