Directorate for Environmental Protection and Water Management of Lower Tisza District

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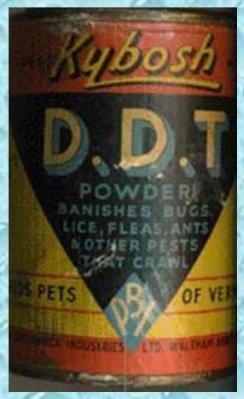
Development of xenobiotic-degrading bioaugmentation products

Lívia Vidács

DKMT-2010

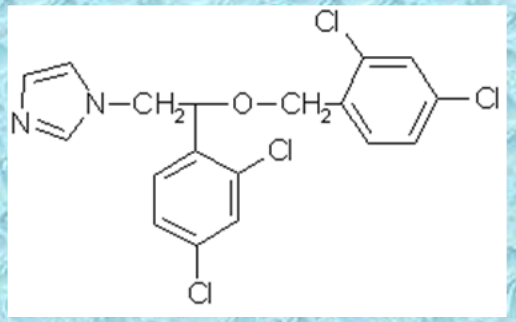
Xenobiotics

- **Xenobiotics are essentially synthetic chemicals that are foreign in nature.**
- **The large-scale production and extensive use of synthetic organic compounds for agricultural, industrial, domestic and military activities has led to the widespread distribution of xenobiotics in the environment.**



Pesticides

Chemotherapeutical compounds



Xenobiotics

Petroleum products

Industrial wastes

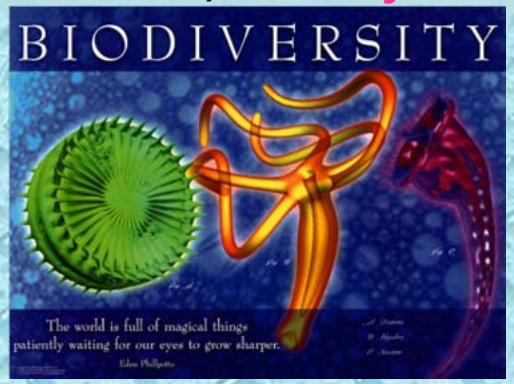
Pesticides

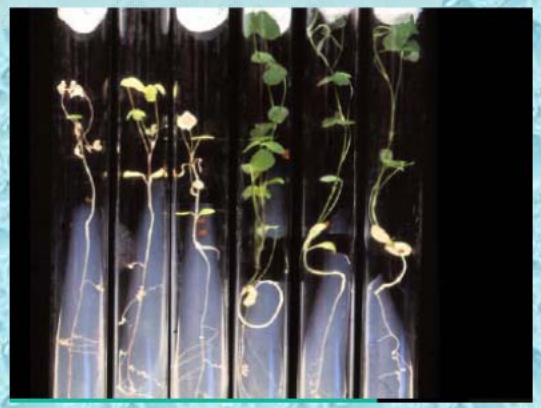
•Pesticides are widely used in agriculture.

• Pesticides may cause acute and delayed health effects. These effects can range from simple irritation of the skin and eyes to more serious effects such as affecting the nervous system, causing reproductive problems, and also causing cancer.

•Pesticide use raises a number of environmental concerns. Over 95% of sprayed herbicides reach destination other than their target species, including non-target species: air, water and soil. Pesticide flow occurs when pesticides suspended in the air as particles are carried by wind to other areas, potentially contaminating them. Pesticides are one of the causes of water pollution, and some pesticides are persistent organic pollutants and contribute to soil contamination.

• In addition, pesticide use reduces biodiversity, nitrogen fixation, pollination, destroys habitat (especially for birds).





