APPLICATION OF ECO-GENOTOXICOLOGICAL AND MICROBIOLOGICAL PARAMETERS FOR THE ASSESSMENT OF THE QUALITY OF WASTEWATER INDUSTRIAL REUSE.

Silvana Caciolli, Ines Lacchetti, Mario Carere, Stefania Marcheggiani, Camilla Puccinelli, Anna Maria D'angelo, Silvia Marchini, Roberto Giuseppetti, Elio Pierdominici, Elisabetta Volpi and Laura Mancini.

Italian Institute of Health - Environmental and Health Department, Rome, Italy

E-mail contact: silvana.caciolli@iss.it and laura.mancini@iss.it

INTRODUCTION AND OBJECTIVES

In industrial sectors, water is used in large amount in production cycles. In the field of Medical Devices (MDs) production, for example, water is used, both as an ingredient and as a necessary element for production.

The re-use and the recycle of the wastewater represents a priority area in the strategic plan of the European Commission for a sustainable water management also considering the scarcity of water resources caused by the climate changes.

The aim of this study is the assessment of treated wastewater quality discharged from Medical Device and pharmaceutical industries, by identifying a suitable set of tools in order to support the potential re-use of treated wastewater, taking into account both microbiological and eco-/geno-toxicological parameters.

MATERIALS AND METHODS
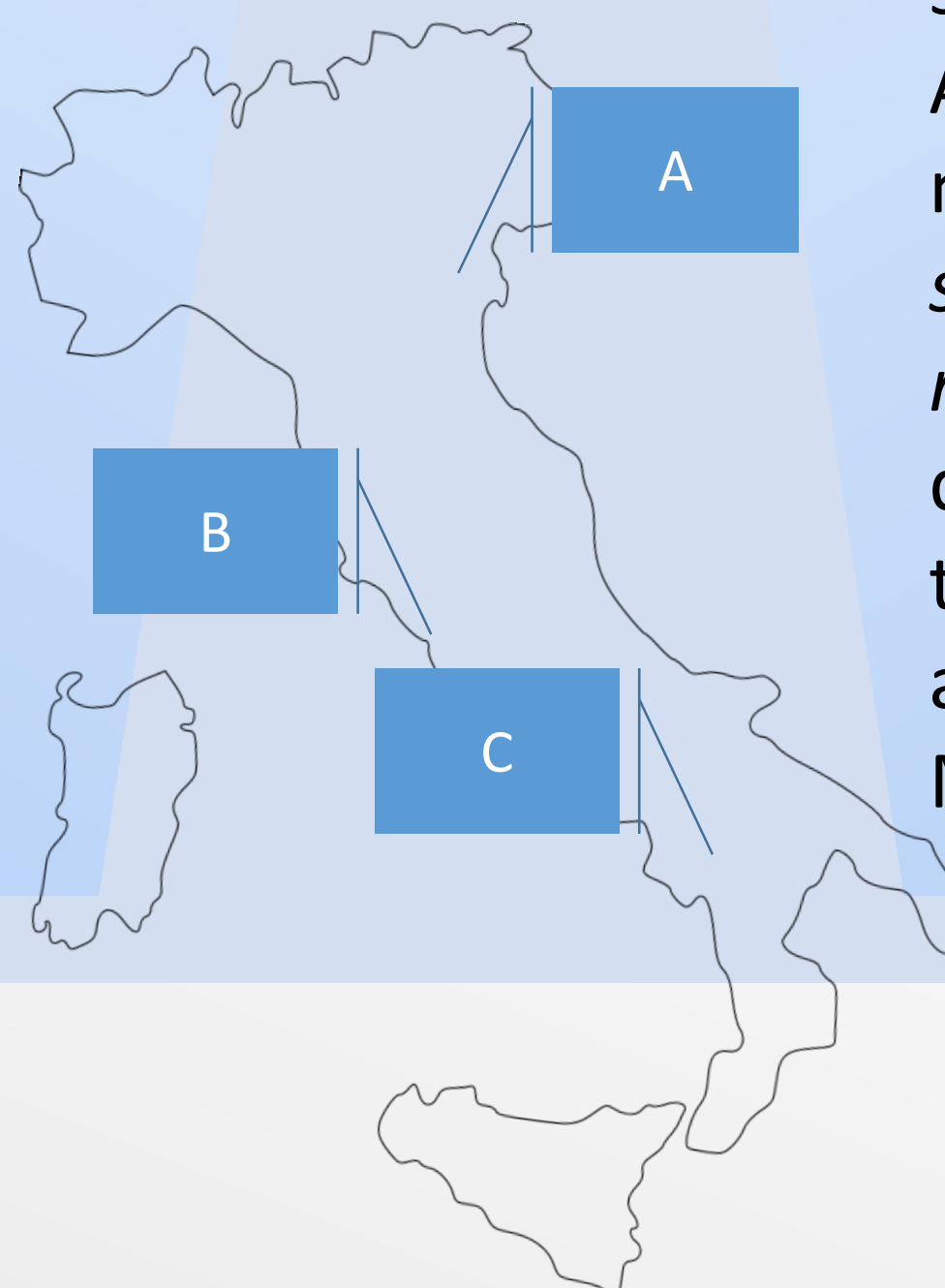
The study was carried out on three MDs industries in Italy, which perform the recycle of the wastewater in their own system processes. Samples were collected inlet and outlet of wastewater treatment plants. The microbiological parameters investigated were Total Microbial Count (TMC) at 22°C and 37°C using European Pharmacopoeia method (2011), *E. coli*, Enterococci, Staphylococcus spp., Pseudomonas spp. using membrane filter technique a standard methods (APHA, 2008).

