

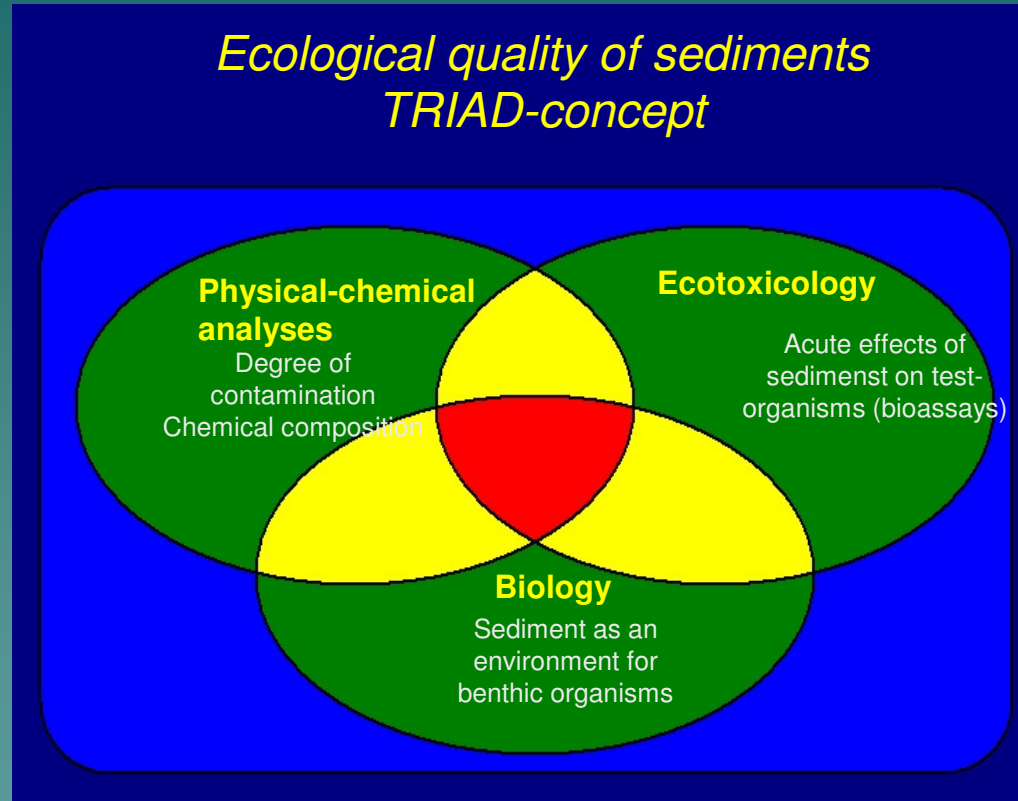
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Microbiotests : an outstanding tool for the evaluation of sediment toxicity

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The TRIAD concept for the evaluation of the ecological quality of sediments



TOXICITY TESTS

*Give a signal for the impact
of **all** the pollutants which
are present in the sample*



Conventional toxicity tests on sediments :

- Algae growth inhibition test (72h)
- *Daphnia magna* immobilisation test (24h-48h)
- Contact test with *Hyalella azteca*
- Contact test with higher plants