Birds

Bees

Effects of pesticides

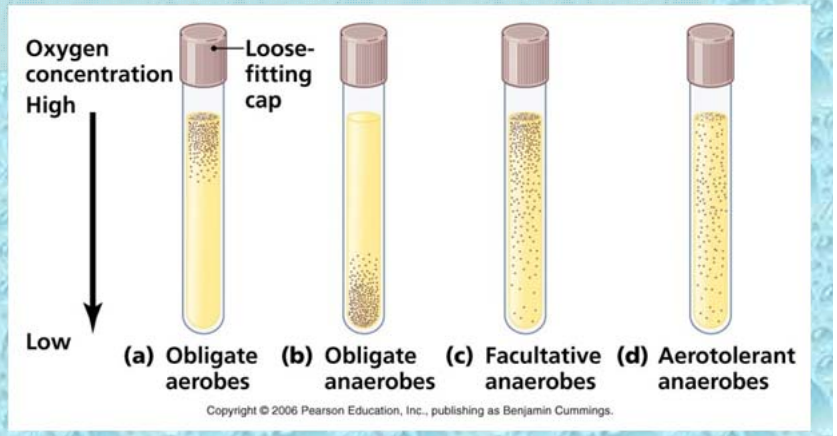
Humans

Plants

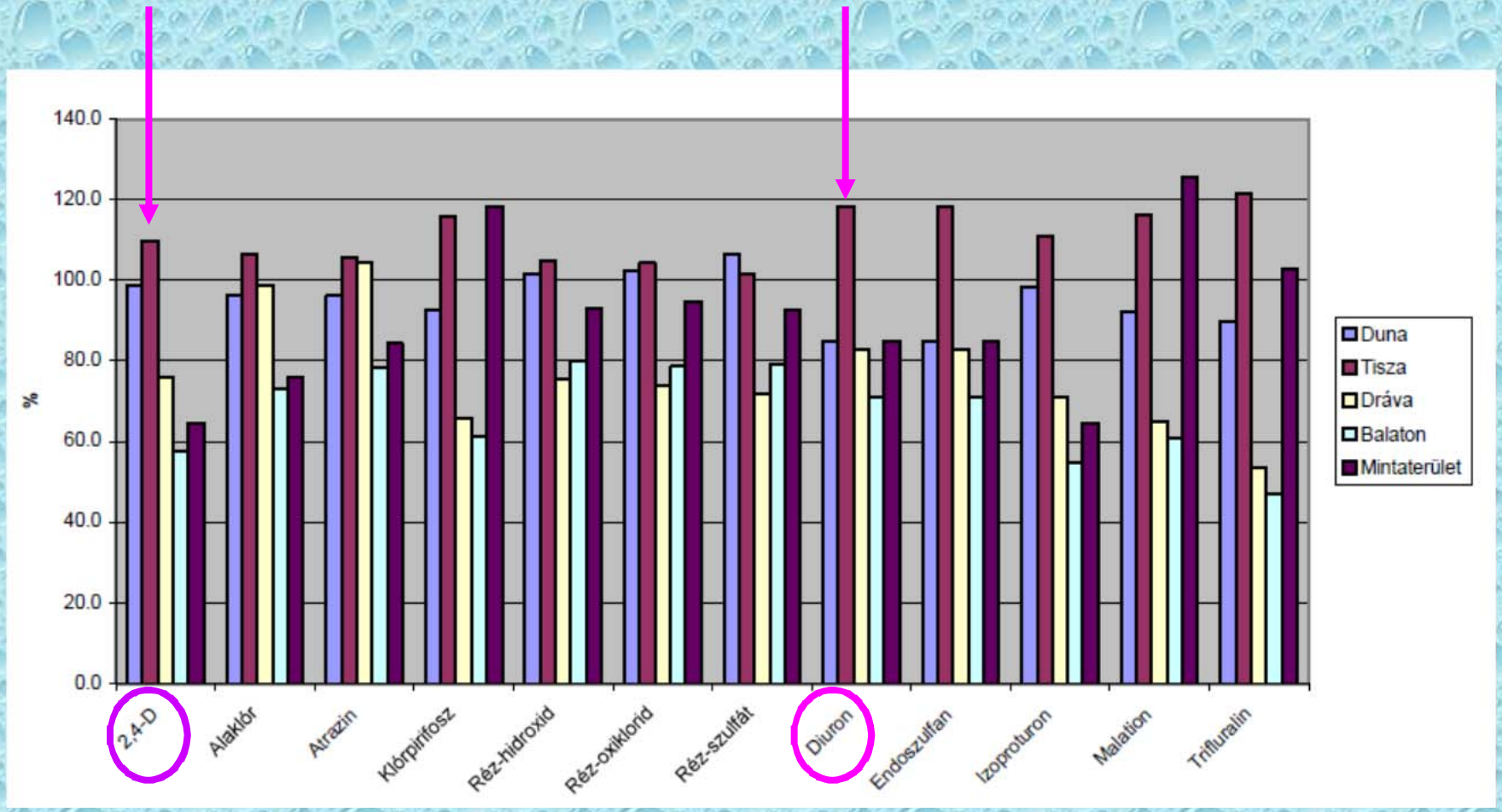
Fishes

Practical approaches to microbial pesticide degradation

- Microbes use the pesticide as an energy and C/N source
- The pesticide is dissolved in an aqueous phase.
- Microbial biodegradation in the water is a result of a complex process of microbial, environmental and chemical interactions.
  - Environmental factors: oxygen, pH, temperature and water content
