Toxicity of oxidation intermediates during water/wastewater treatment

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Outline

- ✓ Fundamentals of ecotoxicology
- ✓ Common standardized or alternative toxicity testing methods
- ✓ Examples
  - ✓ Toxicity of ozonated textile wastewater
  - ✓ Photocatalytic oxidation of pharmaceuticals and urban wastewater spiked with pharmaceuticals
  - ✓ Photocatalytic oxidation of surface waters
- ✓ Conclusion

Mathieu Orfila

- **Toxicology** (from the Greek words *ôïîéêüò* - *toxicos* "poisonous" and *logos*) is a branch of biology, chemistry, and medicine concerned with the study of the adverse effects of chemicals on living organisms.

- It is the study of symptoms, mechanisms, treatments and detection of poisoning, especially the poisoning of people.

From wikipedia

Toxicity of metabolites to human

- Many substances regarded as poisons are toxic only indirectly. An example is "wood alcohol," or methanol, which is chemically converted to formaldehyde and formic acid in the liver.

- It is the formaldehyde and formic acid that cause the toxic effects of methanol exposure.

- As for drugs, many small molecules are made toxic in the liver, a good example being acetaminophen (paracetamol), especially in the presence of chronic alcohol use.

- The genetic variability of certain liver enzymes makes the toxicity of many compounds differ between one individual and the next. Because demands placed on one liver enzyme can induce activity in another, many molecules become toxic only in combination with others.

Ecotoxicology

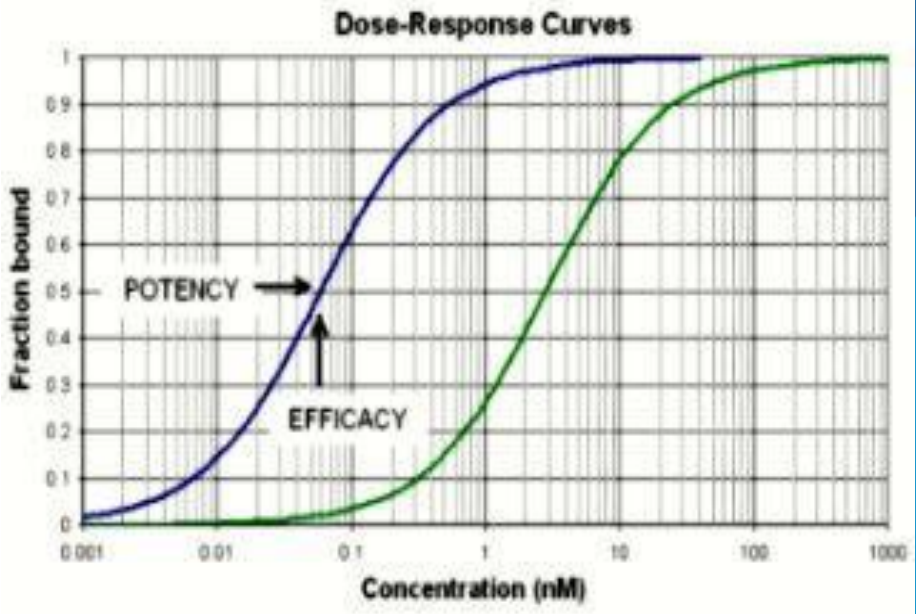
- Ecotoxicology is the study of how chemicals affect the environment and the organisms living in it.

- The goal of ecotoxicity is to understand the concentration of chemicals at which organisms in the environment will be affected. This concentration should be avoided in order to protect the environment.

Dose-Response curve

- The **dose–response relationship**, or **exposure–response relationship**, describes the change in effect on an organism caused by differing levels of exposure (or doses) to a stressor (usually a chemical) after a certain exposure time.[1]
- This may apply to individuals (e.g.: a small amount has no significant effect, a large amount is fatal), or to populations (e.g.: how many people or organisms are affected at different levels of exposure).
- Dose–response relationships generally depend on the exposure time and exposure route (e.g., inhalation, dietary intake); quantifying the response after a different exposure time or for a different route leads to a different relationship and possibly different conclusions on the effects of the stressor under consideration.

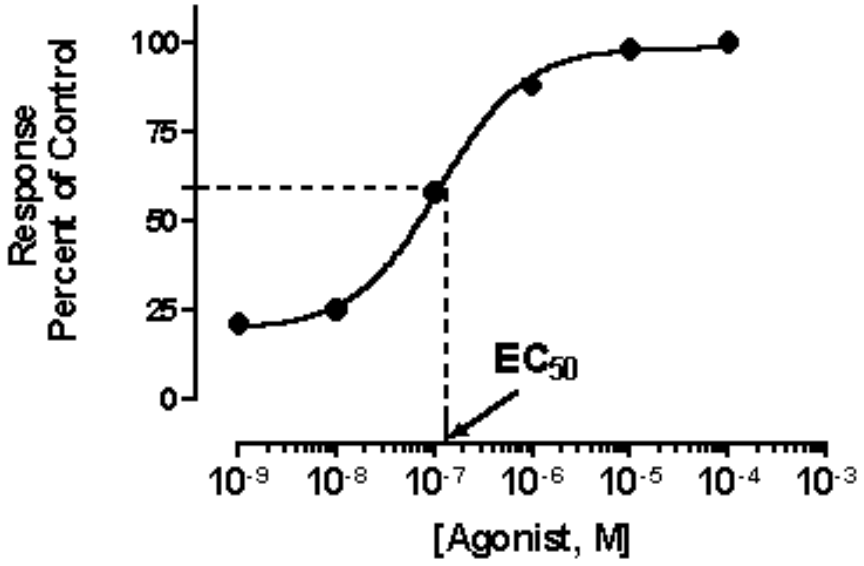
- This limitation is caused by the complexity of biological systems and the often unknown biological processes operating between the external exposure and the adverse cellular or tissue response.