Culturing of micro-algae



Infrastructure
Space
Workload



Inoculation of the test vials
with micro-algae



Incubation of the test vials



Daily counting of the algae



ALGAL TEST PROCEDURE

Infrastructure
Space
Workload



The waterflea *Daphnia magna*

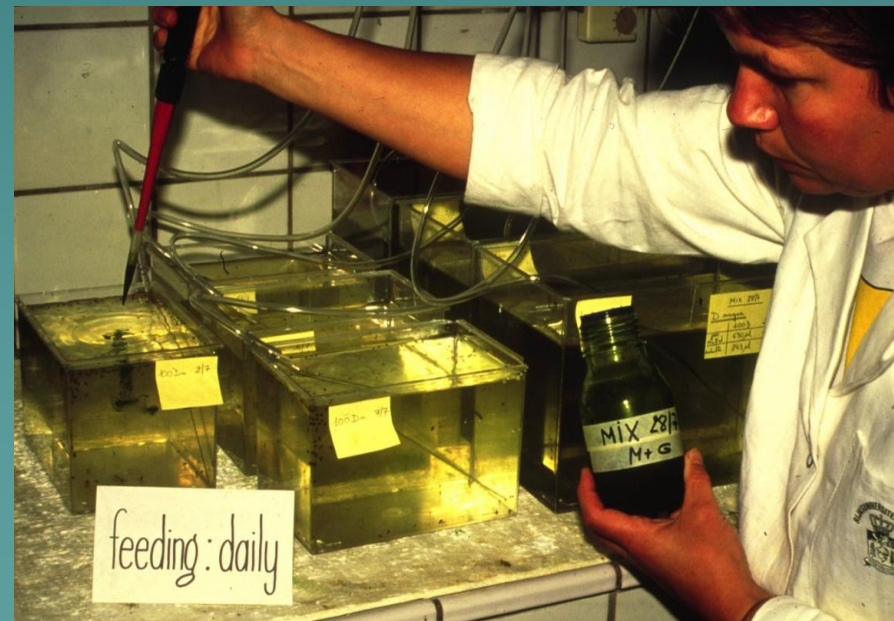


Daphnia culturing

Daily maintenance



Daily feeding with algae



Infrastructure

Space

Workload



Contact test with *Hyaella azteca*



2 mm

Stock culturing of the amphipod
crustacean *Hyaella azteca*

Infrastructure
Space
Workload



Equipment and space



TEST PERFORMANCE

Handlings

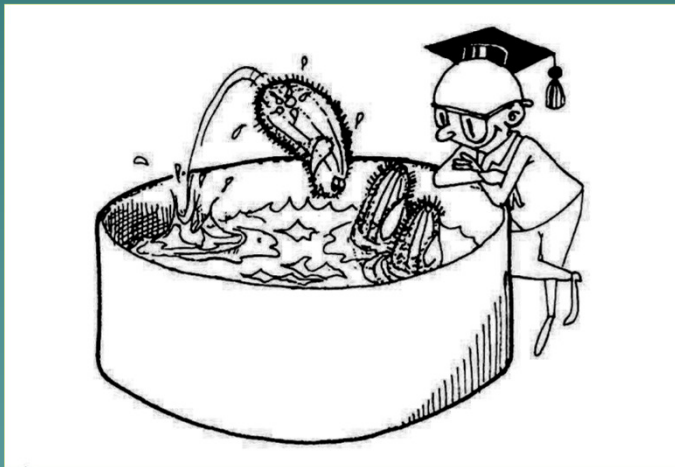


Infrastructure
Space
Workload



Major handicap of conventinal toxicity tests :

Dependence of the culturing and the (daily) maintenance of live stocks of test organisms



Infrastructure

Space

Workload

Costs



Toxicity tests with higher plants

Tests in pots (direct contact tests)

Duration of several weeks

Extensive handlings



Space
Workload
Time



MICROBIOTESTS



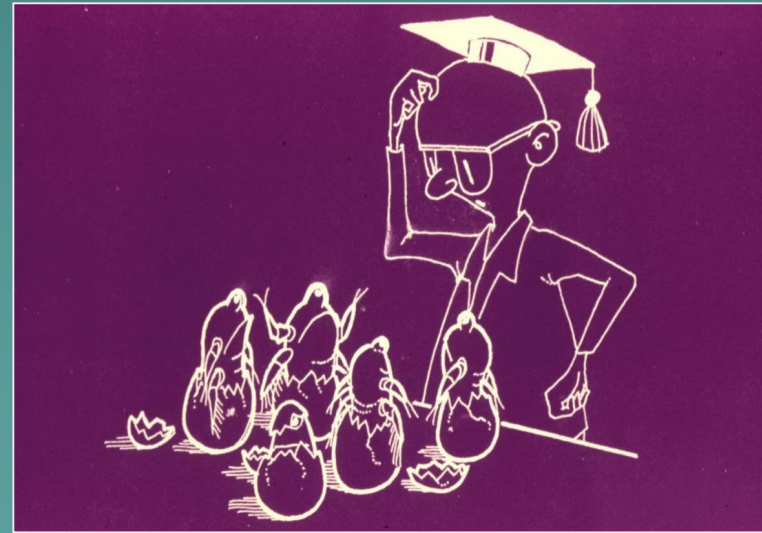
as alternatives for
“conventional” toxicity tests

Development of microbiotests *(1980-2000)* in the Laboratory of Environmental Toxicology and Aquatic Ecology at the Ghent University in Belgium

Fundamental approach :

Use of dormant
or immobilised stages
of selected organisms
to obtain test species

“on demand”