  - Microbial factors: cooperation, growth support, acclimation and competition of degraders
  - Chemical-microbial interactions
  - Environmental-chemical interactions: bioavailability means that a herbicide is in a state that is accessible to microorganisms



Relative load compared to the national average



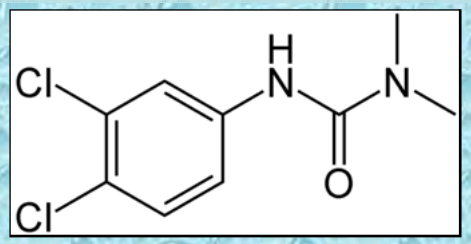
Percentage of pesticide use in the basins in Hungary

Agent	Distribution			
	Danube	Tisza	Drava	Balaton
2,4-D	38,5	52,9	5	3,6
Diuron	33	57,1	5,5	4,4

Microbial degradation possibilities:

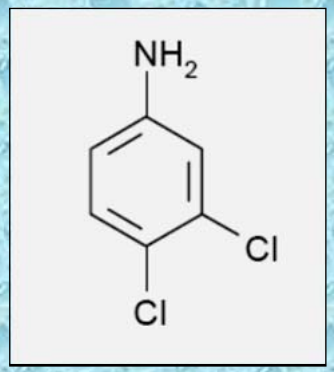
1. Diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea), DCMU

DCMU inhibits photosynthesis, it has a long persistence character. It is highly toxic to aquatic invertebrates and algae, it is teratogenic and known endocrine disruptor.



diuron
(strong ecotoxicity)

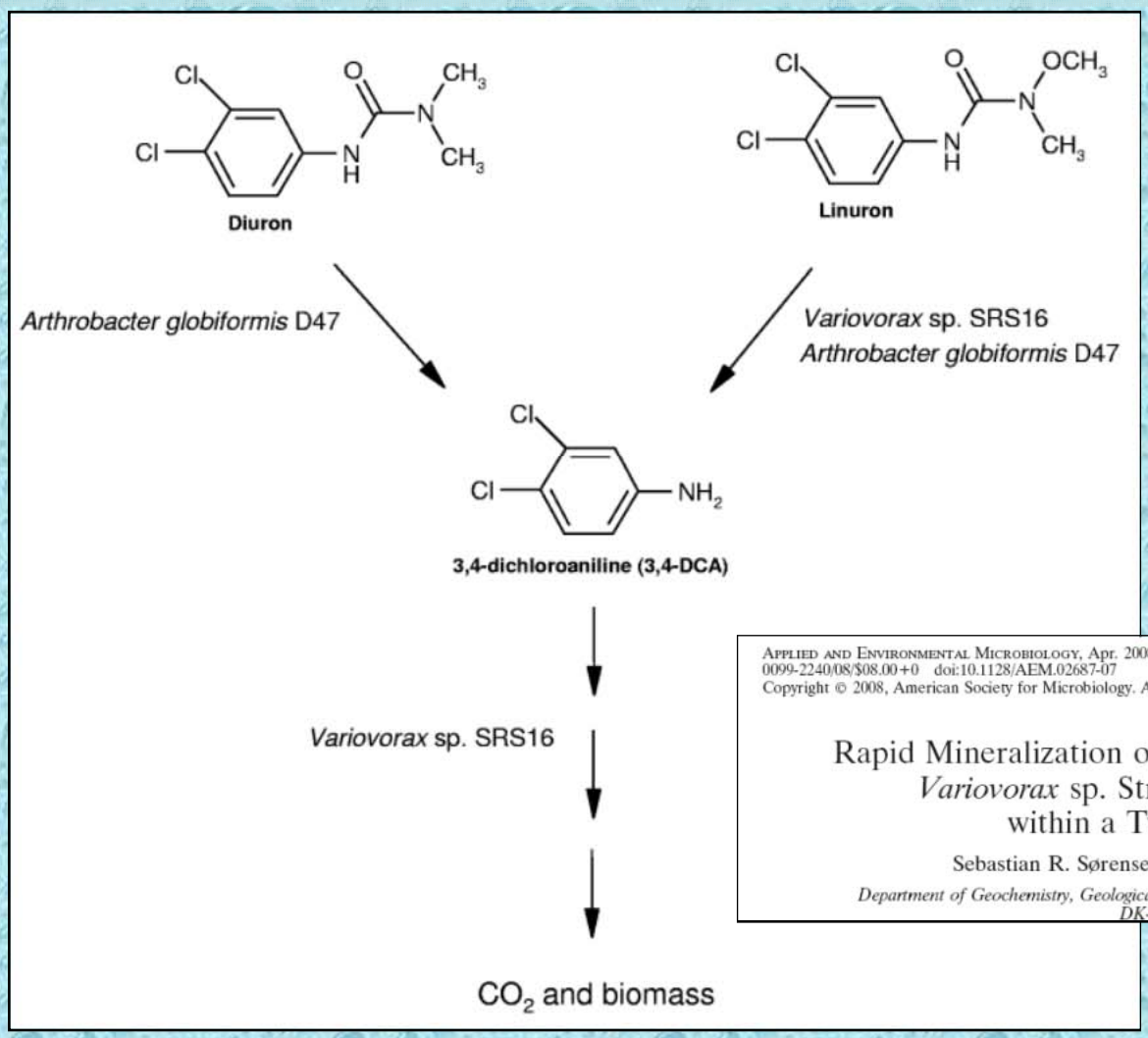
↓ **diuron hydrolase (e.g. *Arthrobacter globiformis*)**