A set of ecotoxicological bioassays was selected for this study, namely the bacterium *Vibrio fischeri*, the algae *Pseudokirchneriella subcapitata*, the crustacean *Daphnia magna*, the fish embyo *Danio rerio* and the plant *Sorghum saccharatum*, in order to represent different trophic levels and thus to assess any potential effects on the aquatic ecosystems. Seeds of *Vicia faba* were exposed for assessing the possible genotoxic effect of wastewater using the Micronucleus test (MN-test).
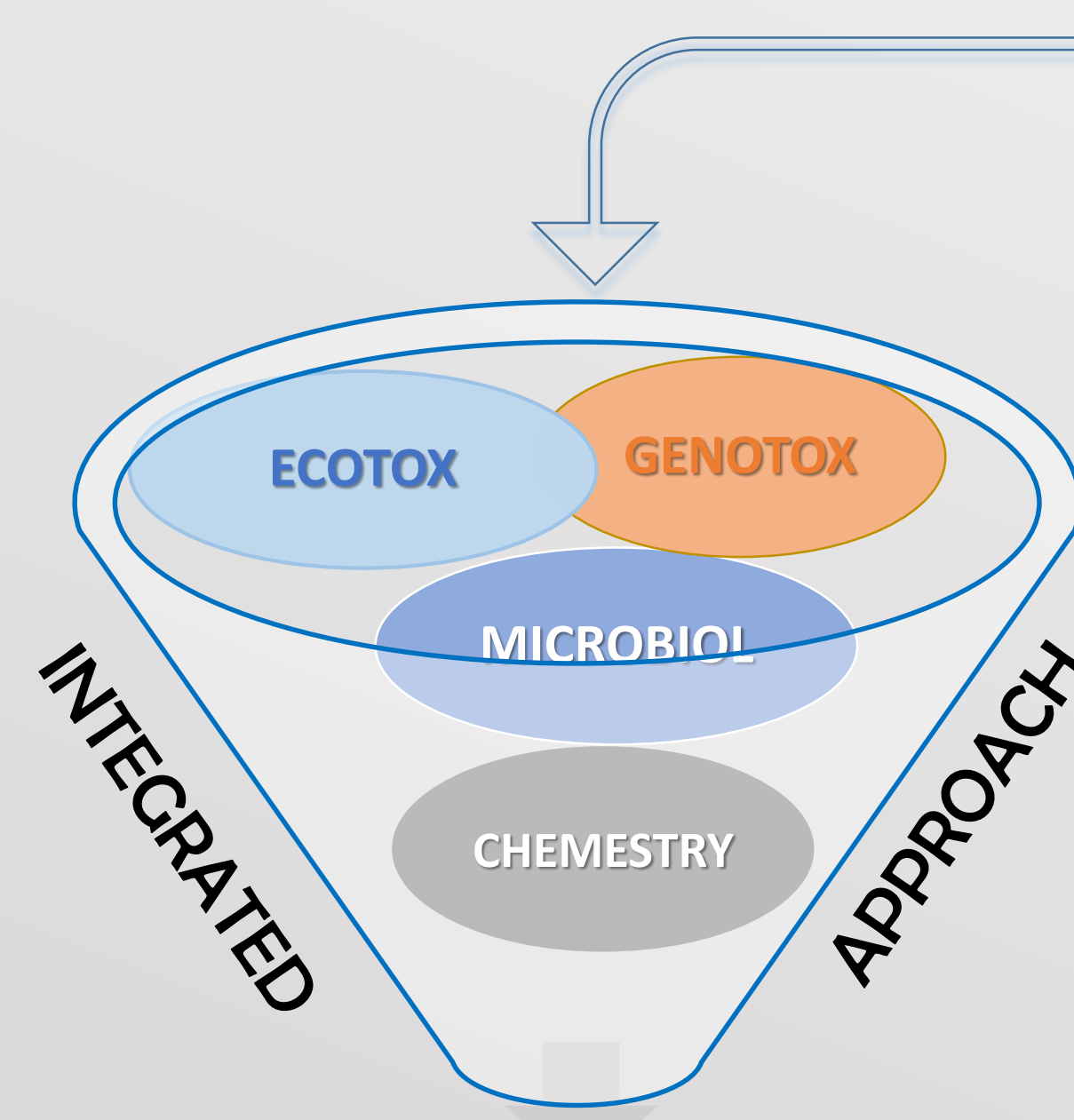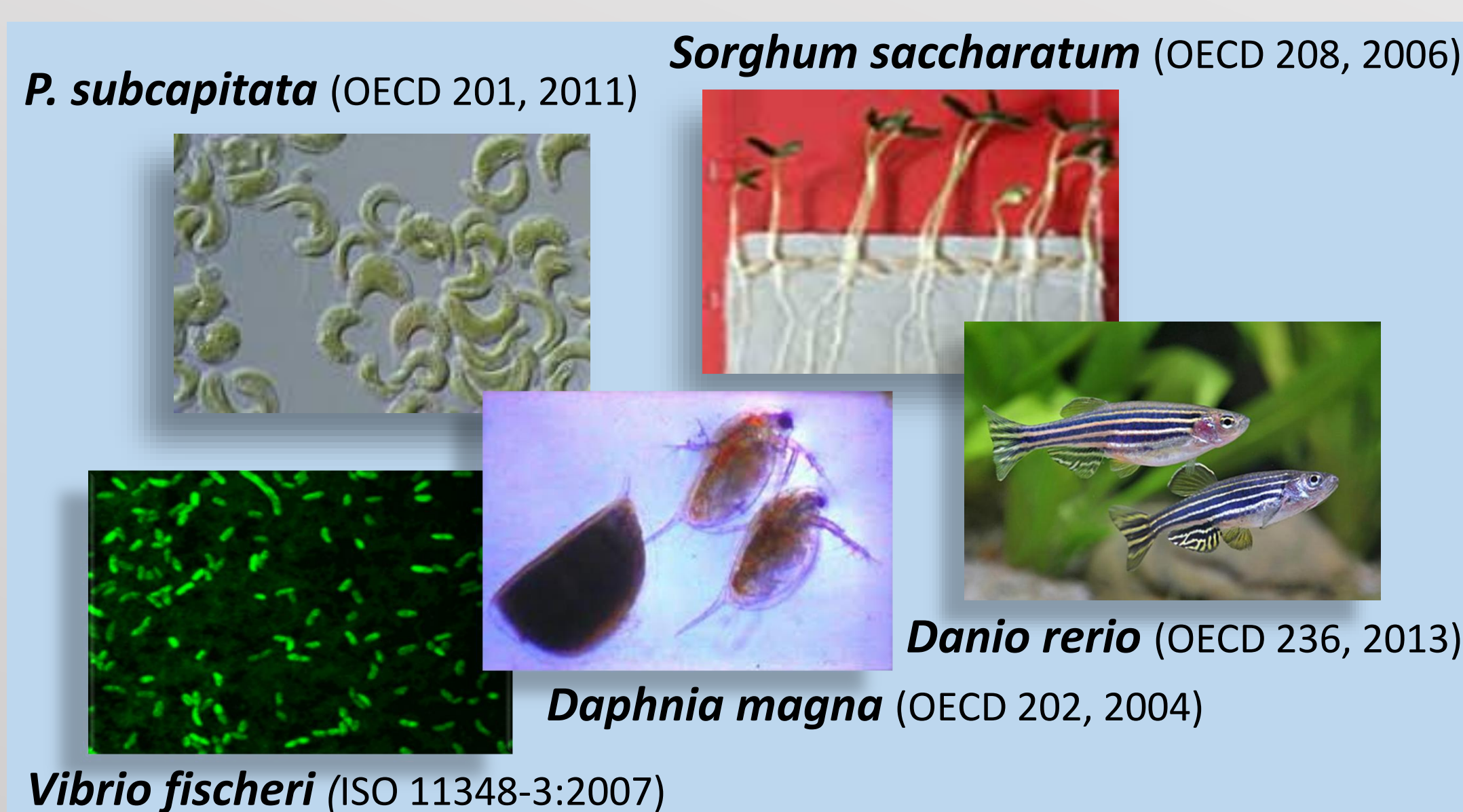