TOXKIT microbiotests

Development of the technology

- User-friendly
- Low cost
- Miniaturised



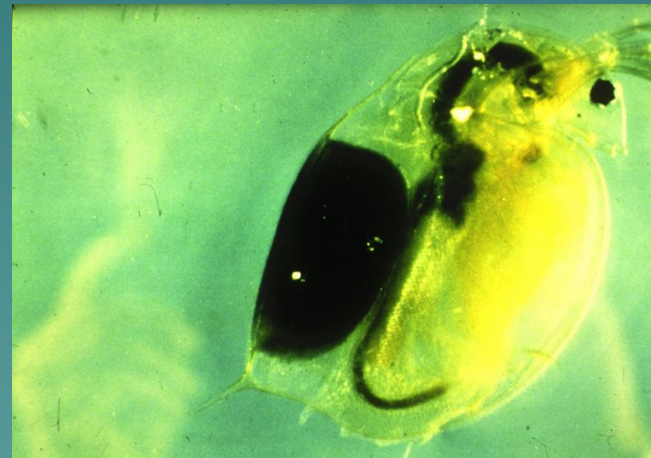
Daphnia magna

Daphnia magna with eggs



Parthenogenetic reproduction

Daphnia magna with capsule (ephippium) containing 2 dormant eggs



Sexual reproduction

Controlled production of dormant eggs – stocking - hatching

Ephippium



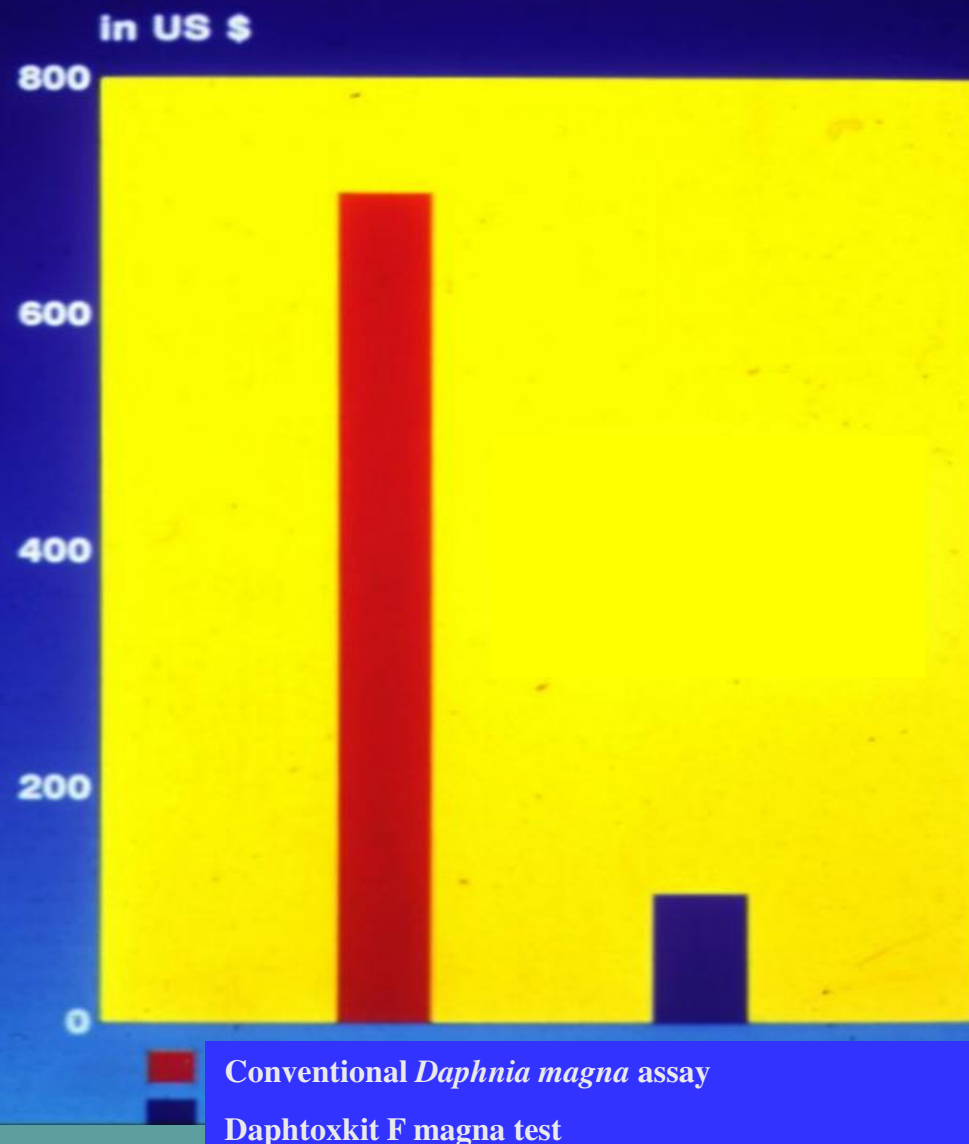
Daphnias hatched from
an ephippium
“and ready for use”



- No need for culturing /maintenance of live stocks of *Daphnia magna*
- The dormant eggs can be stored
- The dormant eggs can be hatched “on demand”