3,4-dichloroaniline (3,4-DCA)
(carcinogenic)

There are many bacteria which have only diuron hydrolase activity, so the 3,4-DCA accumulates in the water.

A *Variovorax* sp. and a *Pseudomonas fluorescens* strain were recently isolated which are able to degrade 3,4-DCA by the two-member consortium.



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Rapid Mineralization of the Phenylurea Herbicide Diuron by *Variovorax* sp. Strain SRS16 in Pure Culture and within a Two-Member Consortium[▽]

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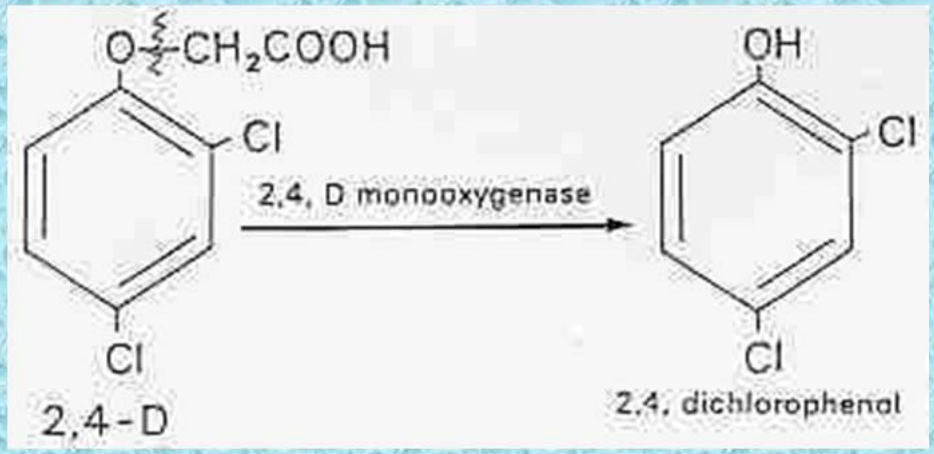
2. 2,4-Dichlorophenoxyacetic acid (2,4-D)

2,4-D has a long persistence character, it is teragenic, carcinogenic and it is a known endocrine disruptor.



Many bacteria are capable of the rapid degradation of 2,4-D to 2,4-dichlorophenol (2,4-DCP).

However, this degradation product is more dangerous than the original herbicide, and even at 20-50 mg/l 2,4-DCP is strongly inhibitory to most fungi and bacteria.

Some *Pseudomonas* strains with the catabolic plasmid pJP4 are capable of full degradation.

