BLOCK 10

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IN SITU IMPLEMENTATION OF TRIALS ON MICROBIOLOGICAL REMEDIATION OF POPS CONTAMINATED SOILS IN KYRGYZSTAN

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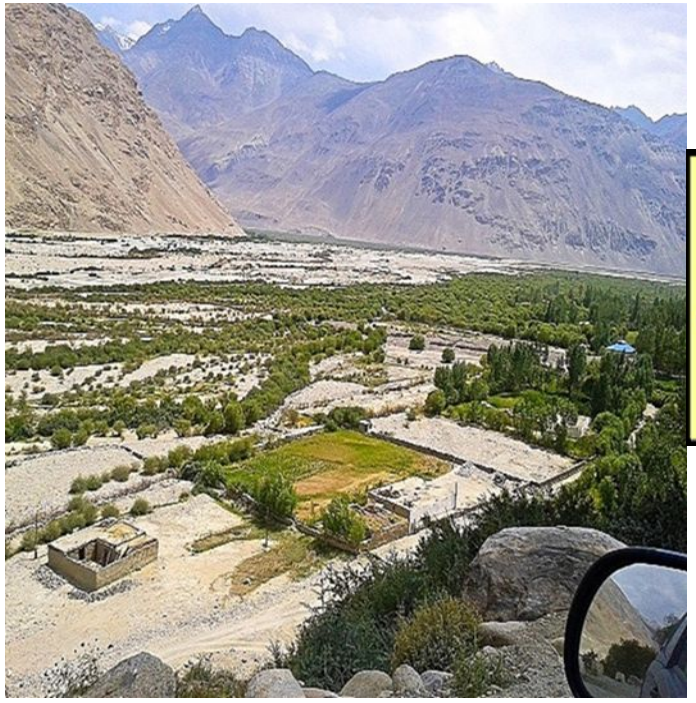
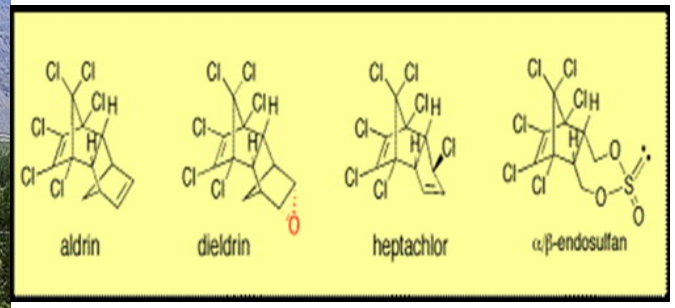
INTRODUCTION.

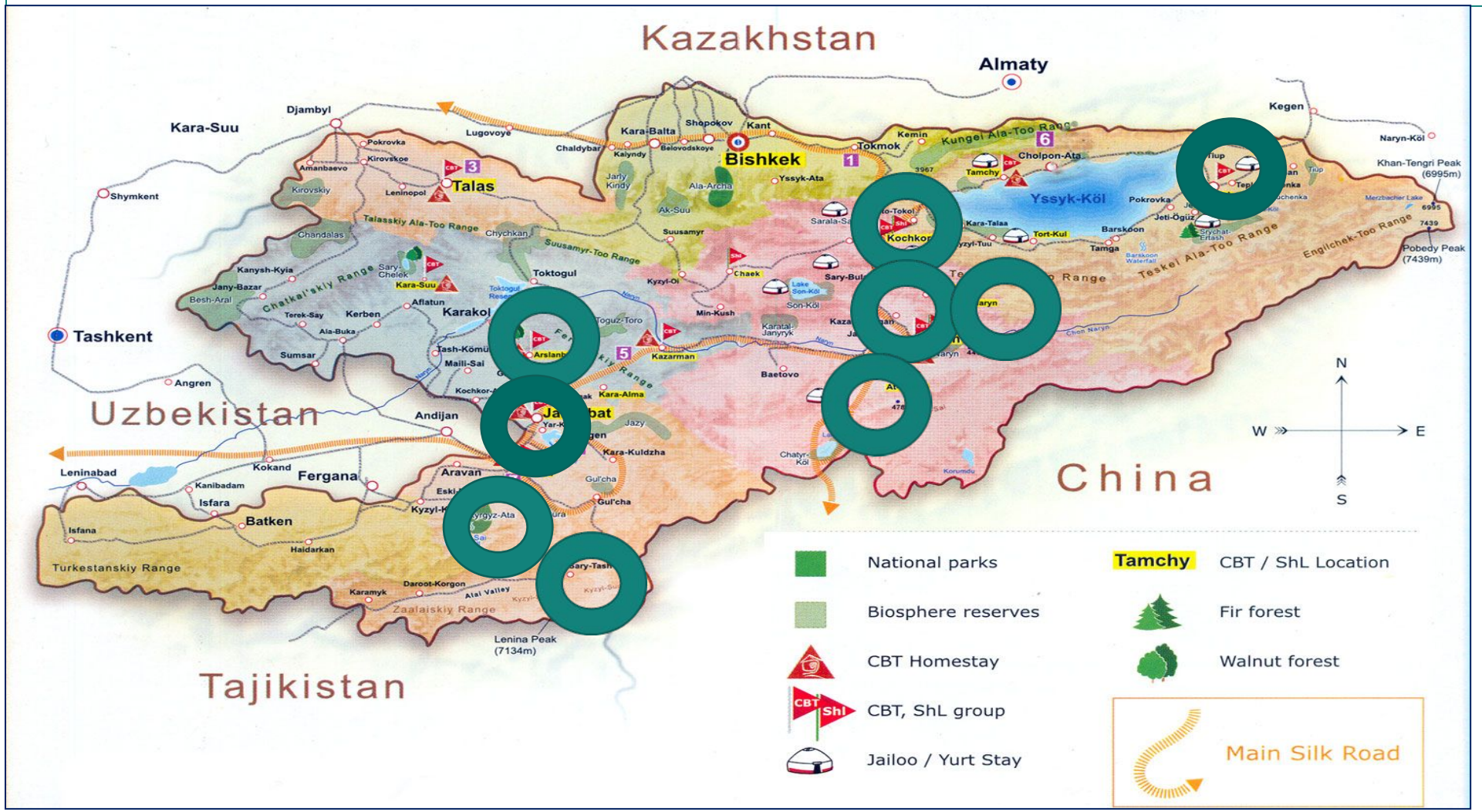
Environmental safety is one of the biggest problems we are facing

- Today, soil pollution by pesticides is a worldwide problem. Organochlorine pesticides are persistent in degradation, so they tend to bio-accumulate in the ecosystem trophic links.
- Currently, 50 storage facilities for obsolete banned pesticides exist in Kyrgyzstan. They hold about > 5.000 tons of these hazardous chemicals and pose a severe threat to the surrounding populations, livestock and environment.