Daphtoxkit F magna

Cost price conventional *Daphnia magna* assay vs. Daphtoxkit F test



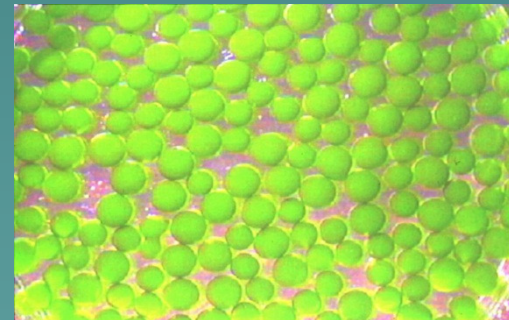
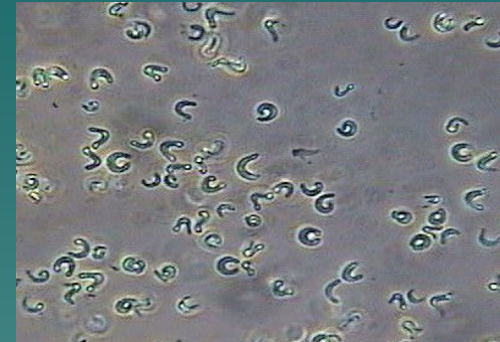
Microbiotest with algae

Micro-algae
(*Pseudokirchneriella subcapitata*)



Algal beads
(1 million cells per ml)

40-50 μm

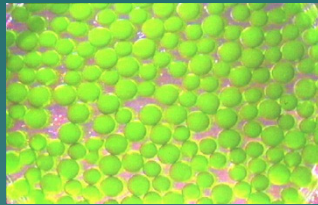


1-2 mm

Microbiotest with algae

Algaltokit

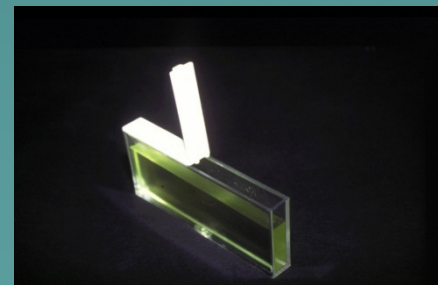
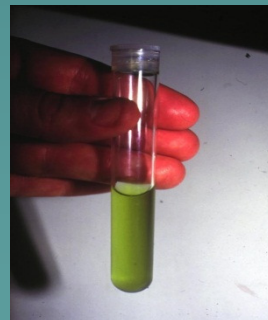
Micro-algae
immobilised in
algal beads



