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Ecotoxicological bioassays for treated wastewater monitoring and assessment

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ECOTOXICOLOGICAL BIOASSAYS FOR TREATED WASTEWATER MONITORING AND ASSESSMENT

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Introduction

The increased need for wastewater reuse arises concern about treated waste water quality assessment.

The quality of reclaimed water should be well established prior to its reuse, in order to prevent potential long-term hazards for humans and environment.

Current legislation on wastewater reuse is based solely on physicochemical and microbiological parameters.

Introduction

Single chemical measurements does not provide alone information on the bioavailability of the substances and on the interactive effects between all the chemicals present

Organic micro-pollutants detection is laborious and difficult to be accomplished and the cost of analysis often hinders the screening of large numbers of samples

The presence and risk of emerging pollutants in waters are only now being recognized, and therefore, they are generally not included in the legislation.

Introduction

The need to improve the efficiency and effectiveness of wastewater quality monitoring and assessment have increased the interest towards innovative methodologies.

Biomonitoring: systematic use of living organisms or their responses to assess the quality of the environment

Introduction

Biomonitoring advantages

- ✓ Direct measurement of the effects of pollutants on biological systems
- ✓ Bioavailability information
- ✓ Integrative measure of the combined effects of multiple contaminants

Bioassays Biomarkers Biosensors

Introduction

laboratory tests in which the toxicity of a sample is measured by exposing a biological system (cells, tissues, or whole organisms) and measuring biological responses

Bioassays

Introduction

molecular or cellular alterations induced in the organism by the exposure to chemical pollulants

Biomarkers

Aim

The aim of the present work was to assess the performance of a suite of ecotoxicological bioassays and biomarkers for monitoring urban treated wastewaters reused for irrigation and for monitoring the irrigated soils.

Aim

Development of ecotoxicological protocols sensitive, informative and low-cost useful for improving the assessment of treated waste water quality.

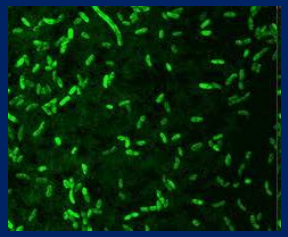
This study has been carried out under the Project In.T.e.R.R.A. (Contract no. 01 01480) cofounded within the Italian Program “PON/Ricerca e Competitività 2007–2013

Methods

Ecotoxicological bioassays for treated wastewater monitoring

Stadardized tests

OECD *Daphnia magna* acute immobilization test



ISO Microtox® Toxicity test

New developed

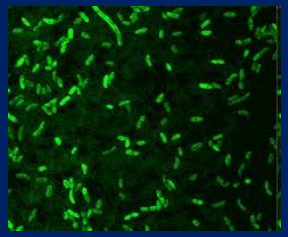
Method for the enzymatic assessment of aqueous environmental matrices (patent n. MI2008A008813)

Methods

Ecotoxicological bioassays for treated wastewater monitoring

Stadardized tests

OECD *Daphnia magna* acute immobilization test



ISO Microtox® Toxicity test

Daphnia magna test and Microtox® Toxicity test are accepted in several countries to monitor wastewater treatment systems and to establish quality criteria