From Wikipedia, the free encyclopedia

Dose-Response curve

- A commonly used dose-response curve is the EC_{50} curve, the **half maximal effective concentration**, where the EC_{50} point is defined as the inflection point of the curve.
- Statistical analysis of dose-response curves may be performed by regression methods such as the probit model or logit model, or other methods such as the Spearman-Karber method.[4] Empirical models based on nonlinear regression are usually preferred over the use of some transformation of the data that linearizes the dose-response relationship.[5]
- Dose–response curves can be fit to the Hill equation to determine cooperativity.



From Wikipedia, the free encyclopedia

Dose-Response curve

No Observed Effect Concentration (NOEC) or The **no observed adverse effect level** (**NOAEL**) denotes the level of exposure of an organism, found by experiment or observation, at which there is no biologically or statistically significant (e.g. alteration of morphology, functional capacity, growth, development or life span) increase in the frequency or severity of any adverse effects in the exposed population when compared to its appropriate control.

The lowest observed effect concentration (LOEC) or the **lowest-observed-adverse-effect level** (**LOAEL**) is the lowest concentration or amount of a substance found by experiment or observation that causes an adverse alteration of morphology, function, capacity, growth, development, or lifespan of a target organism distinguished from normal organisms of the same species under defined conditions of exposure. Federal agencies use set approval standards below this level.

The maximum acceptable toxicant concentration (MATC)

Chronic toxicity tests

- In a toxicity test, the NOEC and LOEC are derived as a comparison from the negative control, or the experimental group that does not contain the chemical in question. The NOEC is the highest concentration that does not cause a statistically different effect than the negative control through statistical hypothesis testing. Likewise, the LOEC is the lowest concentration tested that does cause a statistically different effect than the negative control. The MATC is the geometric mean between these two values, such that: MATC=√(NOEC)(LOEC)
- The MATC is calculated to protect against chronic effects on overall function or health of an organism, not death. A partial life cycle test must be used. This type of toxicity test uses organisms in their most sensitive life stages, usually during times of early reproduction and growth, but not juveniles.[3] The MATC is the highest concentration that should not cause chronic effects, however, for regulatory purposes, a maximum concentration to protect against acute effects must exist as well.

The maximum acceptable toxicant concentration (MATC)

Applying MATC to acutely toxic concentrations

- The MATC can be applied to the results of an acute toxicity test to obtain a concentration that would protect against adverse effects during an acute exposure. An LC_{50}, or the concentration at which 50% of the organisms die during an acute toxicity test is used to derive a value called the acute to chronic ratio (ACR).
- The MATC can be used to calculate the ACR as follows: ACR={LC_{50} \over MATC}
- The ACR is useful for estimating an MATC for species in which only acute toxicity data exists, or for setting regulatory guidelines for the protection of aquatic life through water quality criteria by the US EPA.[3]

Considerations on Acute/Chronic tests?

- Very low concentrations that escape the treatment process one cannot rule out the potential for ***chronic impacts*** or impacts that may develop from the degradation products or ignore the ecological implications that may be caused by mixtures of compounds in nature.

- There are also cases where the method detection limit for a compound (in this case ethinylestradiol) is somewhat higher than the NOEC (No Observable Effect Concentration), and therefore possible ***acute effects*** cannot be excluded.

Bioavailability

- The heteroatom content and multifunctional composition of pharmaceuticals makes them, among other things, polar, ionizable molecules, and influenced by solution pH.
- Pharmaceuticals are multifunctional organic compounds that are ionized in the aquatic environment at environmentally relevant pH levels.
- The degree of ionization of the drug substance at a particular pH will affect its availability to biological organisms, its chemical and physical activity, and its ultimate environmental fate.
- Ionic charge will also affect the potential of a molecule to participate in environmental ion exchange processes. Knowledge of the pK_a can assist experimentalists in their design of appropriate sorption and ecotoxicity studies and in accurately interpreting the results from these studies.

Common standardized or alternative toxicity testing methods

• *Vibrio fisheri*

✓**The marine bacterium *V. fischeri* exists naturally either in a free-living planktonic state or as a symbiont of certain luminescent fish or squid.**

✓ **The basic technology of the Microtox Test System is based upon the use of luminescent bacteria, specifically the strain *Vibrio fischeri* NRRL B-11177, to measure toxicity from environmental samples.**

✓**When properly grown, luminescent bacteria produce light as a by-product of their cellular respiration.**

✓**Cellular activity (toxicity) results in a decreased rate of respiration and a corresponding decrease in the rate of luminescence. The more toxic the sample, the greater the percent light loss from the test suspension of luminescent bacteria.**

✓**The Microtox® system is a screening tool used for a variety of toxicity testing applications. The advantages of this toxicity bioassay are**

its speed, simplicity, relatively low cost, when compared to the cost of chemical analysis.

✓**The Microtox® procedure can be used for testing either water (marine or fresh) or associated sediments.**
The Microtox® assay uses freeze dried luminescent bacteria (Photobacterium phosphoreum) as the test organisms.
✓**Results can be obtained within 5 to 30 minutes.**

$$I\% = \frac{C - S}{C} \times 100$$

Acute toxicity with *Vibrio fischeri*

Reactivation of bacteria (30 minutes)