Algal beads in
a test tube

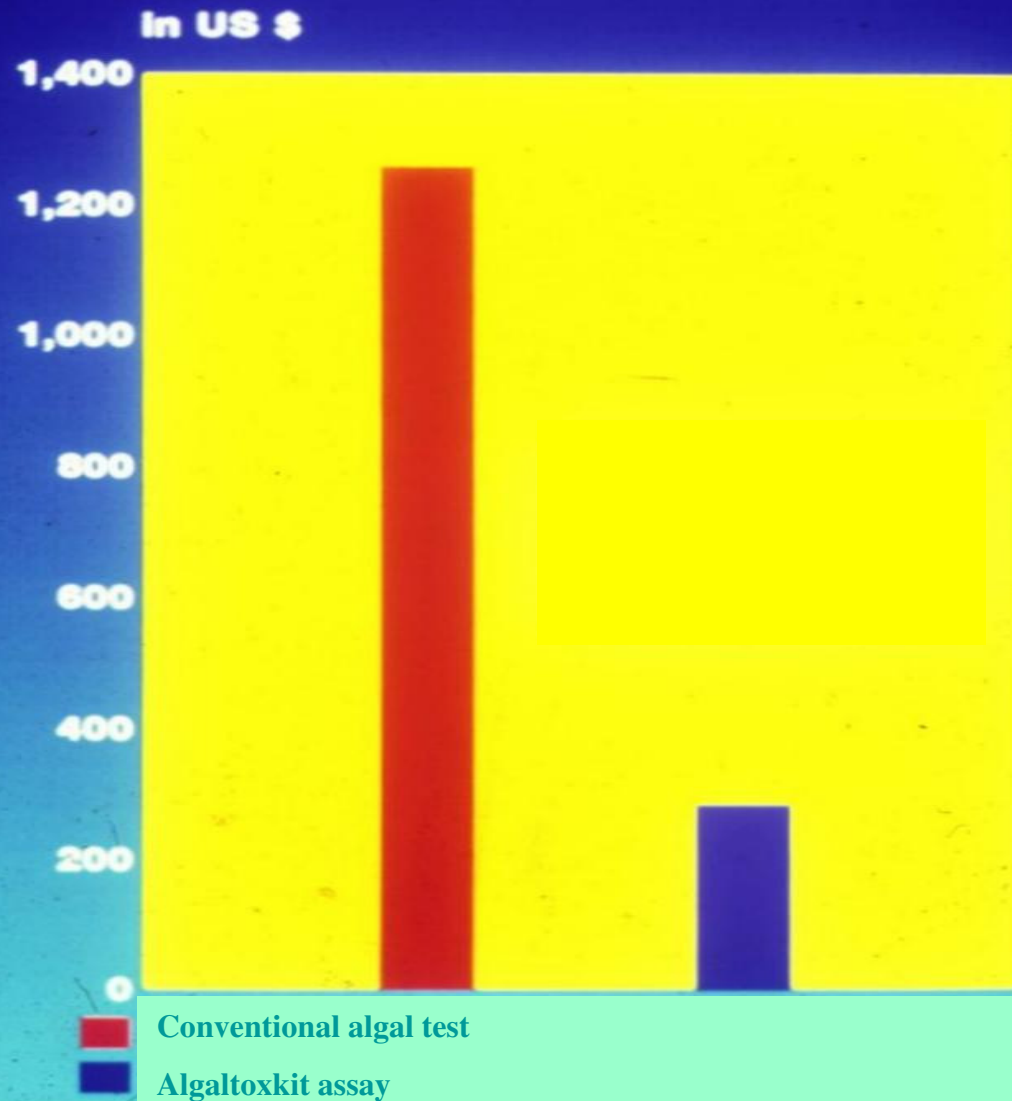


De-immobilisation
of the micro-algae



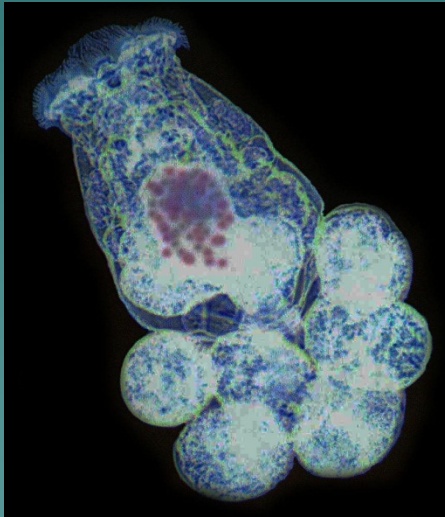
“Long cells” as
test containers

Cost price conventional algal growth inhibition test vs. Algaltoxkit F assay



Rotokit microbiotests

With the rotifers *Brachionus calyciflorus* (freshwater) and *Brachionus plicatilis* (estuarine and marine environments)



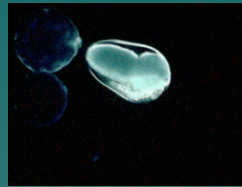
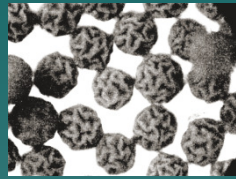
Acute test
(24h)



Chronic test
(48h)



Thamnotoxkit microbiotest



300 µm

Acute test (24h) with larvae of the crustacean *Thamnocephalus platyurus* hatched from cysts (dormant eggs)

Sensitivity similar to that of *Daphnia magna* and frequently used as alternative for the acute toxicity test with *Daphnia magna*



Thamnotoxkit

Ostracodtoxkit microbiotest



150-200 μm

“Subchronic” (6 days) mortality and growth inhibition test with larvae of the ostracod crustacean *Heterocypris incongruens*, hatched from cysts (dormant eggs)



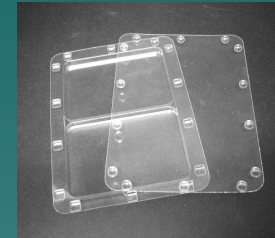
Ostracodtoxkit



Developed specifically for sediments
as “direct contact” test

Microbiotest for “phytotoxicity” measurement

1. Transparent test plates for
“uni-dimensional” growth of
the roots and the shoots of the plants



2. Incubation of the test plates
in vertical position



3. Automatic measurement of the length
of the roots and the shoots,
and data treatment by image analysis



Phytotoxkit microbiotest



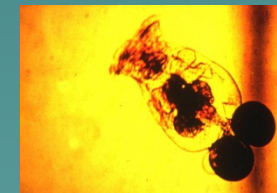
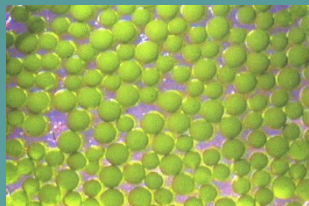
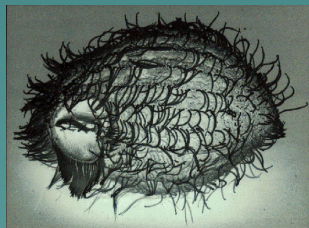
Test plate after 3 days
of incubation



Phytotoxkit

**Contact test for toxicity analysis of soils and sediments
with seeds of 3 higher plants**

Battery of acute and chronic TOXKITS with several test species commercialised by the company MicroBioTests Inc.