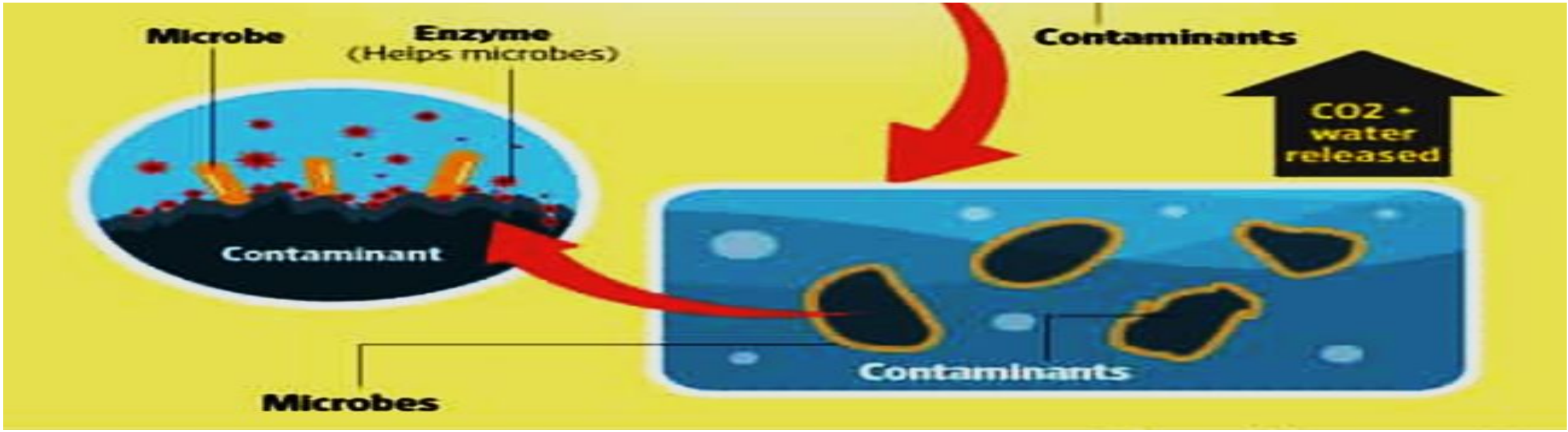
In almost every region of Kyrgyzstan, there are former storehouses and landfills for obsolete pesticides.



Death of a large population of sheep after drinking the water of rivers flowing through these zones (2007)

What is bioremediation?

- **An ecofriendly, cost-effective, rather efficient method, which is an alternative to more expensive and toxic approaches like chemical and physical methods.**
- **Bioremediation exploits the metabolism of certain groups of microorganisms that use pesticides as nutrients for their metabolic reactions and completely mineralise the pesticides or convert them into decomposition products.**
- **In this study, we aimed to develop a bioremediation approach for treating polluted soils that uses agrochemical improvements and selected active degrading bacteria. A bioproduct was tested in in situ field trials.**



Research design

- Former pesticides store in Chem–Korgon village (N 42°49’23.9” and E 75°31’49.8”) was a suitable place for testing the efficacy of bioremediation.
- Three conditions were tested:
  1) Addition of fertile soil and bioproduct ;
  2) Addition of fertile soil but no bioproduct (Control 1);
  3) no fertile soil and no bioproduct (Control 2).
- The treatment was applied directly to the contaminated soil.
- For the bioproduct, three bacterial species were used: *Stenotrophomonas sp.* (Ps-B strain), *Lysinobacillus fusiformis* (SA-4 strain) and *E. cloacae* (SB-2 strain).