Methods

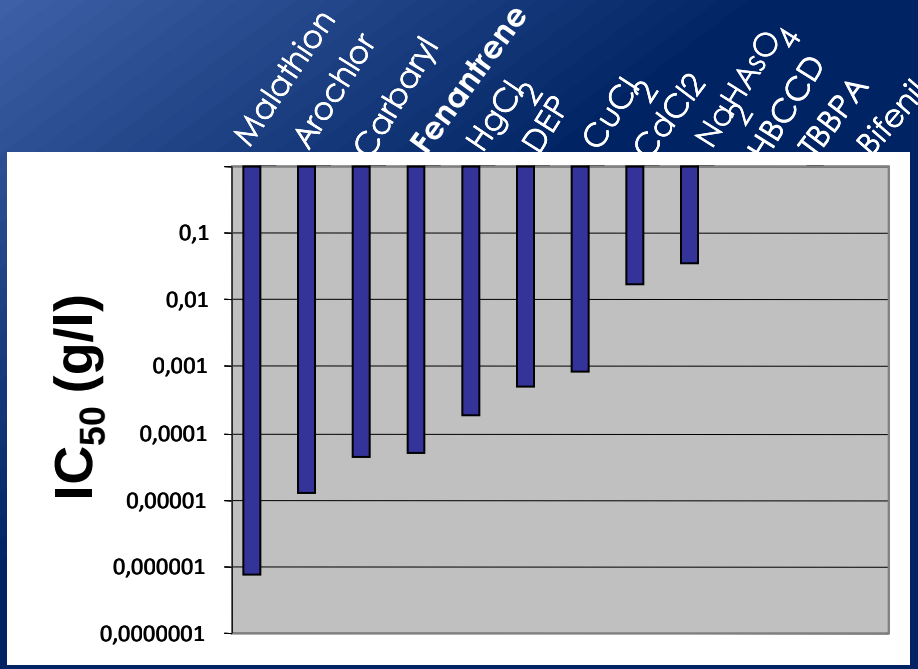
New developed

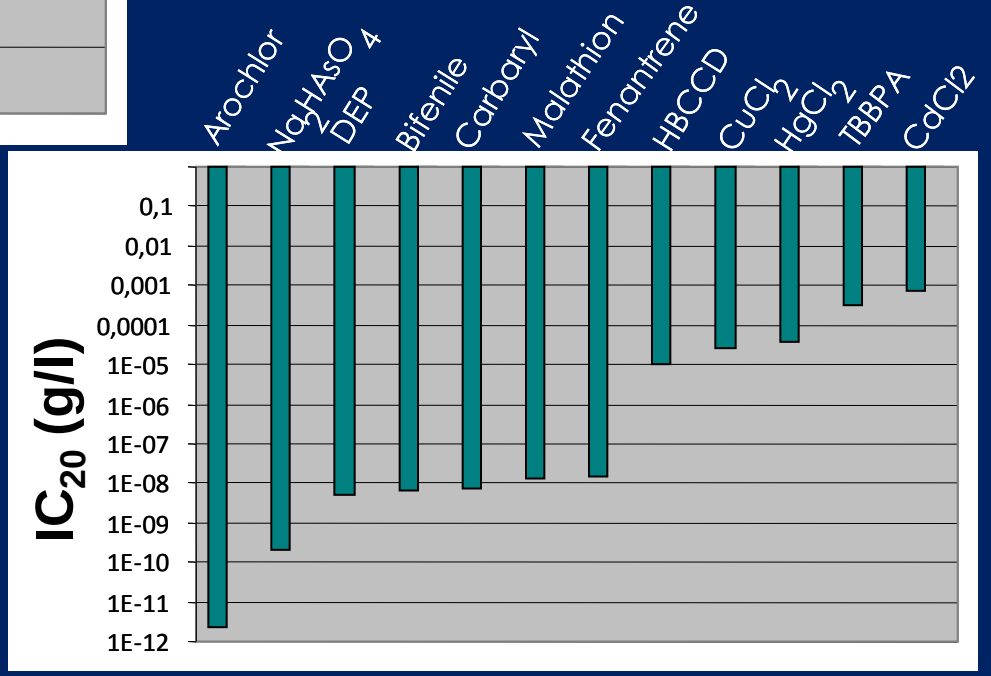
Method for the enzimatic assessment of aqueous environmental matrices (patent n. MI2008A008813)

It is based on the *in vitro* inhibition measurement of the carbonic anhydrase enzymatic activity by multiple contaminants present in aqueous environmental samples. The inhibition of the enzyme is proportional to the toxicity of the sample

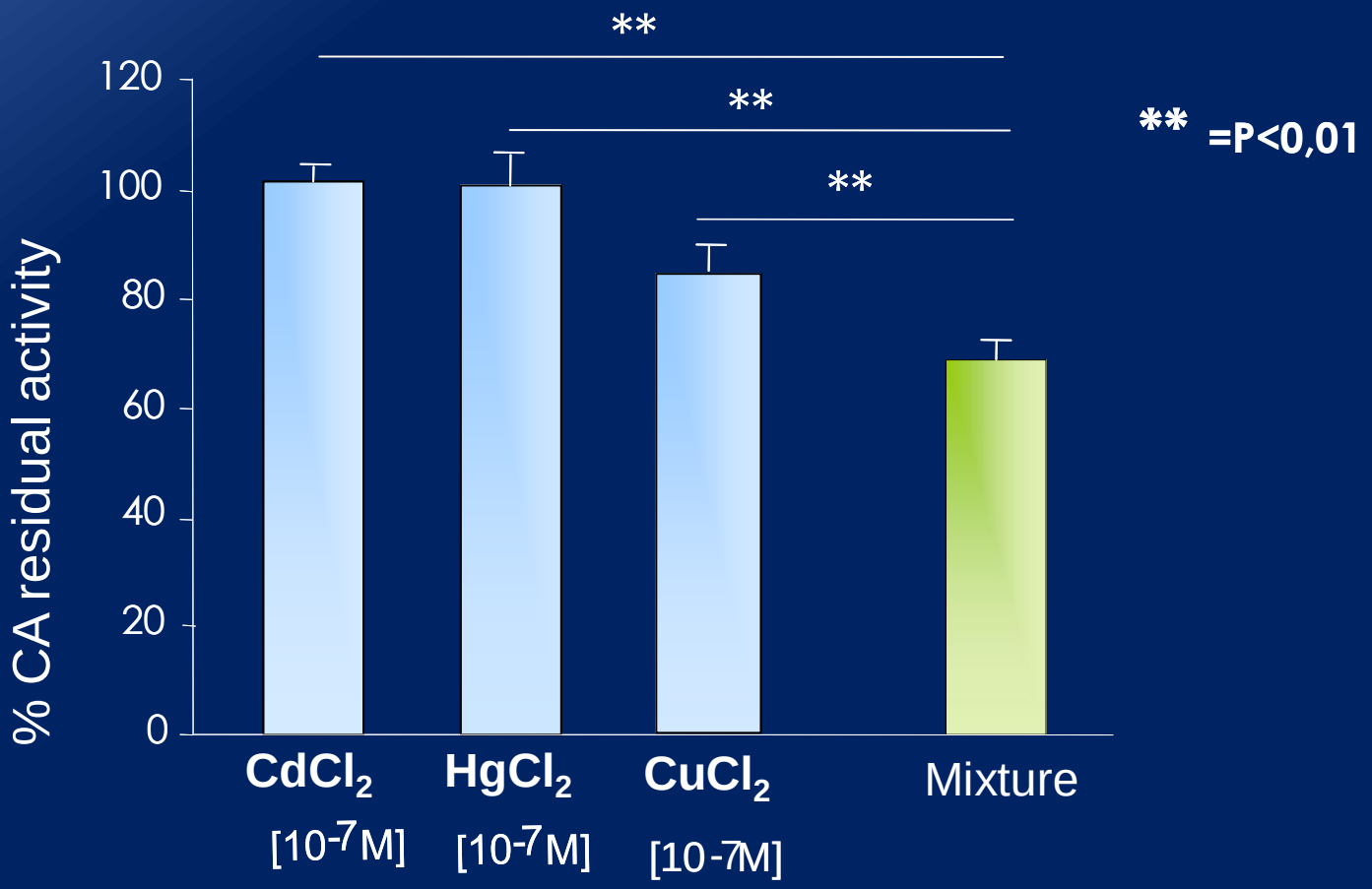
- ✓ sensitive to the major classes of environmental chemical contaminants
- ✓ rapid (time of analysis: 10 min), low cost, non specialized equipment required
- ✓ does not require the use of living organisms

Sensitivity of the patented method to chemical pollutants (EC_{50} and EC_{20})





Sensitivity of the patented method to chemical pollutants (synergic effect)



Methods

Ecotoxicological bioassays for monitoring soil irrigated with treated wastewaters

Stadardized tests

OECD Acute and chronic toxicity test with *Eisenia fetida*

New developed

Biomarker analysis

Methods

Earthworms as bioindicator organisms for of soil ecotoxicity assessment

Earthworms are very important organisms for soil formation and organic matter breakdown.