Application of TOXKIT microbiotests



Chemical compounds

Surface waters

Groundwaters

Sediments

Biotoxins

Solid wastes

Waste waters

Composts

Soils

Sludges

Detoxification/Bioremediation

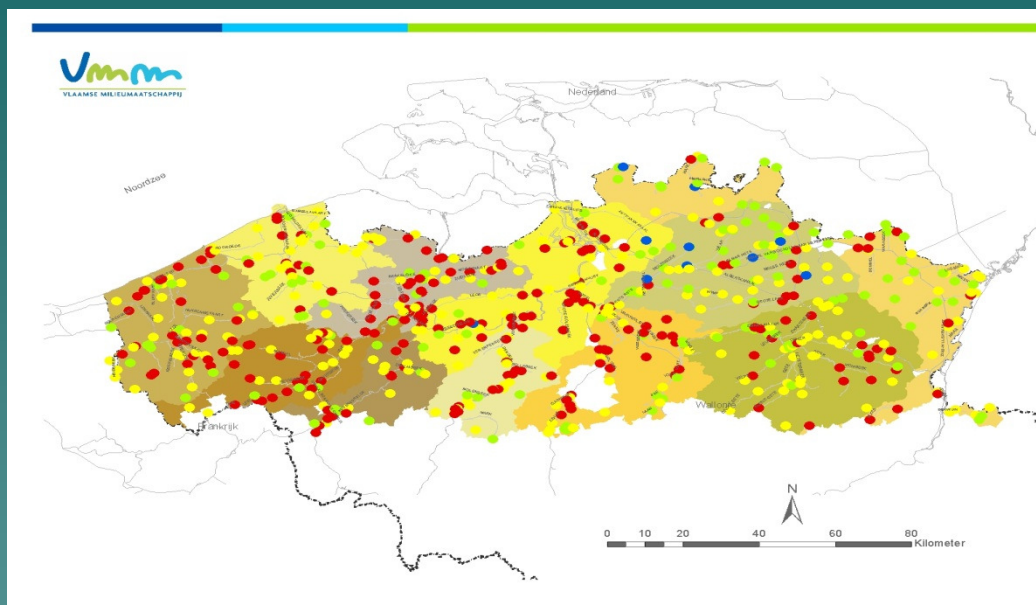
Water contamination emergencies

Marine environment

*Over **400** scientific papers and reports on various applications of Toxkit microbiotests are listed on the website www.microbiotests.be*

*Already more than **200.000** bioessais have been carried out with Toxkits in 40 countries worldwide*

Ecotoxicological analyses of river sédiments in Flanders, Belgium



Over the last ten years, river sediments of the hydrographic basins in Flanders are analysed each year on their “ecological quality” (TRIAD approach)

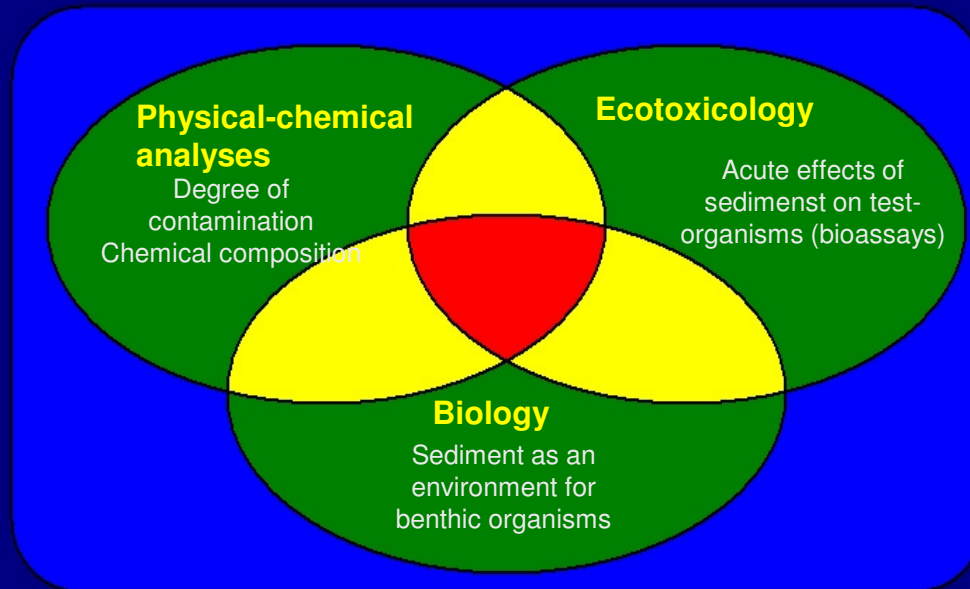
About 1000 samples from 600 sites have already been examined on their toxicity