Preparation of the land for the trials

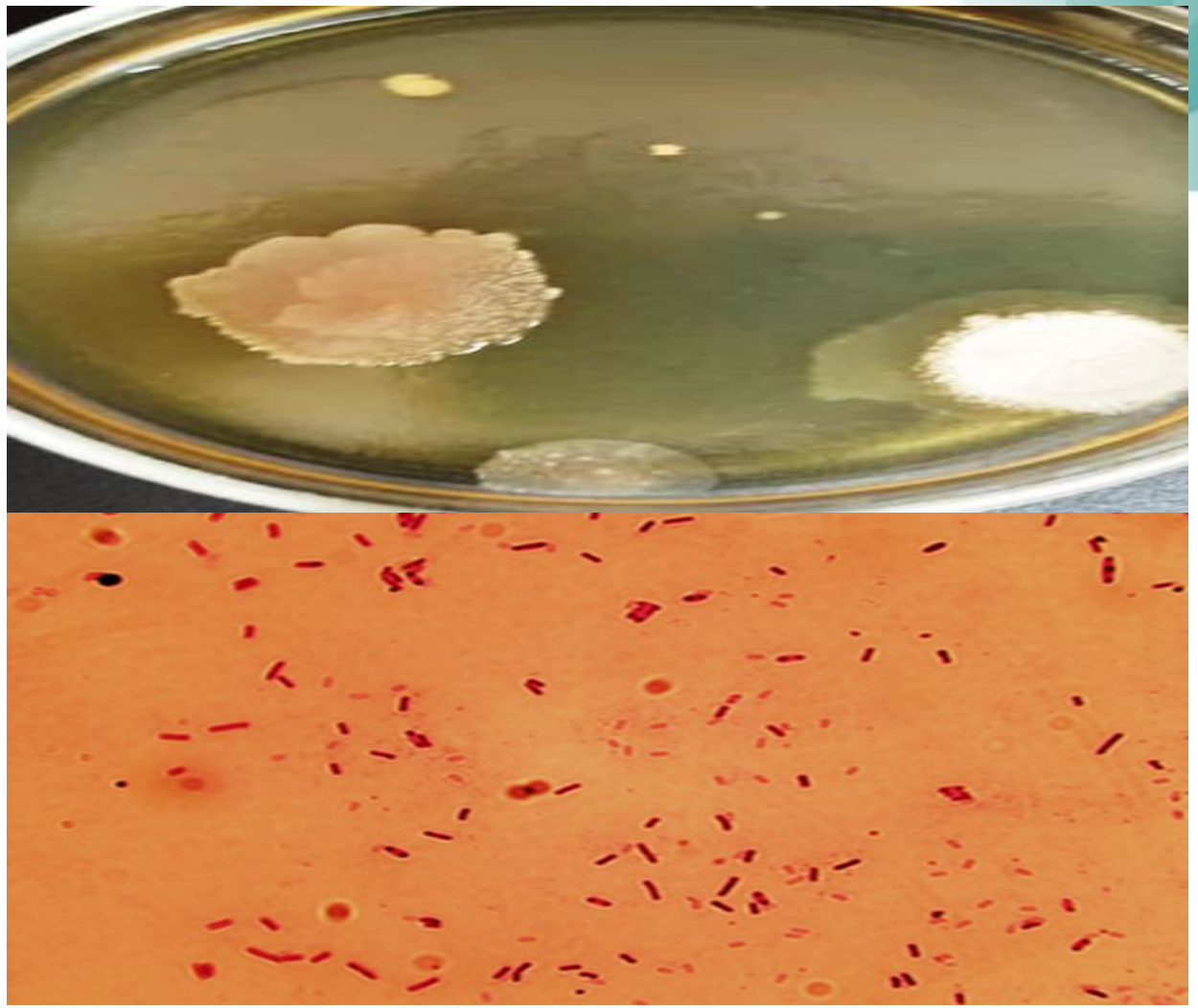
- A tractor was used to plough the top layer to a depth of 25–30 cm.
- Soil samples (100 g each) were separately taken from the 10–12 cm and 25–30 cm layers for chromatographic and microbiological analysis.
- The soil pH was measured across the ploughed area.
- To create optimal conditions for bioremediation, fertile soil was added and mixed with the contaminated soil and irrigated with water to activate the local microflora.
- To reach the needed moisture capacity in the contaminated soil, 18 tons of fertile soil were added to the 40-m^2 plot.

Preparation of the bioproduct used in the bioremediation

- Suspensions of the three selected bacteria (*L.ysinobacillus fusiformis*, *Stenotrophomonas sp.* and *E. cloacae*) were prepared via submerged cultivation in a bioreactor (LAMBDA Laboratory Instruments, The Czech Republic, 7L). The bacterial strains were cultured separately.
- The resultant bacterial suspensions were mixed together to produce a single bioproduct that was then applied to the soil.
- Bioremediation conditions in the soil at the trial sites such as moisture (60–75%), temperature (26–27 °C) and pH (7.4–7.62) were monitored before applying the bioproduct.

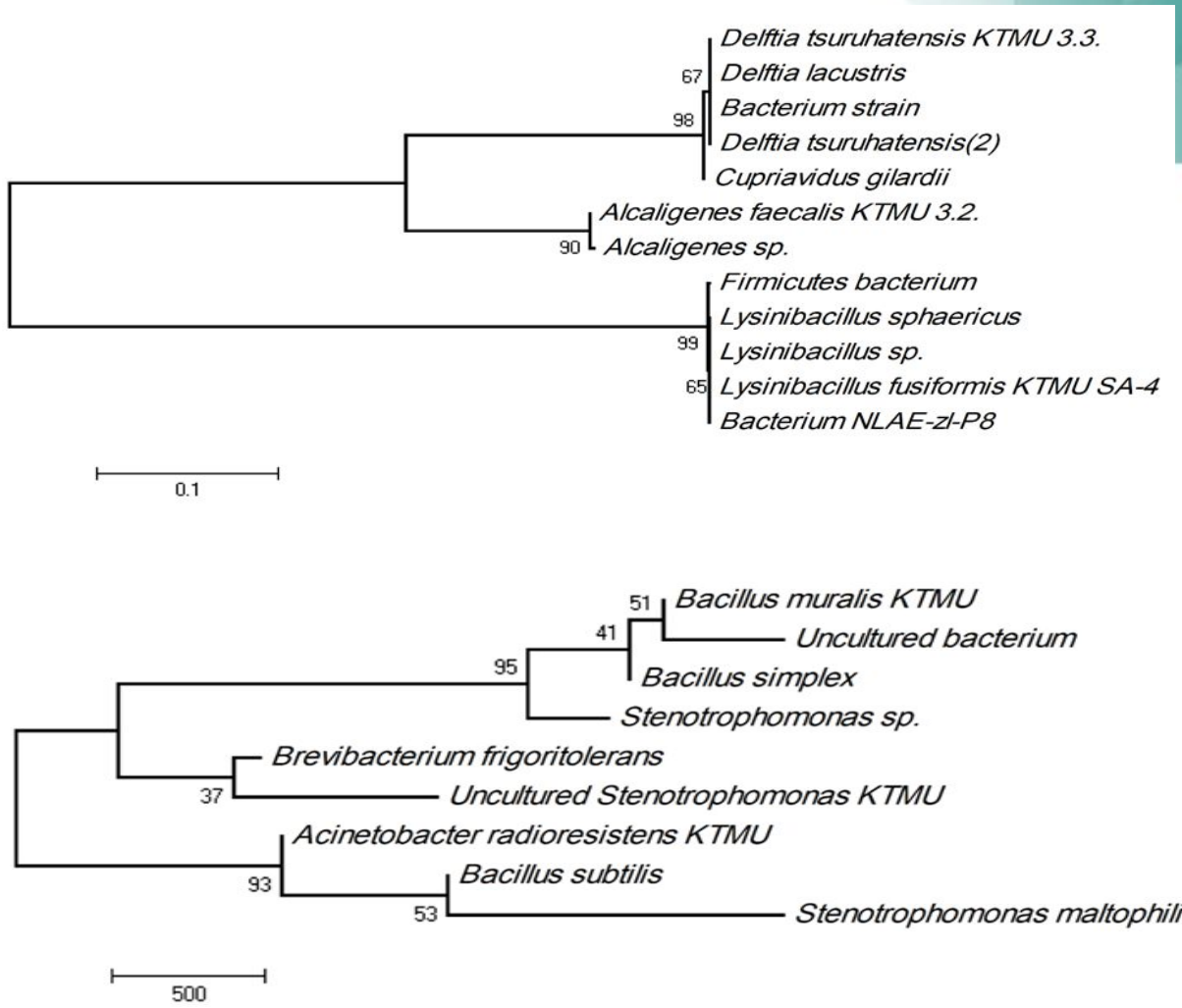
Local soil microflora before bioproduct application