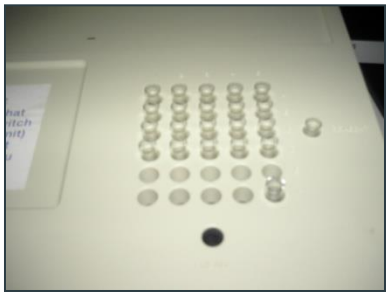
Control test

Bacteria exposed to samples

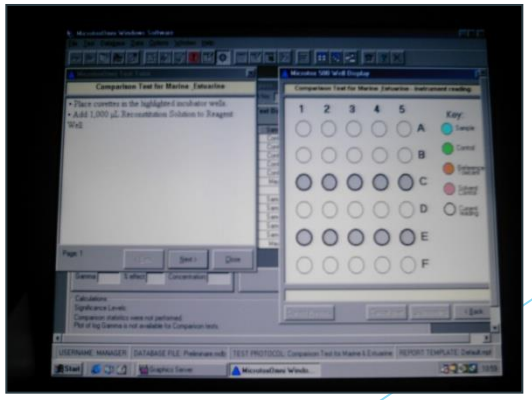
I

II

III



- 15 and/or 30 minutes
- 15° C



% BIOLUMINESCENCE INHIBITION

IRSA-CNR: 8060:1994

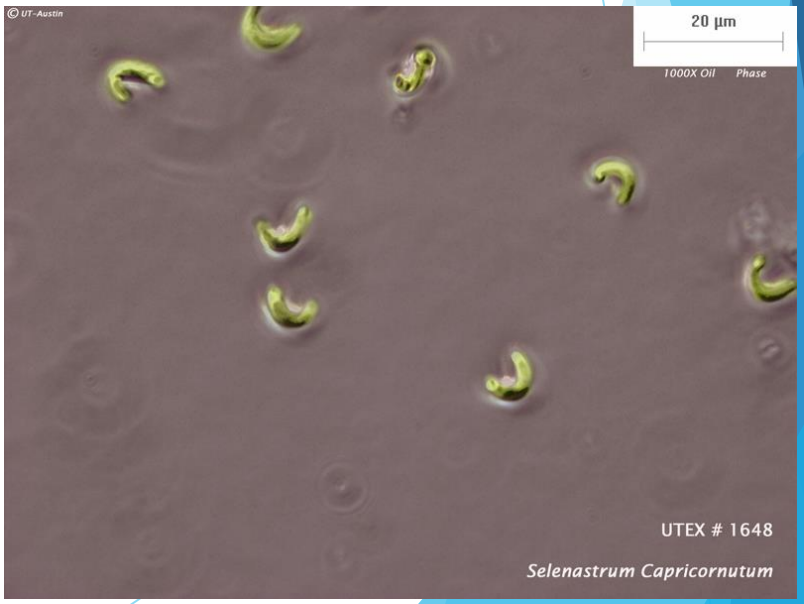
- ***Unicellular green algae cell groth inhibition test Pseudokirchneriella subcapitata***

(Selenastrum capricornutum)

- Spectrophotometric measurements
- Cell counts

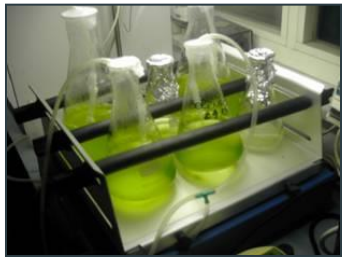
$$I\% = \frac{C - S}{C} \times 100$$

Fig. 1 – Piastre per colture cellulari; test standard con dicromato di potassio a 72 ore.