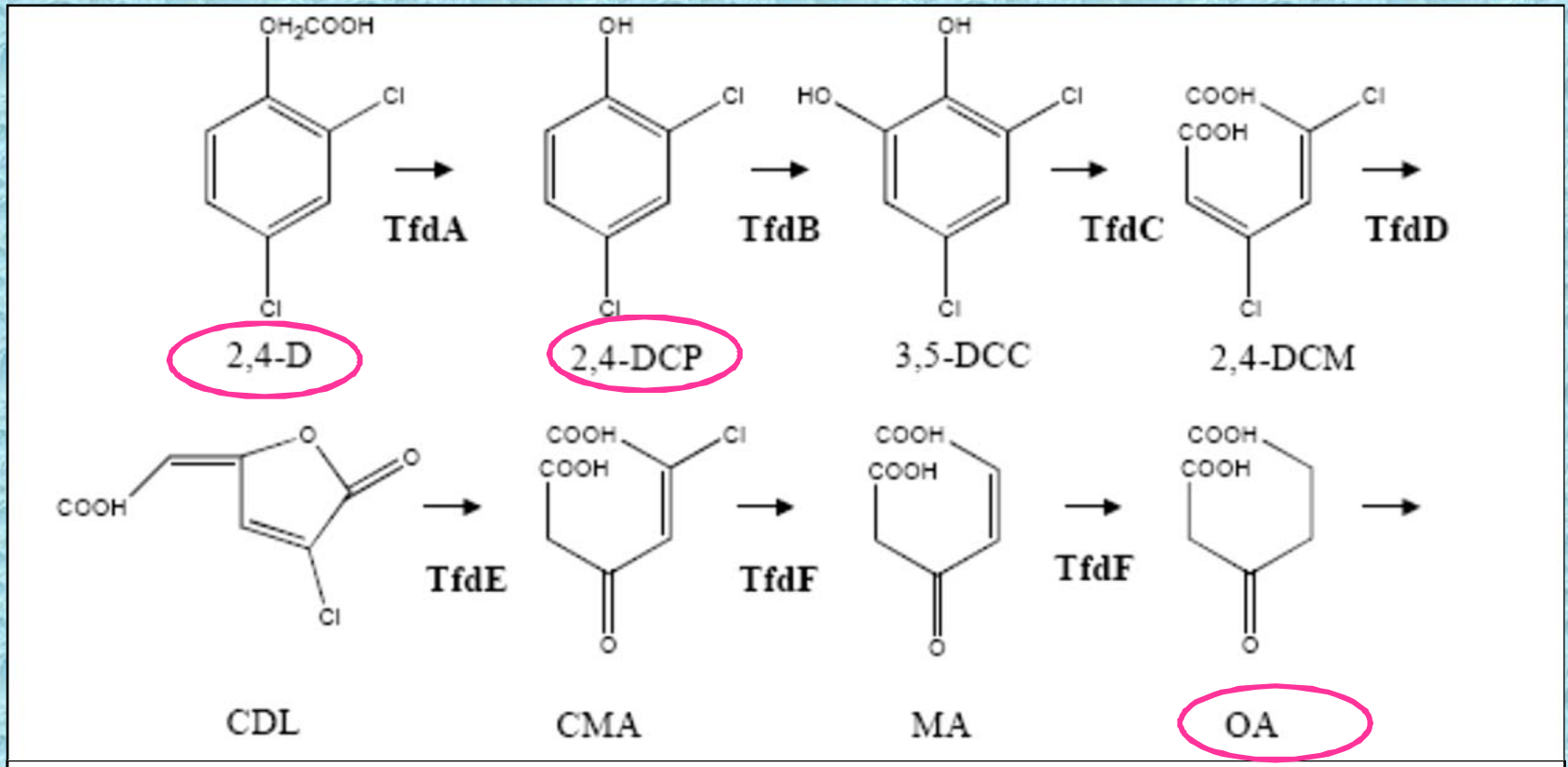
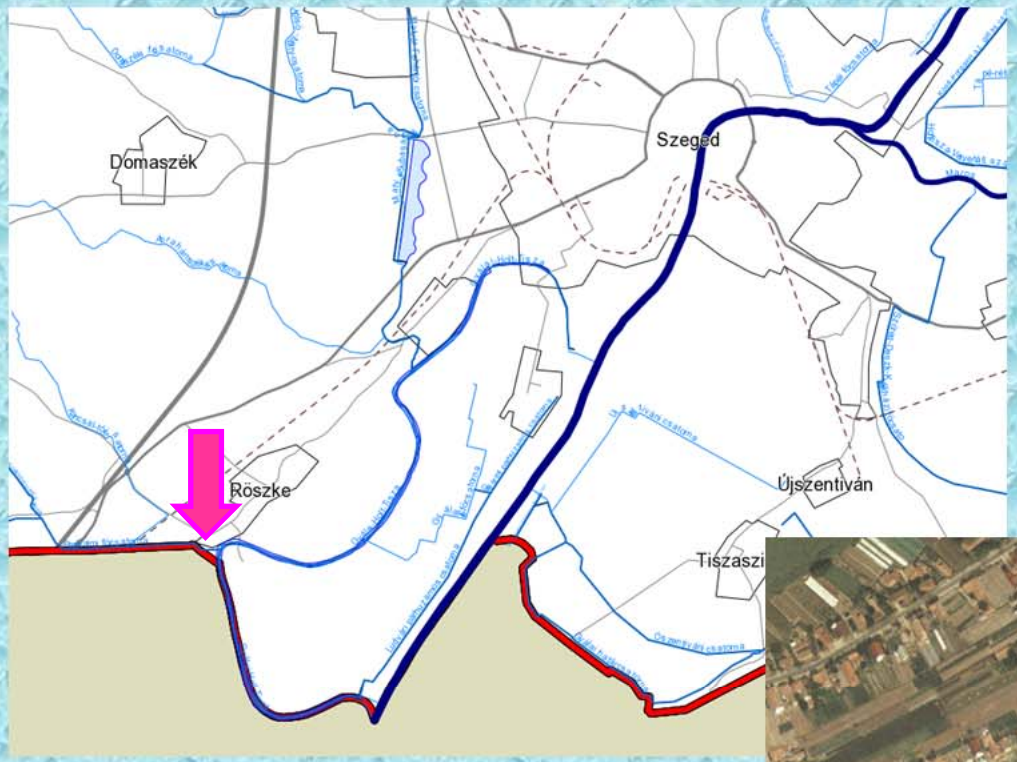