- In the area where the warehouse stored obsolete pesticides for more than 50 years, the natural soil microflora was represented by only two genera: ***Bacillus*** and ***Streptomyces.***
- Both form spores, a feature that supports survival and resistance to adverse conditions.
- Soil samples were found to contain relatively low amounts of colony-forming units (CFU): 30,000-36,000±0.98 CFU/g of soil.



Soil bacteria in the experimental plots after three and six months of bioproduct application

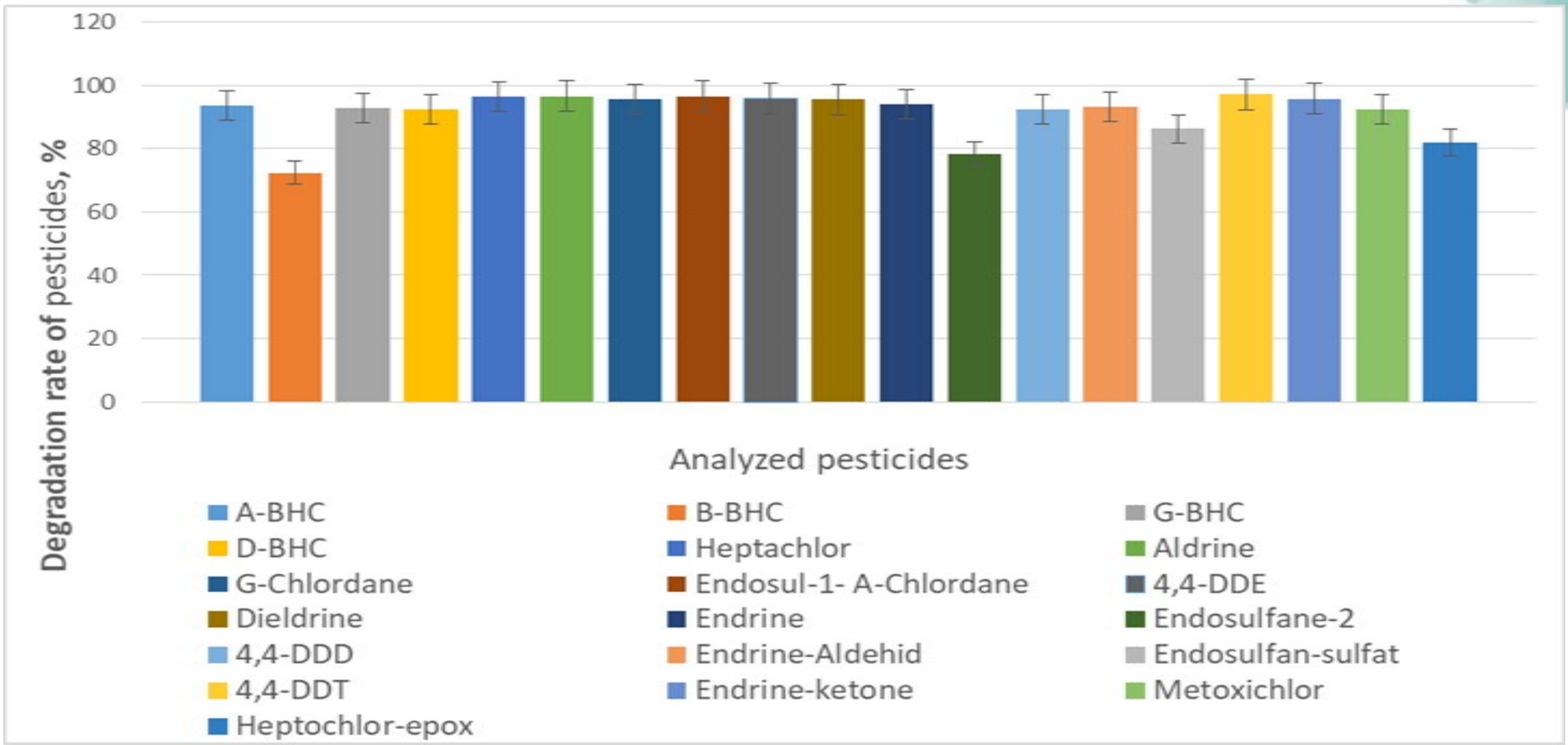
- PCR analysis of the soil bacteria after three and six months of bioproduct application revealed the presence of a bacterial community that was based on the introduced bioproduct species (*L. fusiformis*, *E. cloacae* and *Stenotrophomonas* sp.) + the local microflora.
- The local microflora was found to be richer than before the bioproduct treatment and consisted of *Delfia*, *Alcaligenes*, *Lysinibacillus*, *Bacterium*, *Bacillus* and *Stenotrophomonas* sp.
- These findings indicated that the bioaugmentation not only ensured the survival and functioning of the introduced bacteria, but also the activation of the local microflora.