Cell growth inhibition test with *Pseudokirchneriella subcapitata*

Sospensione algale con densità
300 x 10^3 cells/mL

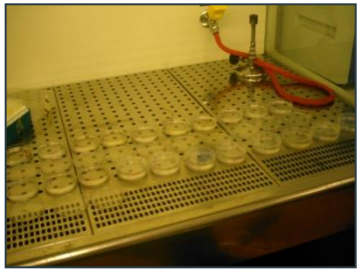


Control

I

II

III



V tot = 10 mL

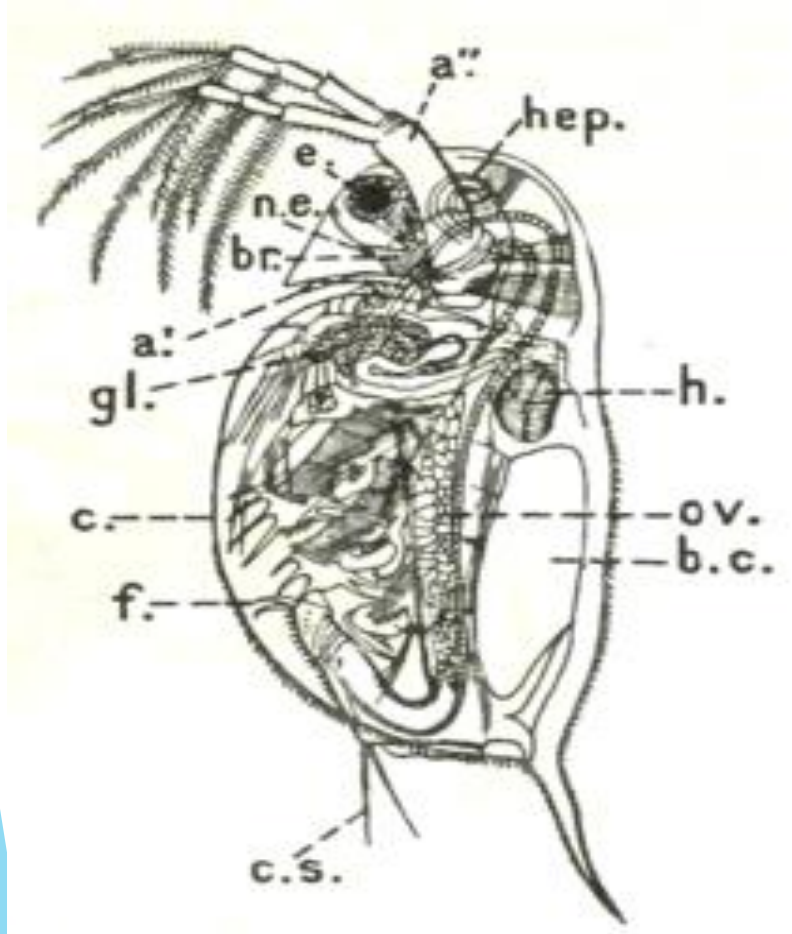
Incubation

- Temperatura 24°C
- Illuminazione continua di circa 8000 lux al piano di lavoro

Dopo un intervallo di incubazione di 96 h

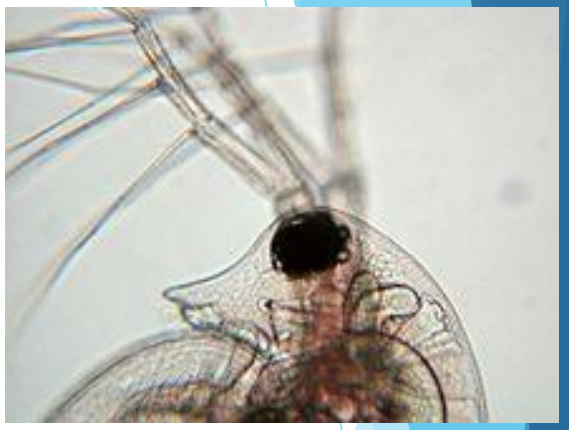
% cell growth inhibition

Appearance and characteristics of *Daphnia magna*



Daphnia, female. a', antennule; a'', antenna; *b.c*, brood-chamber; *br*, brain; *c*, margin of carapace; *c.s*, caudal setae; *e*, compound eyes coalesced into one; *f*, furca; *gl*, maxillary gland; *h*, heart; *hep*, hepatic diverticulum of gut; *n.e*, nauplius eye; *ov*, ovary. (After Claus and Grobben.)

Anatomy of *Daphnia*



Daphnia abdomen

Artemia salina

- The cysts are activated in a standard marine solution (35‰ salinity, Ocean ®) during 48 h .
- *Than new born (<24 h aged) of A. salina* nauplii (<48-h old) are exposed to the samples for 24 h and 48 h using 2 mL of samples.
- Experiments are run 4 replicates using 5 nauplii in each test cell.
- Nauplii are exposed to the samples in dark during test period.
- Both negative control with Ocean ® and positive control with $K_2Cr_0O_7$ tests are performed in parallel. Test results are acceptable if immobilization percentiles are less than 10.

ACUTE TOXICITY TESTING
Daphnia magna, Artemia salina

ISO 6341/2010 – APAT 8040/2003

Feeding media

Selection of new born daphnids (<24 h)

Four replicate with 5 daphnids in each one

R1 R2 R3 R4

Blank

Incubation at room temperature (20-21°C) in dark conditions for 24-48h

Observation of immobilized daphnids after 24, 48 h exposure time

END-POINT

Reporting of immobilization %

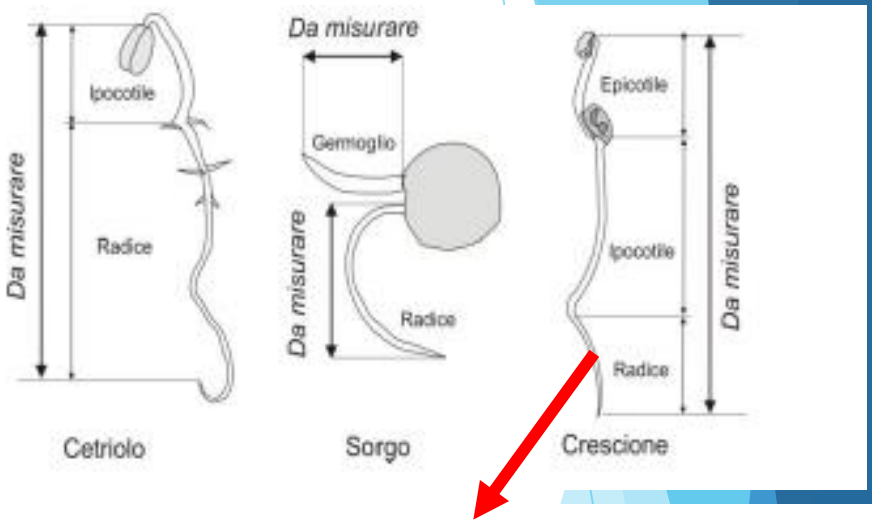
% Immobilization: (number of tested organisms- number of immobilized organisms) *100 / number of tested organisms

Lepidium sativium

The *L. sativum* seeds are germinated in disposable Petri dishes, (100mm in diameter), on Whatmann filter paper moistened with 5mL
of either double-distilled (dd) water (control) .

Tests are run in triplicate, using 10 seeds per dish. Petri dishes are kept in the dark, at 25 ◦C, for 72 h.

The length of the whole plantlet and of the roots are measured with a ruler (against a black background).



End points: Inhibition of Germination and Rooth lenght

Index of inhibition versus blank (IG %)

%IG = (G1*L1)*100/(Gc*Lc)