They are very sensitive to toxic chemicals present in the soil.

These features have led to the use of earthworms as bioindicators of soil pollution

Eisenia fetida

Experimental plan

N° of water samples analyzed: 115 (from four irrigation cycles)
N° of soil samples analyzed: 40 (at the start and the end of the crop cycle)

References values for *Daphnia magna* and Microtox test (ANPA, RTI CTN_AIM 4./2001, D.L. 152/2006)

Mortality or percentage inhibition (I%)*	LC50 o EC50	Toxicity ranking
<20%		No toxic
≥ 20% <50%		Weakly toxic
≥ 50%	100-10	Toxic
> 50%	<10-1	Very toxic
> 50%	<1	Extremely toxic

*referred to the sample as such

Results

Ecotoxicological bioassays on treated waste waters

Treatment plant	data	Water type	*Daphnia*	Microtox (15 min)	AC
Castellana	8-2-’13	primary	**92,0 %**	**75,6 %**	**65,0%**
	8-2-’13	secondary	<20%	<20%	<20%
	8-2-’13	conventional	<20%	<20%	<20%

Summer 2012 irrigation cycle

Treat. plant	Water type	data	Daphnia	Microtox	AC
Noci	**Convenzional**	6-6-'12	<20%	<20%	<20%
	Terziary	6-6-'12	<20%	<20%	<20%
	Convenzional	18-7-'12	<20%	<20%	<20%
	Terziary	18-7-'12	<20%	<20%	<20%
Fasano	Convenzional	4-6-'12	<20%	<20%	<20%
	Terziary	4-6-'12	<20%	<20%	<20%
Trinitapoli	Convenzional	11-6-'12	<20%	<20%	<20%
	Secondary	11-6-'12	<20%	<20%	<20%
	Impoundment	11-6-'12	<20%	**67.4%**	<20%
	Terziary	11-6-'12	<20%	<20%	<20%
	Convenzional	29-6-'12	<20%	<20%	<20%
	Secondary	29-6-'12	<20%	**26.6%**	<20%
	Impoundment	29-6-'12	<20%	**28.3%**	<20%
	Terziary	29-6-'12	<20%	<20%	<20%
	Convenzional	17-7-'12	<20%	<20%	<20%
	Secondary	17-7-'12	<20%	26.6	<20%
	Impoundment	17-7-'12	<20%	**38.3%**	<20%
	Terziary	17-7-'12	<20%	<20%	<20%
Castellana	Convenzional	12-6-'12	<20%	<20%	<20%
	Terziary	12-6-'12	<20%	**38.1%**	<20%
	Convenzional	18-7-'12	<20%	<20%	<20%
	Terziary	18-7-'12	<20%	<20%	<20%

Autumn 2012 irrigation cycle

Treat. plant	Water type	data	Daphnia	Microtox	AC
Noci	Convenzional	2-10-'12	<20%	<20%	<20%
	Terziary	2-10-'12	<20%	<20%	<20%
	Convenzional	14-11-'12	<20%	<20%	<20%
	Terziary	14-11-'12	<20%	<20%	<20%
	Convenzional	18-12-'12	<20%	<20%	<20%
	Terziary	18-12-'12	<20%	<20%	<20%
Fasano	Convenzional	16-10-'12	<20%	<20%	<20%
	Terziary	16-10-'12	<20%	<20%	<20%
	Convenzional	27-11-'12	<20%	<20%	<20%
	Terziary	27-11-'12	<20%	<20%	<20%
Trinitapoli	Convenzional	6-11-'12	<20%	<20%	<20%
	Secondary	6-11-'12	<20%	<20%	<20%
	Impoundment	6-11-'12	<20%	<20%	<20%
	Convenzional	4-12-'12	<20%	<20%	<20%
	Secondary	4-12'-12	<20%	<20%	<20%
	Impoundment	4-11-'12	<20%	**31,2%**	<20%
Castellana	Terziary	2-10-'12	<20%	<20%	<20%
	MBR	2-10-'12	<20%	<20%	<20%
	Terziary	23-10-'12	<20%	<20%	<20%
	MBR	23-10-'12	<20%	<20%	<20%
	FDG	23-10-'12	<20%	<20%	<20%
	Convenzional	20-11-'12	<20%	<20%	<20%
	MBR	20-11-'12	**38,9%**	<20%	**70.2%**
	FDG	20-11-'12	<20%	<20%	<20%

Spring - Summer 2013 irrigation cycle

Treat. plant	Water type	data	Daphnia	Microtox	AC
Noci	Convenzional	22-4-'13	<20%	<20%	<20%
	Terziary	22-4-'13	<20%	<20%	<20%
	Convenzional	21-5-'13	<20%	<20%	<20%
	Terziary	21-5-'13	<20%	<20%	<20%
	Convenzional	11-6-'13	<20%	<20%	<20%
	Terziary	11-6-'13	<20%	<20%	<20%
	Convenzional	2-7-'13	<20%	<20%	<20%
	Terziary	2-7-'13	<20%	<20%	<20%
	Piena scala	22-4-'13	<20%	<20%	<20%
Fasano	Convenzional	22-4-'13	<20%	<20%	<20%
	Impianto pilota	21-5-'13	<20%	<20%	<20%
	Impianto pilota	11-6-'13	<20%	<20%	<20%
	Piena scala	2-7-'13	<20%	<20%	<20%
	Impianto pilota	2-7-'13	<20%	<20%	<20%