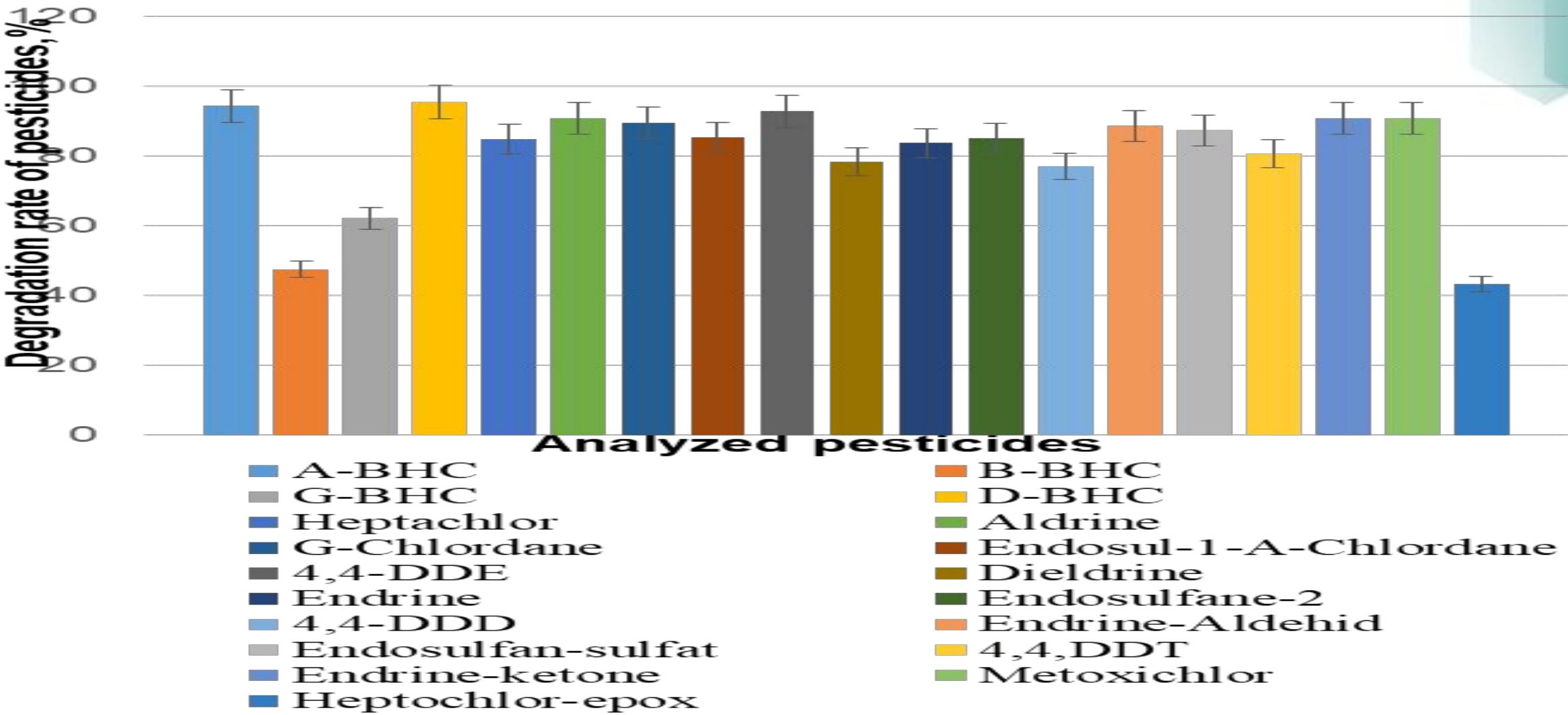
Comparison of the soil pesticide concentrations before the experiment with the maximum permissible concentrations (MPCs)

No.	Pesticide	MPC (mg/kg)	Concentration in polluted experimental plot (mg/kg ± SD)
1	A-BHC	0.1	0.41±0.2
2	B-BHC	0.1	0.38±0.2
3	G-BHC	0.1	0.23±0.2
4	D-BHC	0.1	0.95±0.2
5	Heptachlor	0.05	0.88±0.2
6	Aldrine	0.025	2.40±0.2
7	G-chlordane	0.1	4.40±0.2
8	Endosul-1-A-chlordane	0.01	3.41±0.2
9	4,4 DDE	0.1	3.37±0.2
10	Dieldrine	0.005	4.35±0.2
11	Endrine	0.01	3.68±0.2
12	Endosulfan e-2	0.1	2.24±0.2
13	4,4 DDD	0.1	15.21±0.2
14	Endrine- adehid	0.01	3.29±0.2
15	Endosulfan-sulphate	0.1	6.21±0.2
16	4,4 DDT	0.1	6.09±0.2
17	Endrine- ketone	0.01	3.47±0.2
18	Metoxichl or	1.6	1.74±0.2