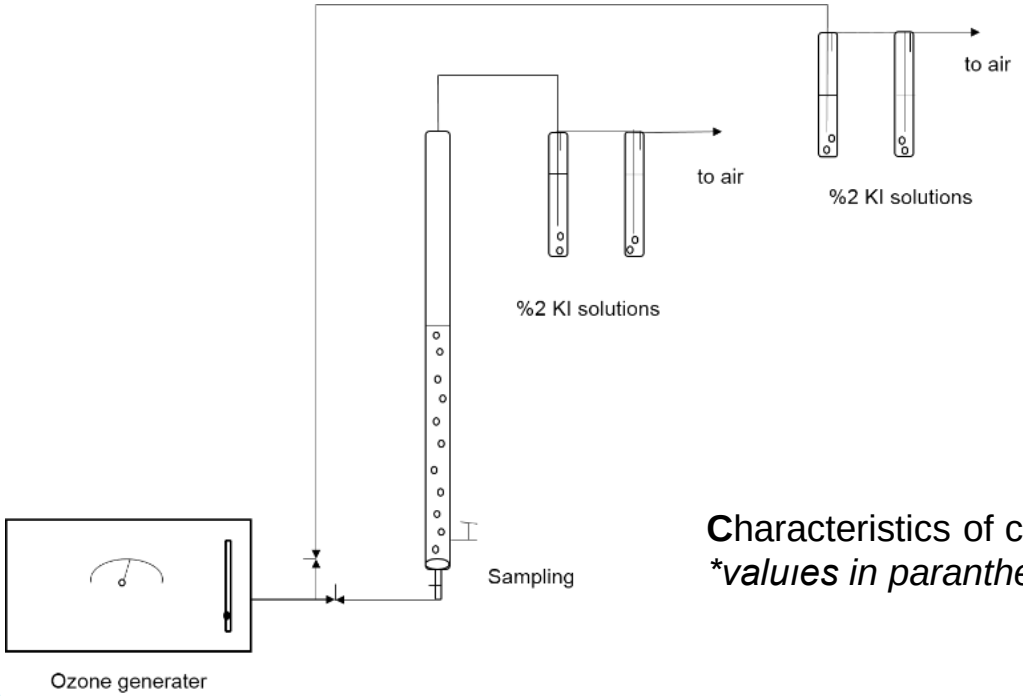
where G1: germinated seed number exposed to sample, and Gc: germinated seed number exposed to negative control medium, L1: length of roots exposed to sample and Lc: length of root exposed to negative control medium.

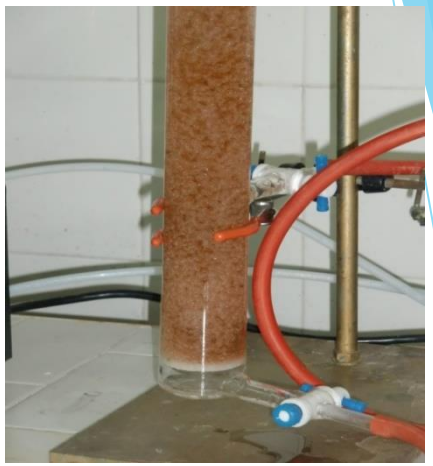
Methods recommended to test pharmaceuticals	Recommended Protocol
Adsorption - Desorption Using a Batch Equilibrium Method	OECD 106/ OECD 121/OPPTS, 835.1110*
Ready Biodegradability Test	OECD 301
Aerobic and Anaerobic Transformation in Aquatic Sediment Systems	OECD 308
Algae, Growth Inhibition Test	OECD 201
Daphnia sp. Reproduction Test	OECD 211
Fish, Early Life Stage Toxicity Test	OECD 210
Activated Sludge, Respiration Inhibition Test	OECD 209

* One study is generally sufficient

Examples

Ozonation of textile wastewater

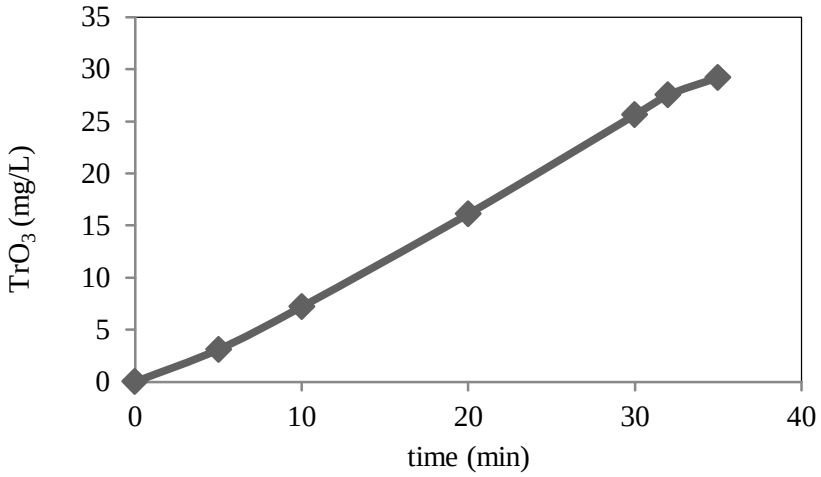


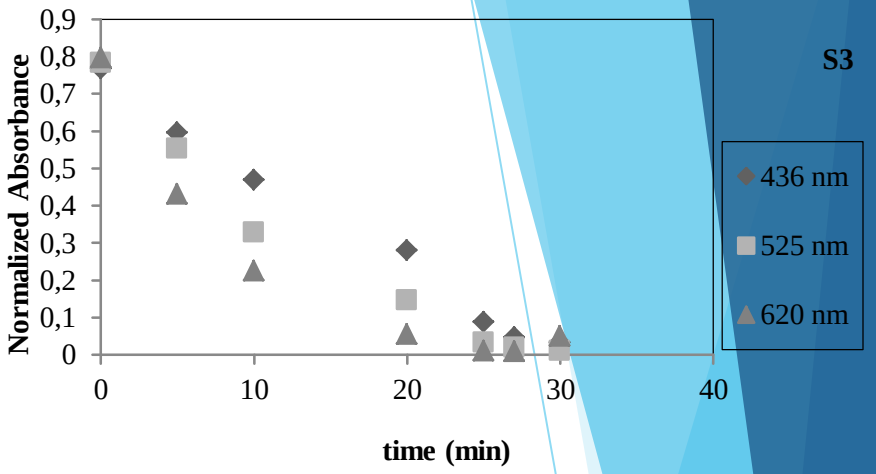


Characteristics of collected textile industry wastewater samples
**valuıes in paranthesis indicate German Wastewater Discharge Limits.*