Spring - Summer 2013 irrigation cycle

Treat. plant	Water type	data	Daphnia	Microtox	AC
Trinitapoli	Conventional	4-6-'13	<20%	<20%	<20%
	Secondary	4-6-'13	**45%**	**43%**	**27%**
	terziary	4-6-'13	<20%	<20%	<20%
	Impoundment	4-6-'13	<20%	**43.5%**	**27.8%**
	Convenzional	4-7-'13	<20%	<20%	<20%
	Secondary	4-7-'13	**45.5%**	<20%	**40%**
	Terziary	4-7-'13	<20%	<20%	**40%**
	Impoundment	4-7-13	<20%	**30,6%**	<20%
	Convenzional	23-7-'13	<20%	31,2	<20%
	Secondary	23-7-'13	**100%**	**30,6%**	**64,2%**
	Terziary	23-7-'13	<20%	<20%	<20%
	Impoundment	23-17'13	<20%	<20%	<20%
Castellana	Convenzional	28-5-'13	<20%	<20%	<20%
	FDG	28-5-'13	<20%	<20%	<20%
	MBR	28-5-'13	<20%	<20%	<20%
	convenzional	30-5-'13	<20%	<20%	<20%
	FDG	30-5-'13	<20%	<20%	<20%
	MBR	30-5-'13	<20%	<20%	<20%
	Terziary	30-5-'13	<20%	<20%	<20%

Autumn 2013 irrigation cycle

Treat. plant	Water type	data	Daphnia	Microtox	AC
Noci	Convenzional	10-10-'13	<20%	<20%	<20%
	Terziary	10-10-'13	<20%	<20%	<20%
Fasano	Convenzional	3-9-'13	<20%	<20%	<20%
	Impianto pilota	3-9-'13	<20%	<20%	<20%
	Piena scala	3-9-'13	<20%	<20%	<20%
Trinitapoli	Convenzional	24-9-'13	<20%	<20%	<20%
	Terziary	24-9-'13	<20%	<20%	<20%
	Convenzional	13-11-'13	<20%	<20%	<20%
	Secondary	13-11-'13	<20%	**28.4%**	<20%
	Terziary	13-11-'13	<20%	<20%	<20%
	Impoundment	13-11-'13	<20%	**52,5%**	<20%
Castellana	Convenzional	11-9-'13	<20%	<20%	<20%
	MBR	11-9-'13	<20%	<20%	<20%
	FDG	11-9-'13	<20%	<20%	<20%
	Terziary	11-9-'13	<20%	<20%	<20%

Comparison between tests

Agreement between tests

Test B \ Test A	+	-
+	a	b
-	c	d

AC

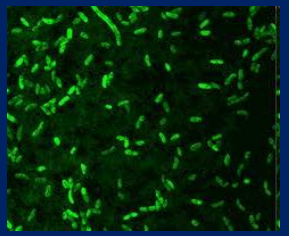
vs

Daphnia

= 95.4 %

AC

vs



Vibrio fisheri

= 85.7 %

Ecotoxicological bioassays on soil irrigated with treated wasterwaters

Eisenia fetida

Exposure of an *Eisenia fetida* homogeneous population to the soil irrigated with treated waste-waters

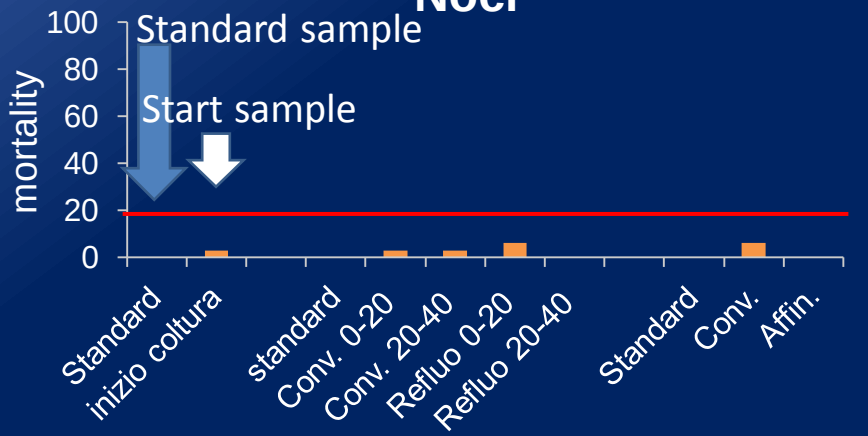
- Acute toxicity test (**14 days exposure)**
- Chronic toxicity test (60 days exposure)
- Biomarker analysis (30 days exposure)

Ecotoxicological bioassays on soil irrigated with treated wasterwaters

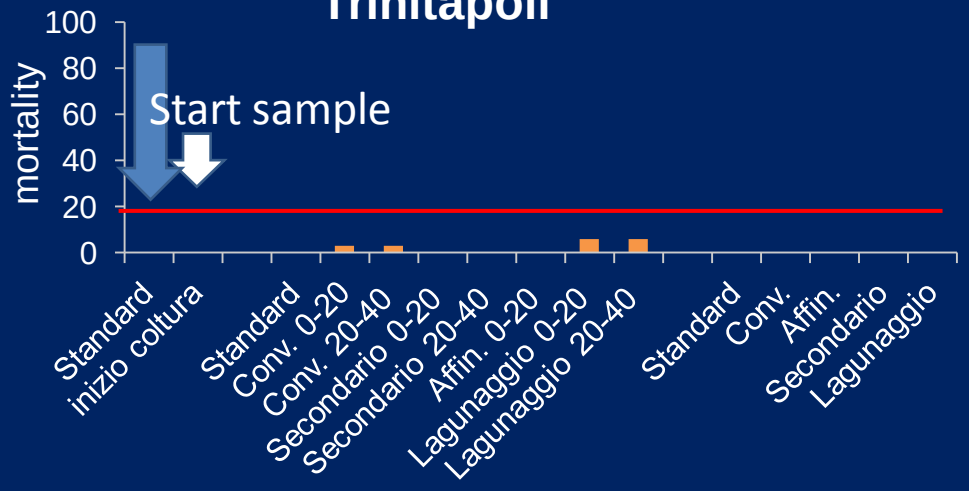
Eisenia fetida

Acute toxicity test (14 days exposure)
(OECD, 1984)