TRIAD-approach

Fysico-chemical parameters:

Fractions,
Organic matter,
Mineral oil,
Heavy metals,
OCP's,
PAH's,
PCB's.

Ecological quality of sediments TRIAD-concept



Ecotoxicological parameters:

Solid-phase test:

- *Hyalella azteca*



Liquid-phase test:

- *Raphidocelis subcapitata*



Liquid-phase test:

- *Thamnocephalus platyurus*



Biological parameter:

Benthic macro-invertebrates



Biotic Sediment Index (BSI): presence of indicator organisms and taxonomic diversity



Comparison by the Environmental Agency of Flanders (VMM) of the *Hyaella azteca* contact test with the Ostracodtoxkit microbiotest



Complicated and expensive



Hyaella azteca

(1800-2000µm)



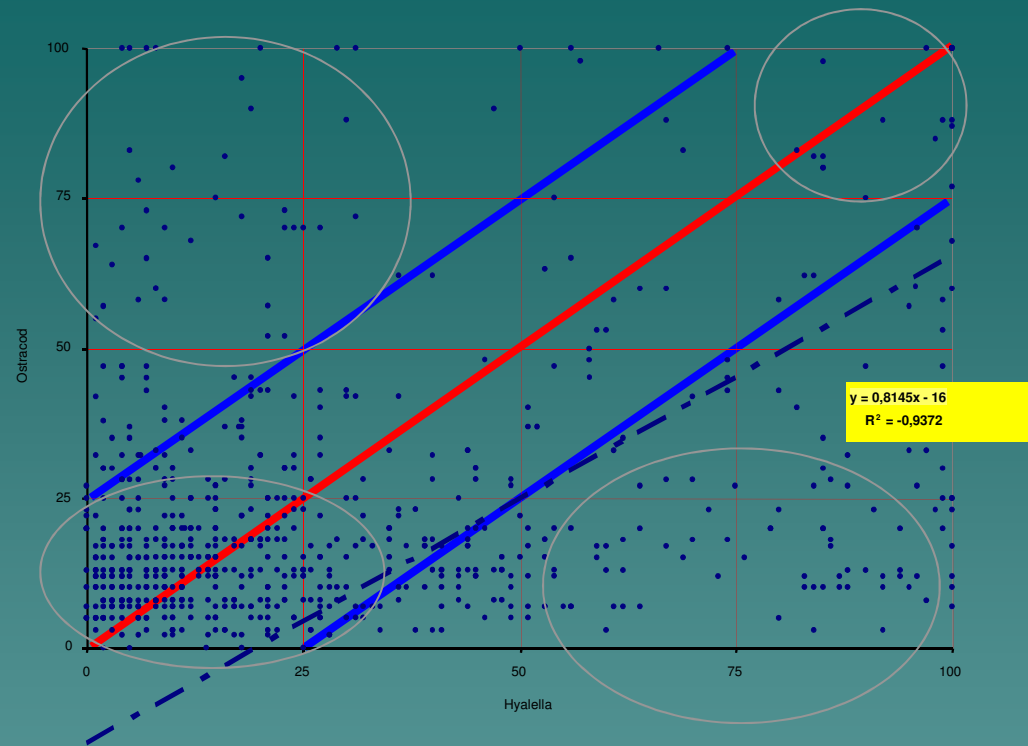
Simple, practical and low cost



Heterocypris incongruens

(150-200 µm)

Statistical comparison of nearly 1000 data pairs



The magnitude of the effects is similar for the two bioassais for the majority of the samples.