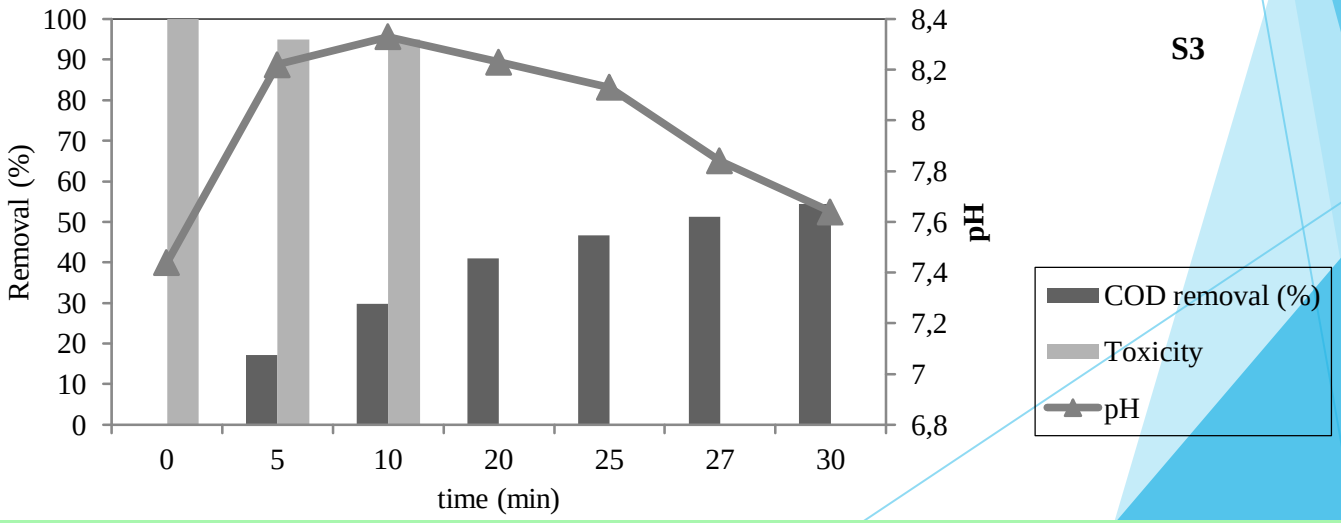
Origin of the wastewater	Prior ozonation	Post ozonation	Ozone dose applied (mg/L.min)
Biological treated cotton-polyster dyeing textile finishing wastewater	S30	S3	10, 8

Parameter	Birim	S30
pH	--	7,44
KOİ	[mg/L]	205,9
NH_4-N^+	[mg/L]	1,96
TKN	[mg/L]	8,12
AKM	[mg/L]	65
T-P	[mg/L]	8,75
UV absorbans	436 (0,07)*	0,655
	525 (0,05)*	0,695
	620 (0,03)*	0,56





TrO_3 (mg/L) = input O_3–O_3 in off gas - residual O_3 in the reactor

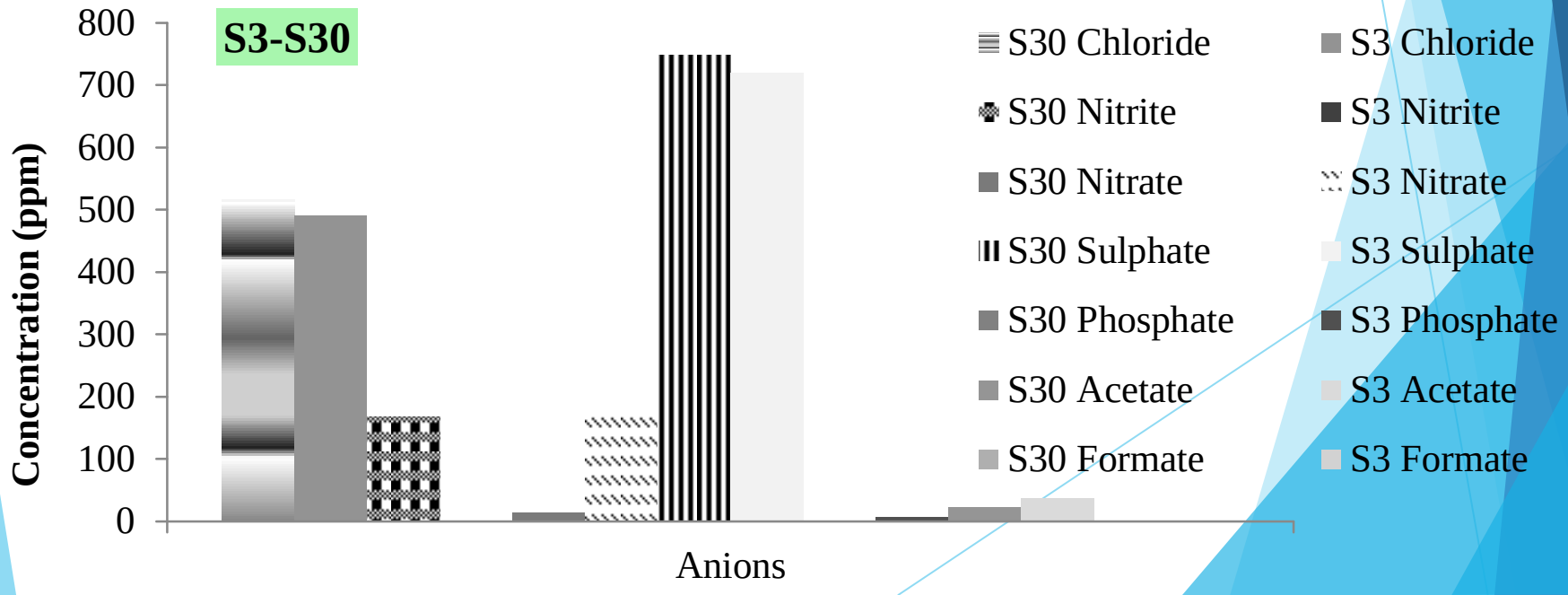


NKU Scientific Research Project Office funded porject research (NKUBAP:00.17.12.02)