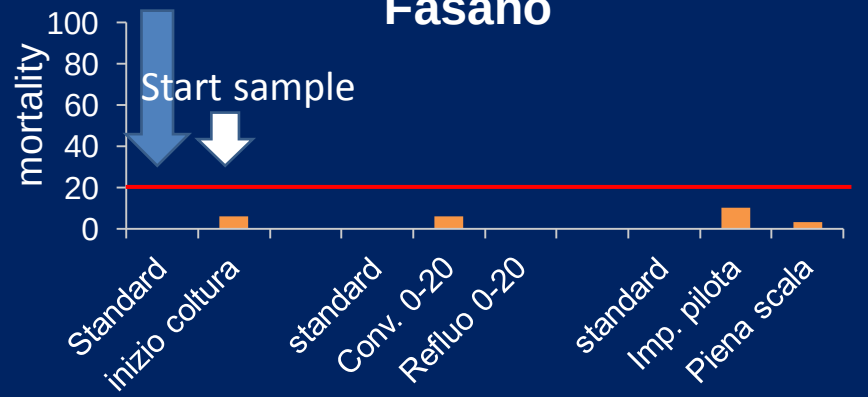
Noci



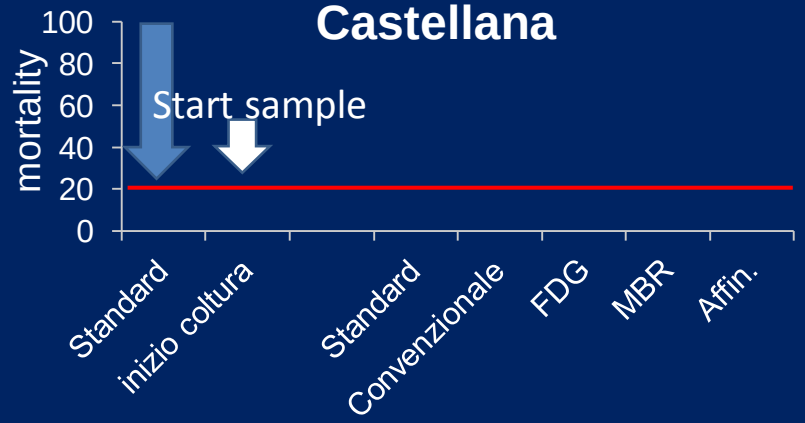
Trinitapoli



Fasano



Castellana

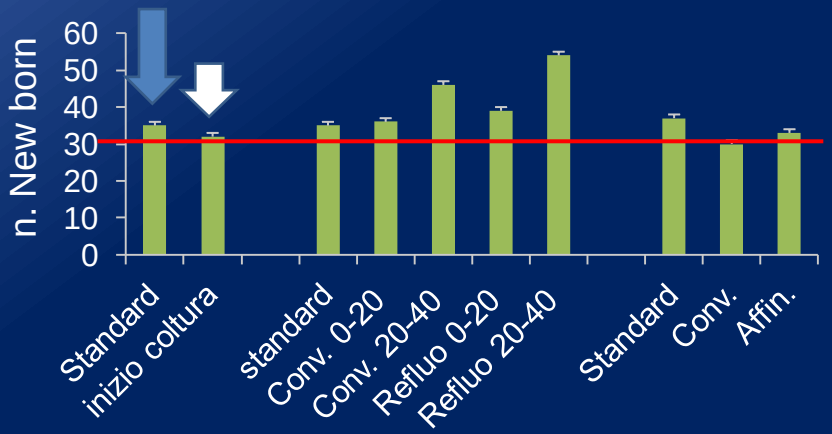


Ecotoxicological bioassays on soil irrigated with treated wasterwaters

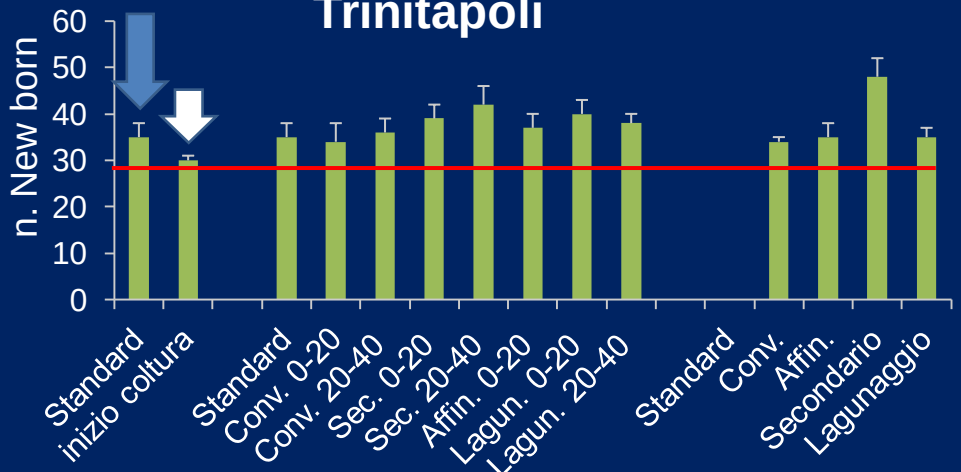
Eisenia fetida

Chronic toxicity test (60 days exposure)
(OECD, 2004)