Fig. 1. Pathway for 2,4-D degradation of 2,4-D. Genes within plasmid pJP4 encode the first six steps in the pathway (Laemmli et al., 2000). TfdA, 2,4-D α-ketoglutarate dioxygenase; TfdB, chlorophenol hydroxylase; TfdC, chlorocatechol 1,2-dioxygenase, TfdD, chloromuconate cycloisomerase; TfdE, diene-lactone hydrolase; TfdF, (chloro)maleylacetate reductase. Abbreviations: 2,4-D, 2,4-dichlorophenoxyacetic acid; 2,4-DCP, 2,4-dichlorophenol; 3,5-DCC, 3,5-dichlorocatechol; 2,4-DCM, 2,4-dichloromuconate; CDL, *cis*-chlorodiene lactone; CMA, chloromaleylacetate; MA, maleylacetate; OA, 3-oxoadipate.

Rensing et al. (2002) Soil Biol. Biochem 34: 285-296.


Domaszék
Szeged
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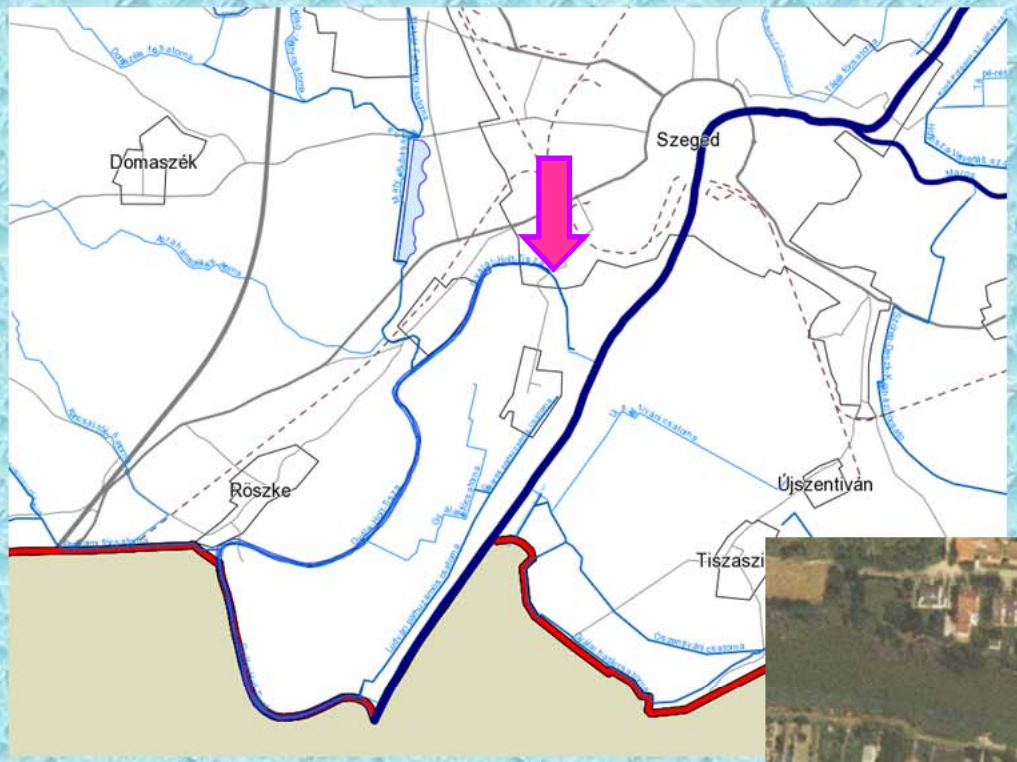
Tiszaszi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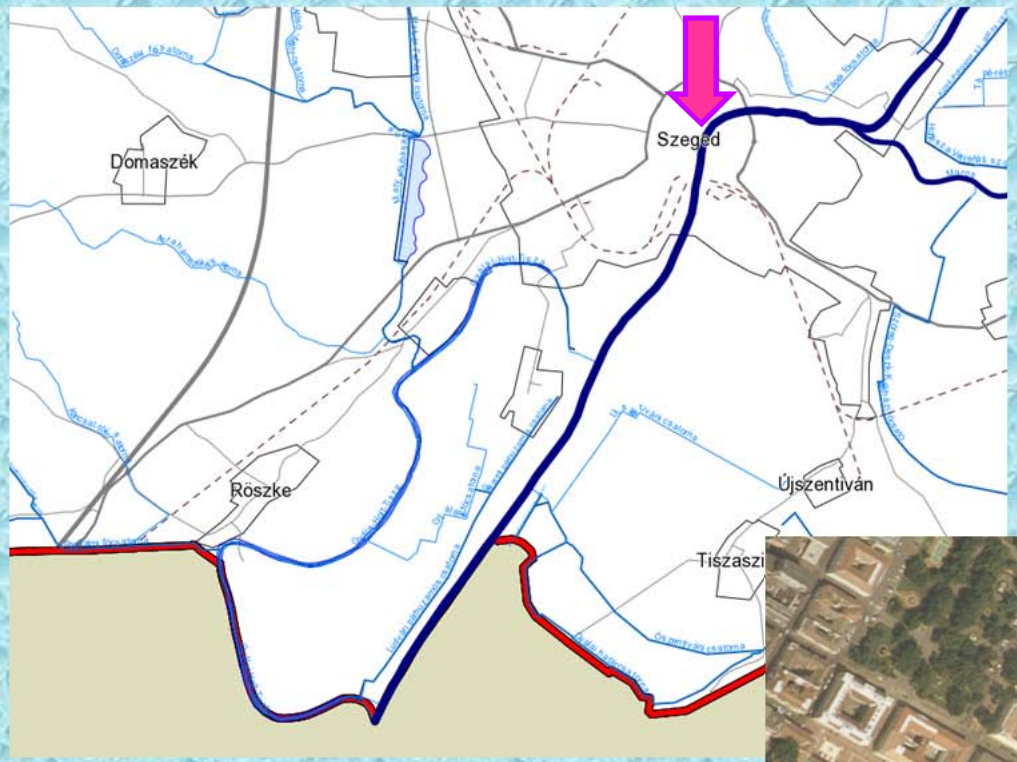