GC-MS scanning results in the sample prior and after ozonation (Hexzan:methylene chloride (1:1) extraction)

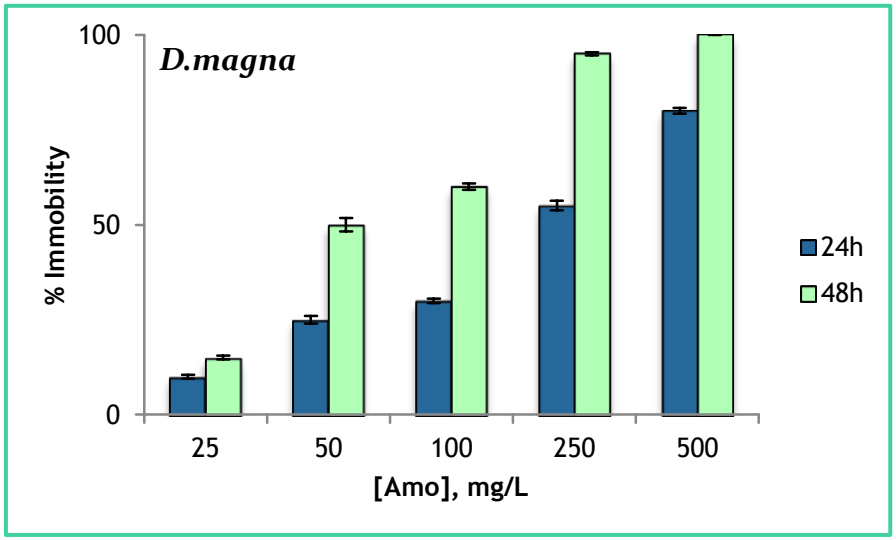
Sample	Compounds
S30	2,2,4,6,6-pentamethyl-Heptane, 2-methyl-Propanoic acid
S3	2,2,4,6,6-pentamethyl-Heptane, 2,2,3,4-tetramethyl-Pentane, 2-methyl-Propanoic acid, Tetradecanamide, (Z)-9-Octadecanamide

Ion chromatography results of the prior and post ozonation

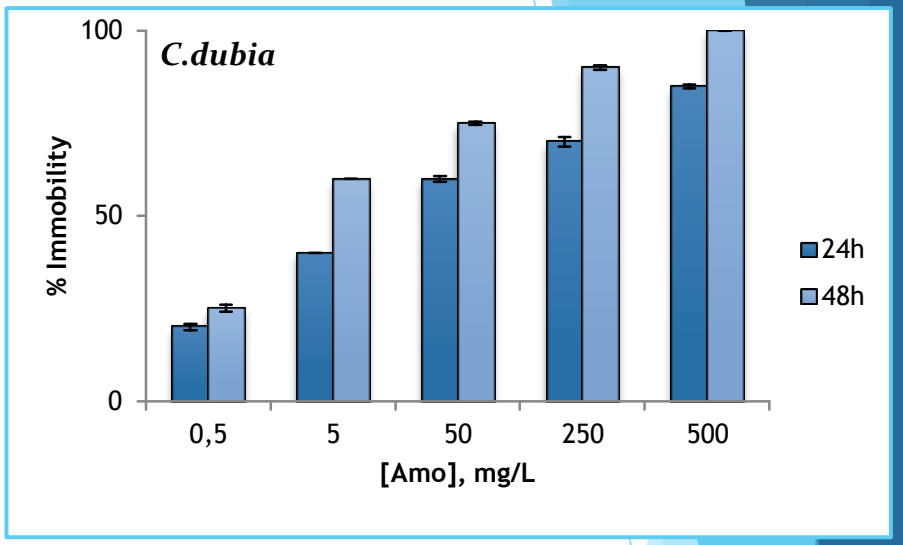


Photocatalytic treatment of pharmaceuticals

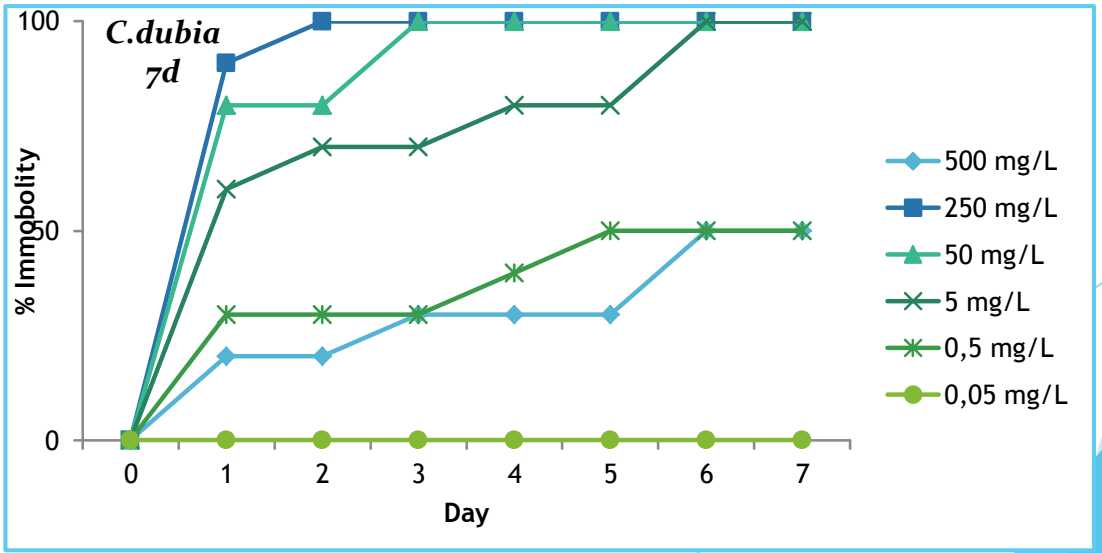
Toxicity of Amoxicillin to *Daphnia magna* and *Ceriodaphnia dubia* prior photocatalysis