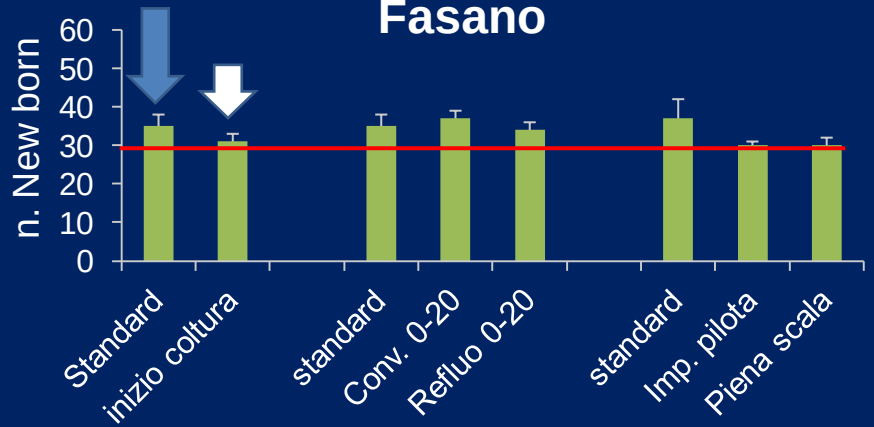
Noci



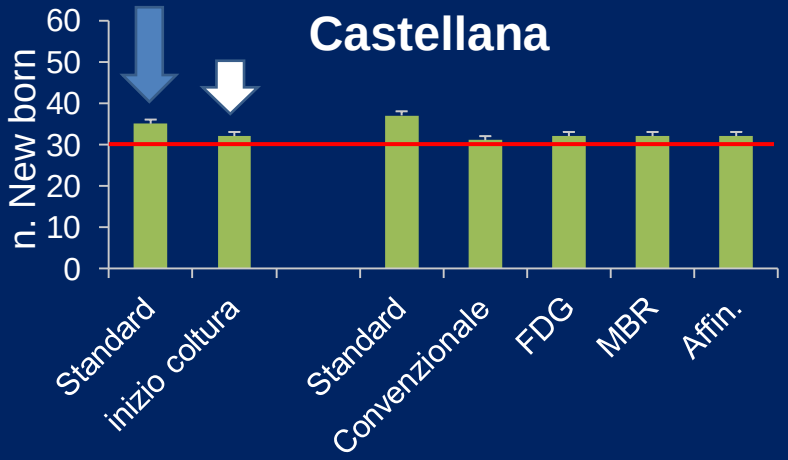
Trinitapoli



Fasano



Castellana

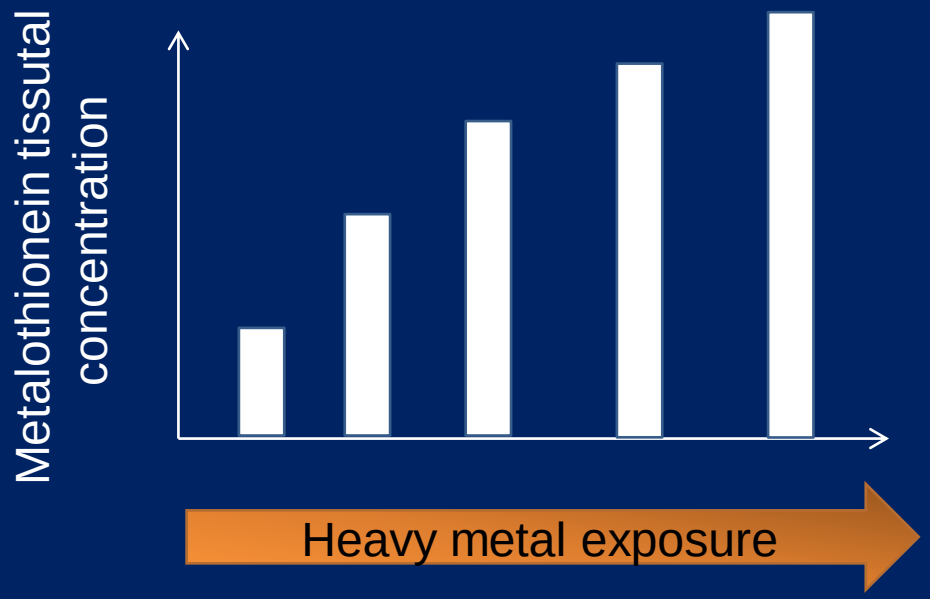


Biomarkers in bioindicator organisms exposed to soil irrigated with treated wastewaters

Eisenia fetida

Biomarker analysis (30 days exposure)

Metallothioneins



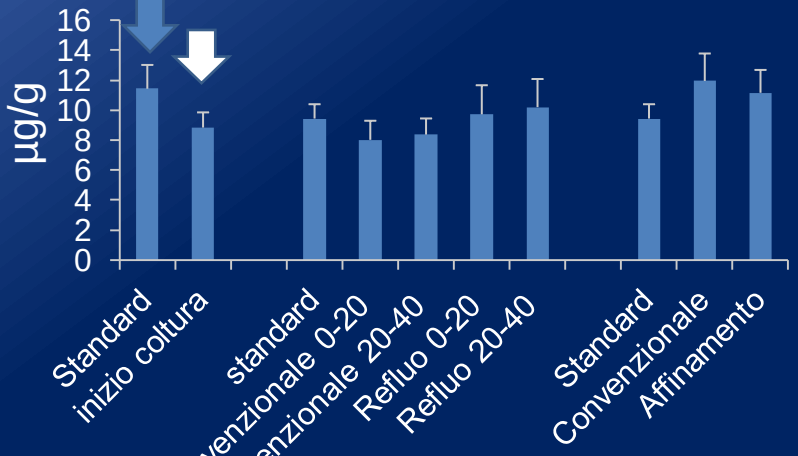
Metallothionein: cytosolic proteins able to chelate hevy metal cations (Hg^{2+}, Cd^{2+}, Cu^{2+}, Zn^{2+}). Metallothionein expression increases following exposure to heavy metals.

Biomarkers in bioindicator organisms exposed to soil irrigated with treated wastewaters