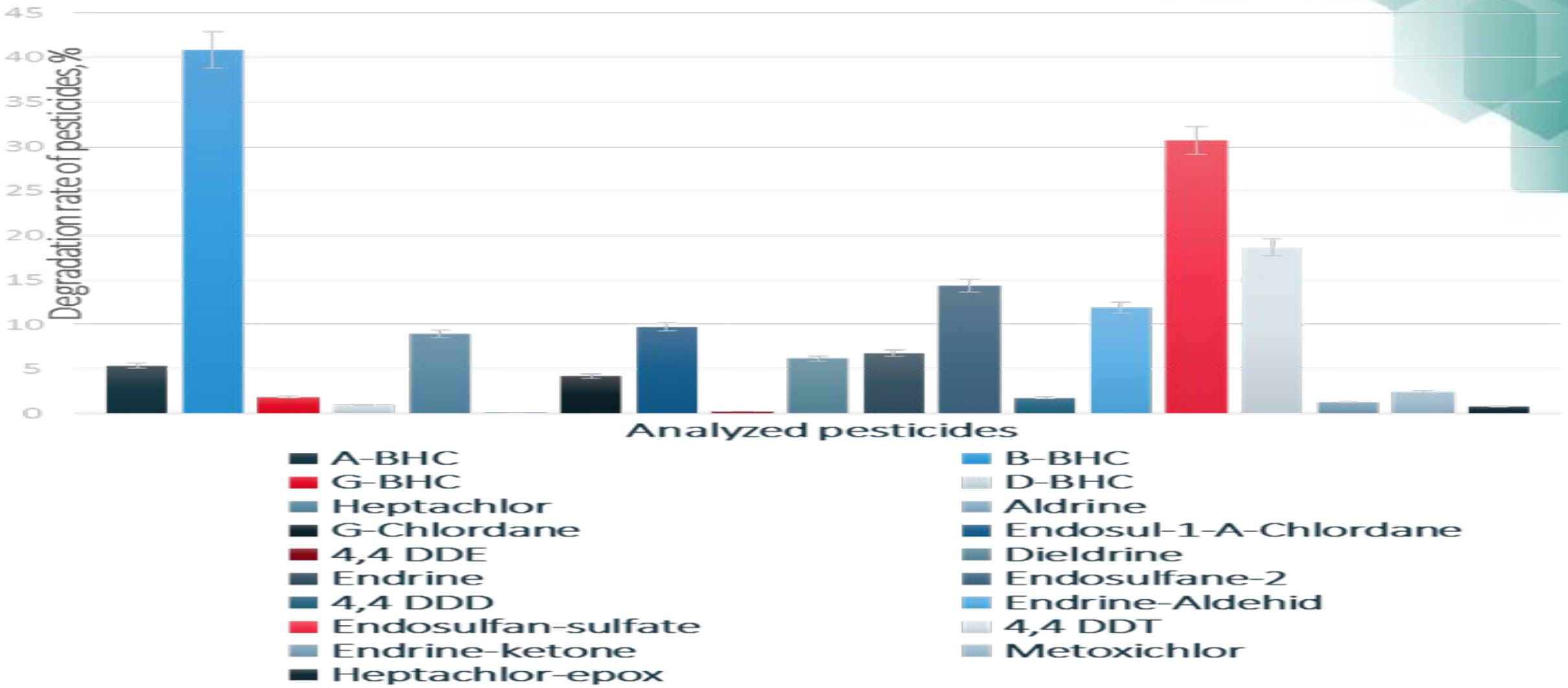
Degradation of pesticides in soil (10-12 cm layer) that received fertile soil and the bioproduct (after 3 months)



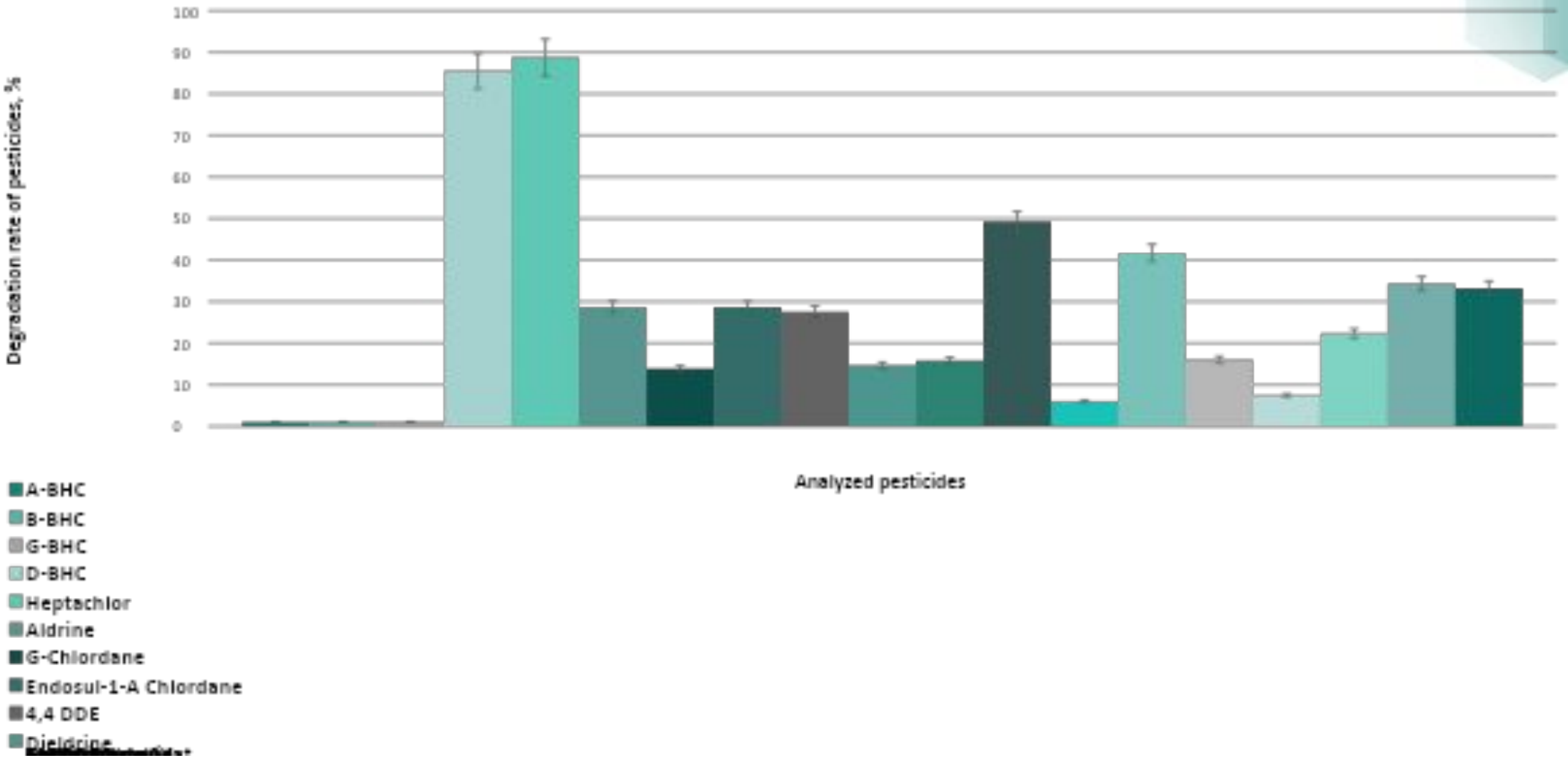
Degradation of pesticides in soil (10–12 cm layer) that received fertile soil but no bioproduct (control plot 1) (after 3 months)