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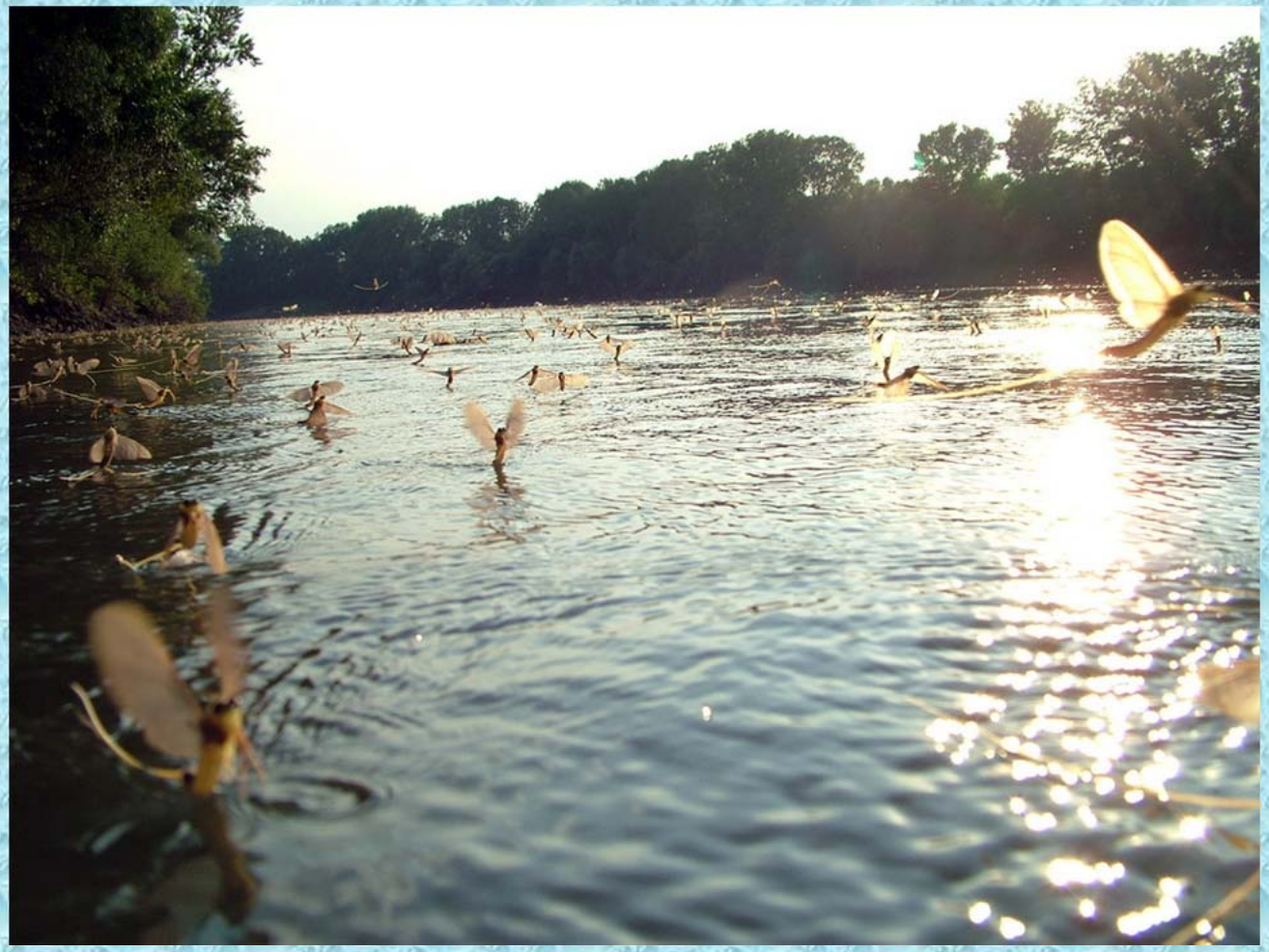
Újszentiván

- **For evaluation of the bioaugmentation potential of the xenobiotic-degrading strains, accurate and rapid molecular diversity methods (RISA and community-ARDRA) will be used to monitor the microbial community structure and population sizes of the degraders.**
- **The efficient xenobiotic-degrading bacteria will be taxonomically identified by partial sequencing of their 16S rDNA and *rpoB* genes.**
- **Optimized methods for pesticide analysis will be developed.**
- **Their basic physiological parameters: pH, water activity, temperature tolerance, etc. will be determined.**
- **Degradation kinetics and products will be determined.**

Final aim: Development of optimized, harmonically working bacterial mixtures which are capable of the rapid and complete degradation of 2,4-D and diuron.



Thank you for your attention!