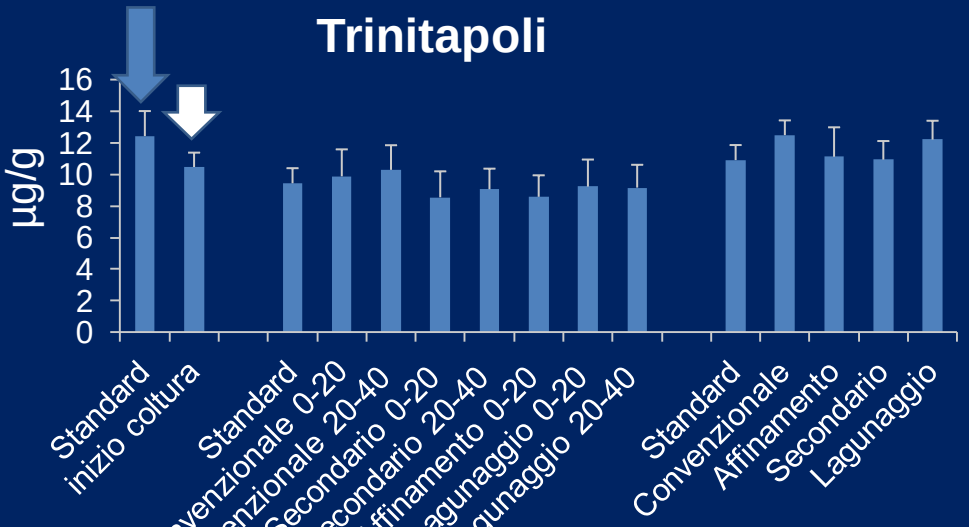
Eisenia fetida

Metallothioneins

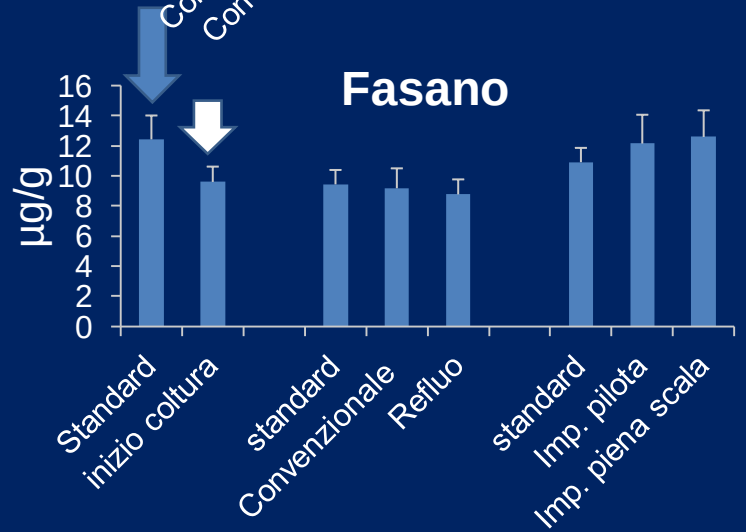
Noci



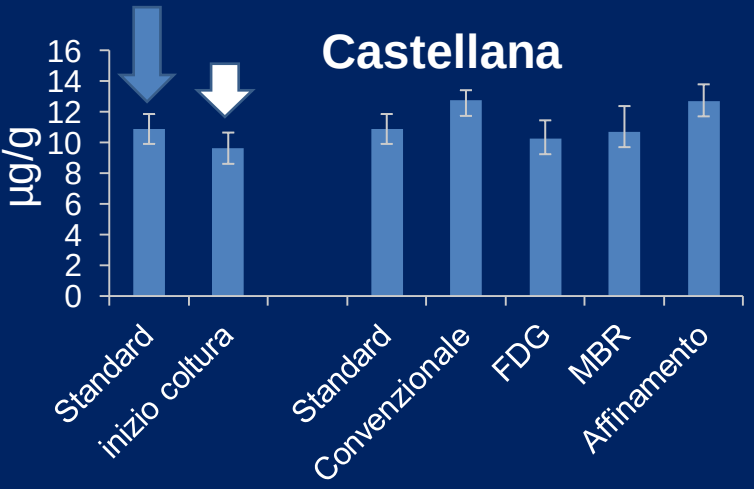
Trinitapoli



Fasano



Castellana

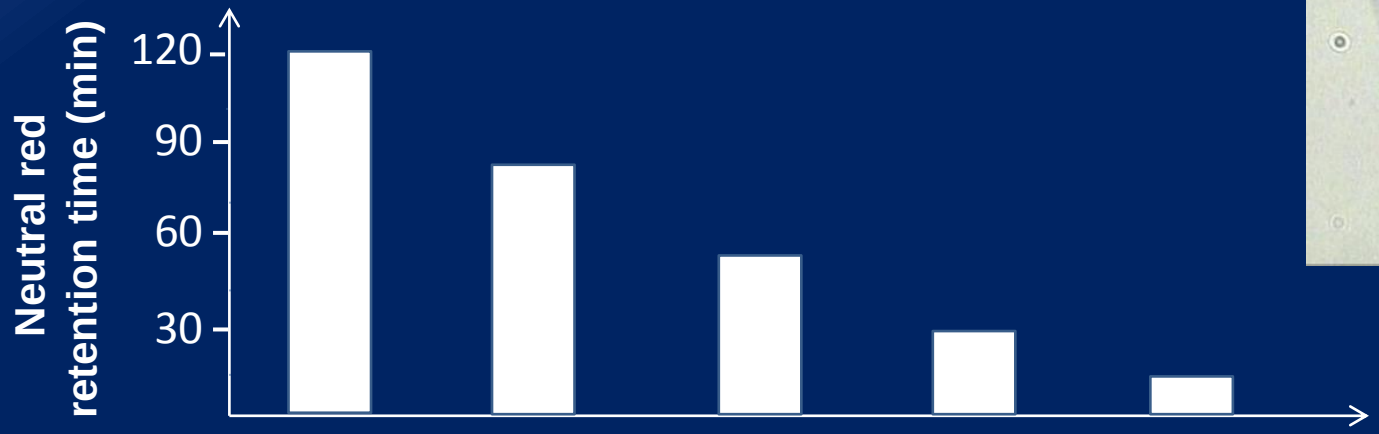


Biomarkers in bioindicator organisms exposed to soil irrigated with treated wastewaters

Eisenia fetida

Biomarker analysis (30 days exposure)

Lysosomal membrane stability



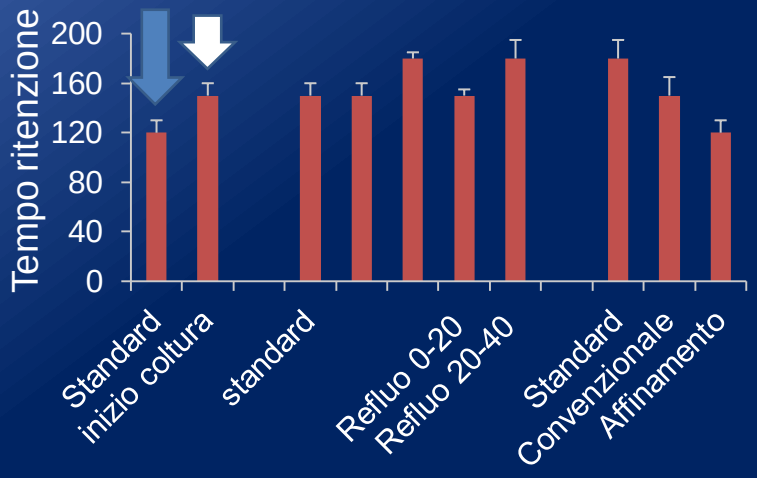
Exposure to chemical pollutants (organic and inorganic)