Degradation of pesticides in soil (10–12 cm layer) that did not receive fertile soil nor the bioproduct (control plot 2) (after 3 months)



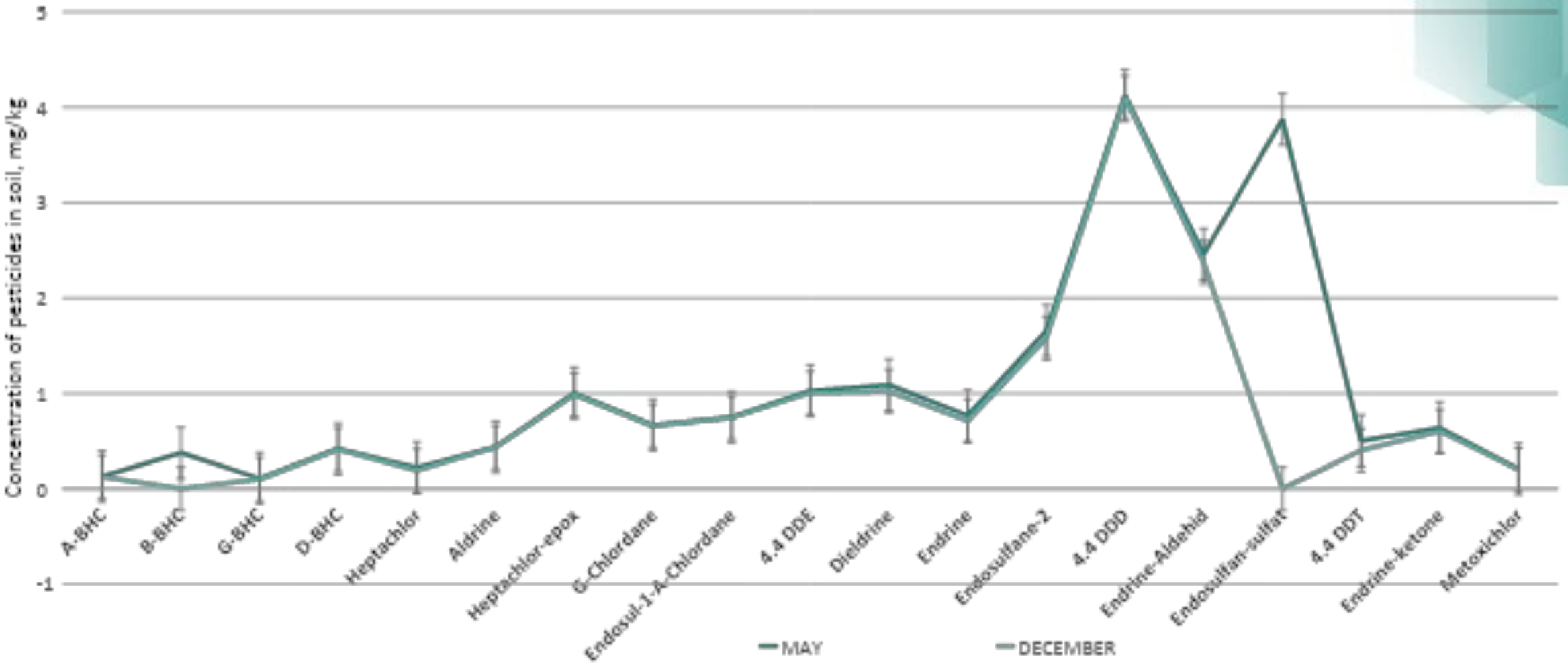
Degradation of pesticides in soil (30 cm depth) that received fertile soil and the bioproduct (after 3 months)



Pesticides concentration detected in soil (12- 35 cm deep) six months after the start of treatment

No	Pesticide	Concentration in control soil (fertile soil + no bioproduct) (mg/kg ± SD)		Concentration in experimental soil (fertile soil + bioproduct) (mg/kg ± SD)	
		May, 2021	December, 2021	May, 2021	December, 2021
1	A-BHC	0.231±0.05	0.019±0.03	0.270±0.03	0.047±0.02
2	B-BHC	0.225±0.02	0.014±0.01	0.380±0.03	0.043±0.04
3	G-BHC	0.240±0.02	0.007±0.02	0.170±0.03	0.015±0.05
4	D-BHC	0.926±0.03	0.008±0.02	0.686±0.04	0.046±0.03
5	Heptachlor	0.476±0.05	0.016±0.03	0.550±0.05	0.005±0.05
6	Aldrine	0.903±0.02	0.146±0.02	1.418±0.01	0.044±0.02
7	Heptachlor-epox	0,878±0.03	0,779±0.01	2.544±0.02	0.039±0.02
8	G-Chlordane	1.439±0.03	0.259±0.02	2.625±0.03	0.011±0.03
9	Endosul-1-A-Chlordane	0.916±0.05	0.365±0.03	2.048±0.03	0.009±0.03
10	4.4 DDE	1.062±0.04	0.260±0.05	2.060±0.02	0.138±0.02
11	Dieldrine	1.350±0.04	0.216±0.01	4.347±0.04	0.035±0.03
12	Endrine	**1.022±0.05**	**0.452±0.02**	**2.223±0.05**	**0.105±0.05**
13	Endosulfane-2	**2.615±0.03**	**1.584±0.02**	**2.088±0.02**	**0.079±0.03**
14	4.4 DDD	3.662±0.02	0.028±0.04	9.706±0.3	0.060±0.03
15	Endrine-Aldehid	1.128±0.04	0.774±0.03	1.876±0.03	0.273±0.04
16	Endosulfan-sulfat	2.174±0.01	1.974±0.03	5.079±0.02	0.064±0.03
17	4.4 DDT	1.734±0.02	0.101±0.02	3.298±0.02	0.248±0.02
18	Endrine-ketone	1.314±0.01	0.130±0.03	2.057±0.02	0.002±0.03
19	Metoxichlor	0.733±0.03	0.307±0.02	0.974±0.03	0.006±0.03

Pesticides concentration six months after the initiation of bioremediation at the control site (no fertile soil + no bioproduct applications) of the Chym–Korgon store



Results of germination tests 12 months after the initiation of bioremediation at the polluted sites

Experimental plot with germinated plants in spring period.