For the other samples, it is either the ostracod or the amphipod which is more sensitive



Ecotoxicological analyses of river sediments in France

Résultats du groupe de travail MEEDDM « Dangersité des sédiments »

Critère H14 (écotoxicité)

Critères H7 (C), H11 (M) et H10 (R)

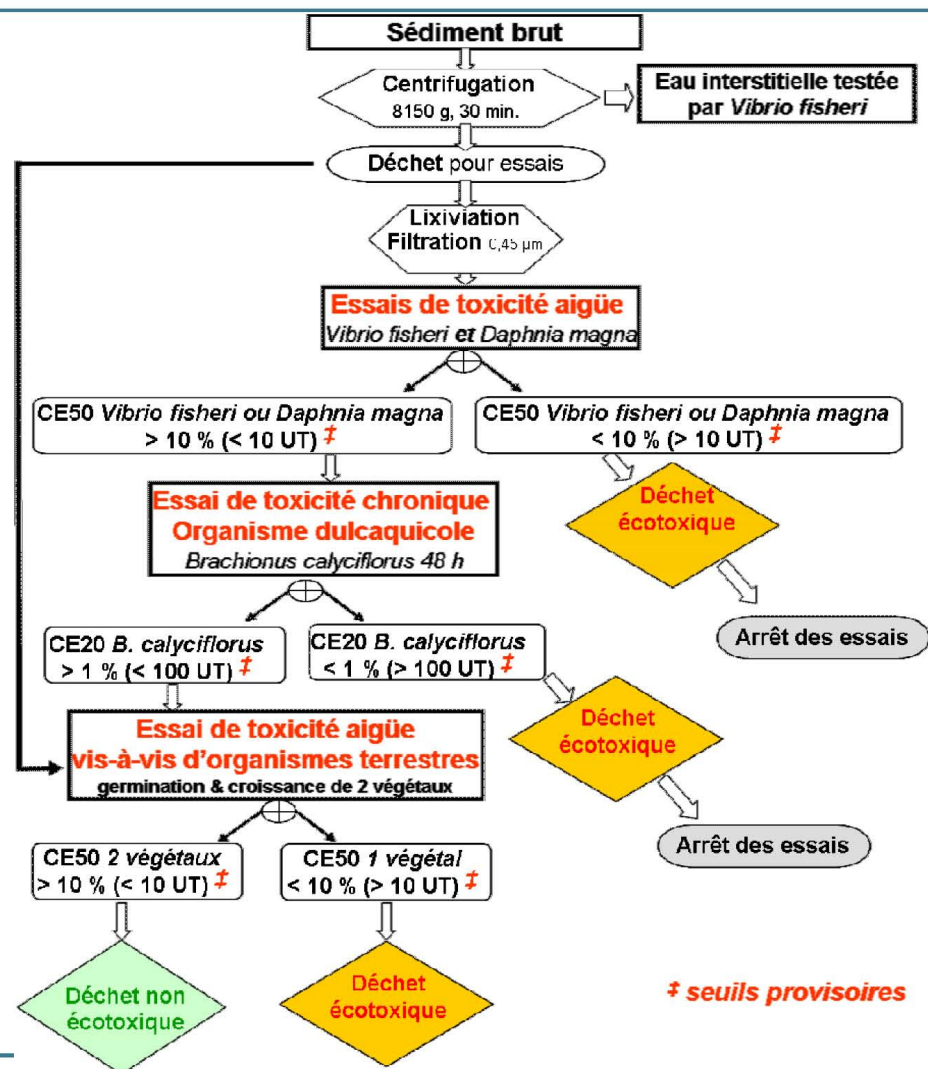
C. Mouvet

BRGM/EPI/SSP



C. Mouvet, Journées Nationales Sédiments, Environord, 10 juin 2010

Le
protocole
H14
MEEDDM
octobre 2009



Use of Toxkit microbiotests in a regulatory framework



Five Toxkit microbiotests are totally conform with,
and have been accepted as ISO standards



Rotoxkit F chronic



Daphtoxkit F magna



Algaltoxkit



Thamnotoxkit



Ostracodtoxkit

The **Phytotoxkit** will be submitted shortly to ISO to become
an ISO standard for the ecotoxicological analysis of soils and
sediments



Phytotoxkit

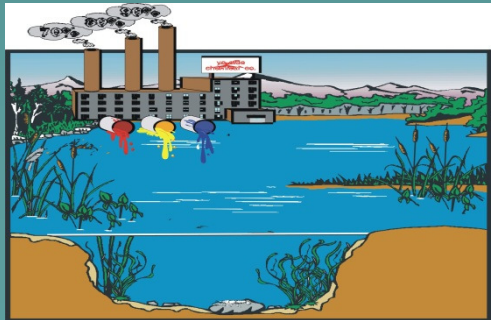
TOXKIT microbiotests



- Independent of culturing and maintenance of test organisms
- Miniaturised
- User friendly
- Standardised
- Validated by interlaboratory testing
- Highly reproducible
- Low cost

Practical and reliable tools for

Environmental biomonitoring



Research