REUSE

MEDICAL DEVICE INDUSTRIES

WASTE WATER → TREATMENT PLANT

P. subcapitata (OECD 201, 2011)
Sorghum saccharatum (OECD 208, 2006)
Danio rerio (OECD 236, 2013)
Daphnia magna (OECD 202, 2004)
Vibrio fischeri (ISO 11348-3:2007)

ECOTOX — GENOTOX — MICROBIOL — CHEMESTRY
INTEGRATED APPROACH

Vicia faba (MN test)
(Rapporti ISTISAN 13/27)

RESULTS AND DISCUSSIONS

MICROBIOLOGICAL PARAMETERS

INDUSTRY	TREATMENT	E. coli CFU/100ml	Enterococci CFU/100ml	Staphylococcus spp. CFU/250ml	CBT 37° C CFU/ml	CBT 22° C CFU/ml
A	Before	5,8x10³	>300x10⁴	>300x10⁴	>300x10⁴	>300x10⁴
A	After	0	17	22	>300x10²	>300x10²
B	Before	35x10⁶	620x10⁶	>300x10⁶	40x10⁶	94x10⁶
B	After	0	10x10³	81x10⁴	60x10³	80x10³
C	Before	70x10⁴	34x10⁴	320x10⁶	5x10⁶	30x10⁶
C	After	0	0	688x10⁴	0	14x10³

All microbiological parameters show a significant decrease in treated water samples and *E. coli* is absent.

ECOTOXICOLOGICAL BIOASSAYS

INDUSTRY	TREATMENT	V. fischeri % light emission inhibition (30')	D. magna % immobilization 48h	S. saccharatum % growth inhibition 72h Shoots Length	S. saccharatum Roots Length	P. subcapitata % growth inhibition 72h	FET Mortality %	FET total sublethal
A	Before	95,26	100	19.6	9,7	<20		
A	After	<0	30	-2.6	-10.4	<20		
B	Before	100	100	28.6	50,8	<20		
B	After	25,37	25	29.4	19,1	<20		
C	Before	18,07	55	21,5	-10,3	21	5%	53%
C	After	<0	40	63,1	47,3	42	5%	16%

The ecotoxicological assays highlight a significant toxicity of the wastewater before the treatment while an evident decrease has been recorded after it; sublethal effects for *Danio rerio* embryos are also recorded in sample C.
(in red sample with significant toxicity)

MICRONUCLEUS TEST (*Vicia faba*)

INDUSTRY	TREATMENT	MN frequency 4h+20h	4h+68h	24h	72h
A	Before	0,4 (±0,2)	1,2 (±0,5)	0,6 (±0,3)	0,3 (±0,2)
A	After	0,2 (±0,1)	0,5 (±0,3)	0,4 (±0,2)	0,3 (±0,2)
B	Before	0,4 (±0,2)	2* (±0,9)	2,7** (±1,5)	n.d.
B	After	4,7** (±2.0)	2** (±0,7)	1,9** (±0,5)	2,1** (±0,8)
C	Before	3*** (±0,4)	0,7 (±0,4)	2,2** (±0,8)	9,2*** (±3,7)
C	After	1* (±0,3)	2,7** (±0,8)	3,3*** (±0,9)	2,2** (±0,9)
Negative control	Tap water	0,1 (±0,1)			
Positive control	Maleic Hydrazide	102,3 (±11,7)			

MN test shows a mixed situation at different exposure times. An highly significant of MN frequencies have been reported for sample B before and after treatment, sample C shows a genotoxicity effect especially after.
(* significant, ** very significant, *** highly significant)

CONCLUSIONS

This study confirms that the Ecotoxicological bioassays are a valid tool to provide useful informations complementary to the chemical analysis especially for the detection of mixtures and unknown substances and underlines the importance of the selection of a suitable eco-genotoxicological battery in order to detect the possible effects of different wastewater treatment.

In conclusion, the results of this study suggest the need to add eco-genotoxicological tests in the legislative parameters in order to have more appropriate evaluation of the treated wastewater. This approach can be considered a useful and promising tool in the reuse management of industrial wastewater and an initial support to the policy in this field.