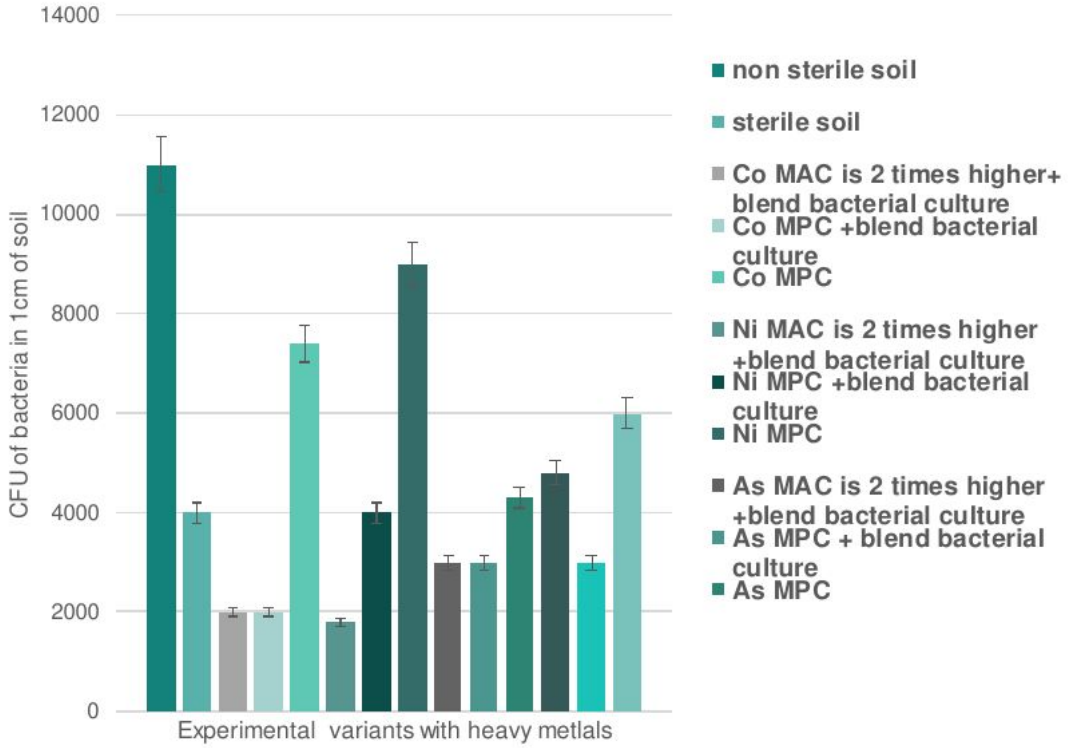
Experimental plot with germinated and growing plants in summer period

In vitro and in vivo screening tests to select bacteria resistant to heavy metal compounds

- Microorganisms can transform heavy metals through oxidation, recovery, methylation and demiling
- Lab trials were made in soil contaminated with lead (Pb), arsenic (As), cobalt (Co), and nickel (Ni) at levels of 1 resp. 2 maximum permissible concentrations (MPC)
- Bacterial association (108 cells/ml) Alcaligenes faecalis 3.2; Delftia tsuruhatensis 3.3. and Stenotrophomonas sp.PSB was added
- Microbiological parameters of the soil and HM concentrations were assessed over 30, 60, 90 days
- Results suggest decrease of concentration of metals in soil due to adsorption on the bacteria cellular surface or immobilization in bacteria cell

In vitro and in vivo screening tests to select bacteria resistant to heavy metal compounds

- Microbiological analyses showed that in addition to Alcaligenes faecalis, Delfia tsurhatensis and Stenotrophomonas sp also local soil microflora (especially Lysinibacillus fusiformis) was actively involved in process of metal transformation
- Lysinibacillus fusiformis can be used as a universal biotransformation tool for many metal ions.
- Brevibacillus parabrevis can be considered to remove lead (Pb) ions
- Brevibacillus reuszeri and Bacillus safensis can be used for removing arsenic (As) ions



Bacterial concentration in soils with 1 resp. 2 MPC after 90 days

Conclusions

- Three conditions were tested: application of fertile soil and bioproduct, application of fertile soil but no bioproduct (Control 1), and no fertile soil and no bioproduct (Control 2).
- The most effective condition, in terms of pesticide degradation, was the application of fertile soil and the bioproduct. The degradation using this condition was 1.5 to 2 times higher than when only fertile soil was added (and no bioproduct) and five times higher than when no fertile soil and no bioproduct were added.
- Using the association of active degrading bacteria and improving the agrochemical conditions of the soil made it possible to remove obsolete pesticides within 6 months, their concentrations ranging from 0.41 mg minimum to 15.21 mg maximum per kg of soil.
- The trials showed that degradation of pesticides by microbes depends not only on the bacterial enzyme system but also on the conditions like temperature, pH of soil, moisture contents and nutrients.
- Further research will optimize the degradation conditions in a variety of soil types, ex situ conditions with high concentration of pesticides and/or with presence of heavy metals, using the bacterial strains selected for this study. Also, the products of degradation will be studied in more detail.

Acknowledgment

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THANK YOU FOR YOUR ATTENTION

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