Biomarkers in bioindicator organisms exposed to soil irrigated with treated wastewaters

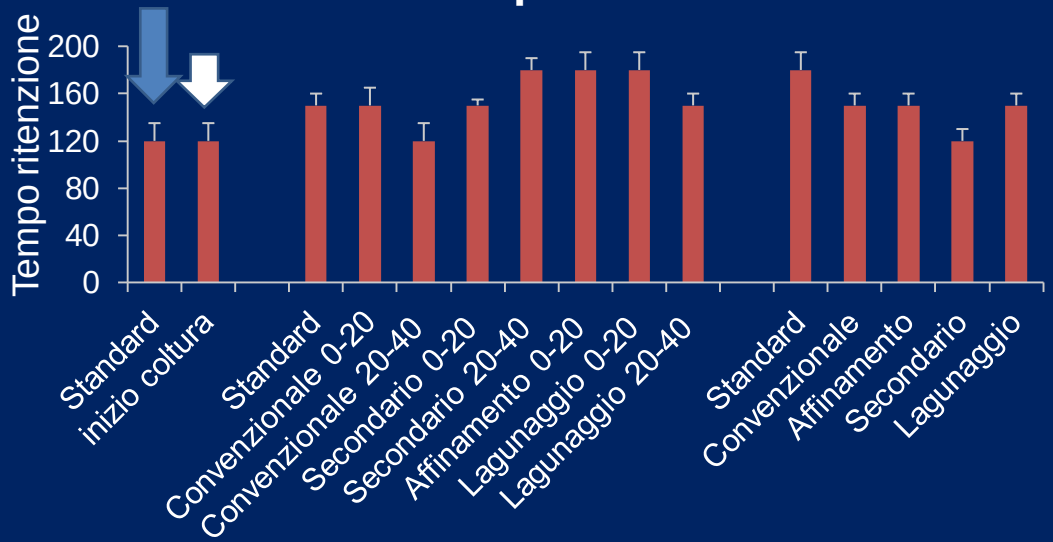
Eisenia fetida

Lysosomal stability

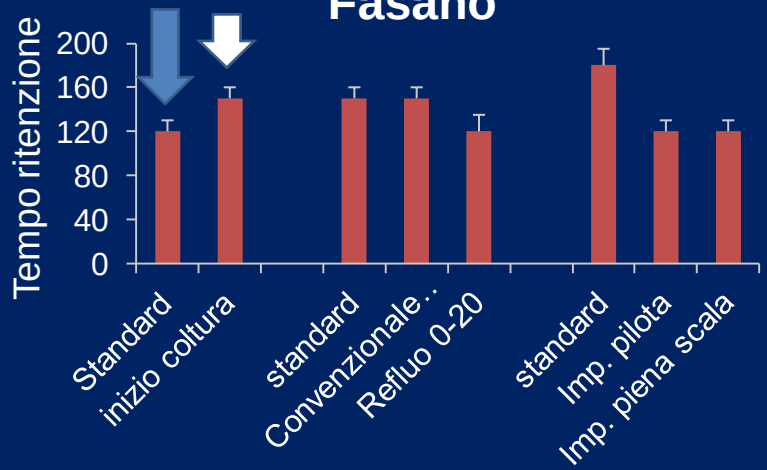
Noci



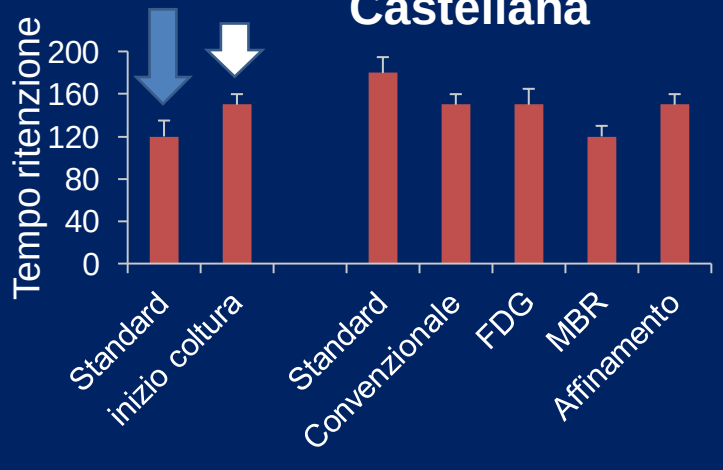
Trinitapoli



Fasano



Castellana



Conclusions

1. The obtained results demonstrate the applicability of *in vitro* and *in vivo* ecotoxicological bioassays for the toxicity assessment of treated wastewaters and for the assessmsent of eventual toxicity phenomena in soils irrigated with treated wastewaters.
They provide an integrated measure of the biological effects of bioavailable chemicals that can be present in waste waters.

Conclusions

2 The new *in vitro* patented method showed highly applicability to waste waters monitoring.

Advantages:

- it shows a high agreement with the standardized tests
- It allows more rapid and cheaper analysis of ecotoxicity.
- It allows to overcome some limits of living organism based bioassays

Conclusions

3 A protocol for the ecotoxicity assessment of the soils irrigated with treated wastewaters has been standardized.

It is based on the integrated use of standardized bioassay and sensitive biomarker analysis on earthworms as soil bioindicator organisms.

Perspectives

The possibility of large-scale use of ecotoxicological methods will improve the process of re-use of wastewater for irrigation in terms of assessment of the toxicological safety of the waters for the environment, for traders and consumers.

Acknowledgement

The authors wish to thank the Italian Ministry of University and Research (MIUR) for its financial support under the Project In.T.e.R.R.A. (Contract no. 01 01480) cofounded within the Italian Program “PON/Ricerca e Competitività 2007–2013