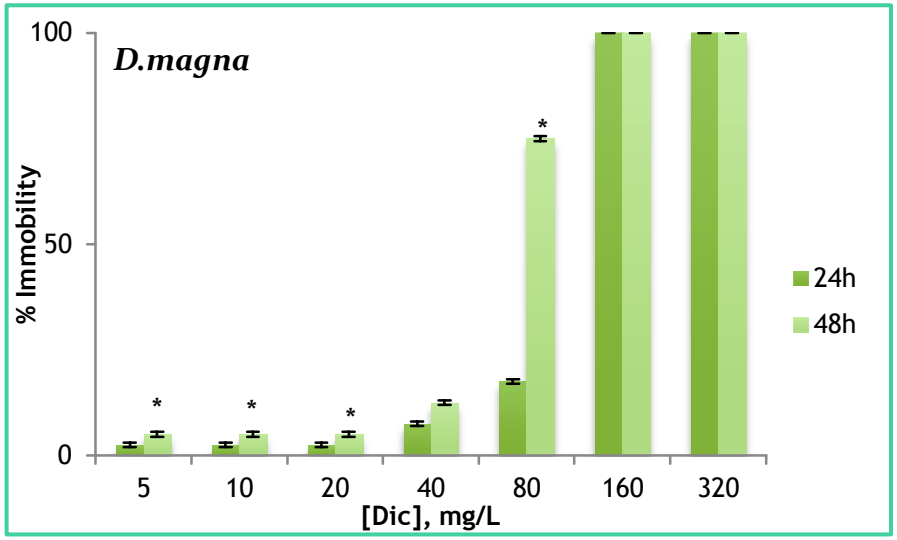
Results are expressed as mean value ± SD of four replies.



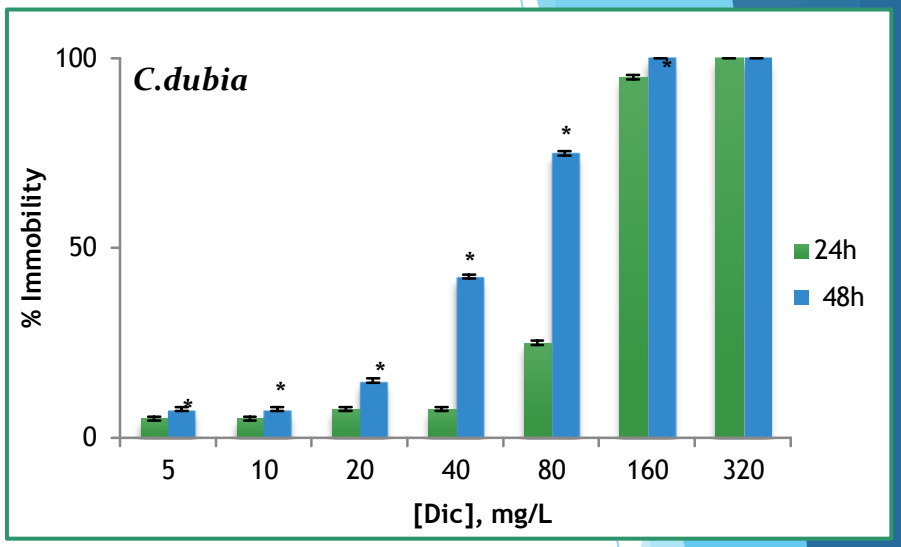
Results are expressed as mean value ± SD of four replies.



Toxicity of Diclofenac-Na to *Daphnia magna* and *Ceriodaphnia dubia prior photocatalysis*



Results are expressed as mean value ± SD of four replies.
*: indicates significant differences vs. 24h. (t-test, $p<0.05$).



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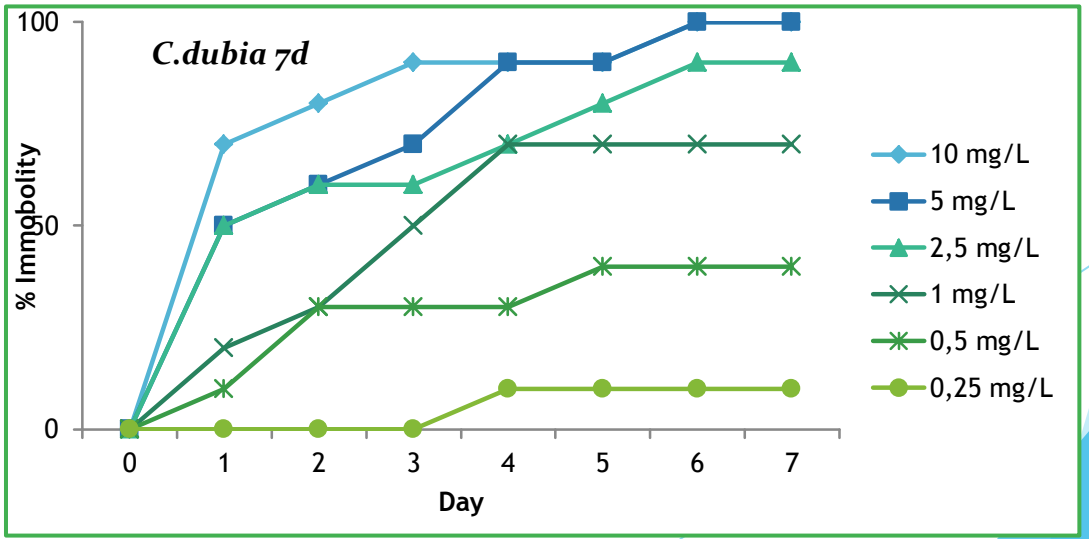
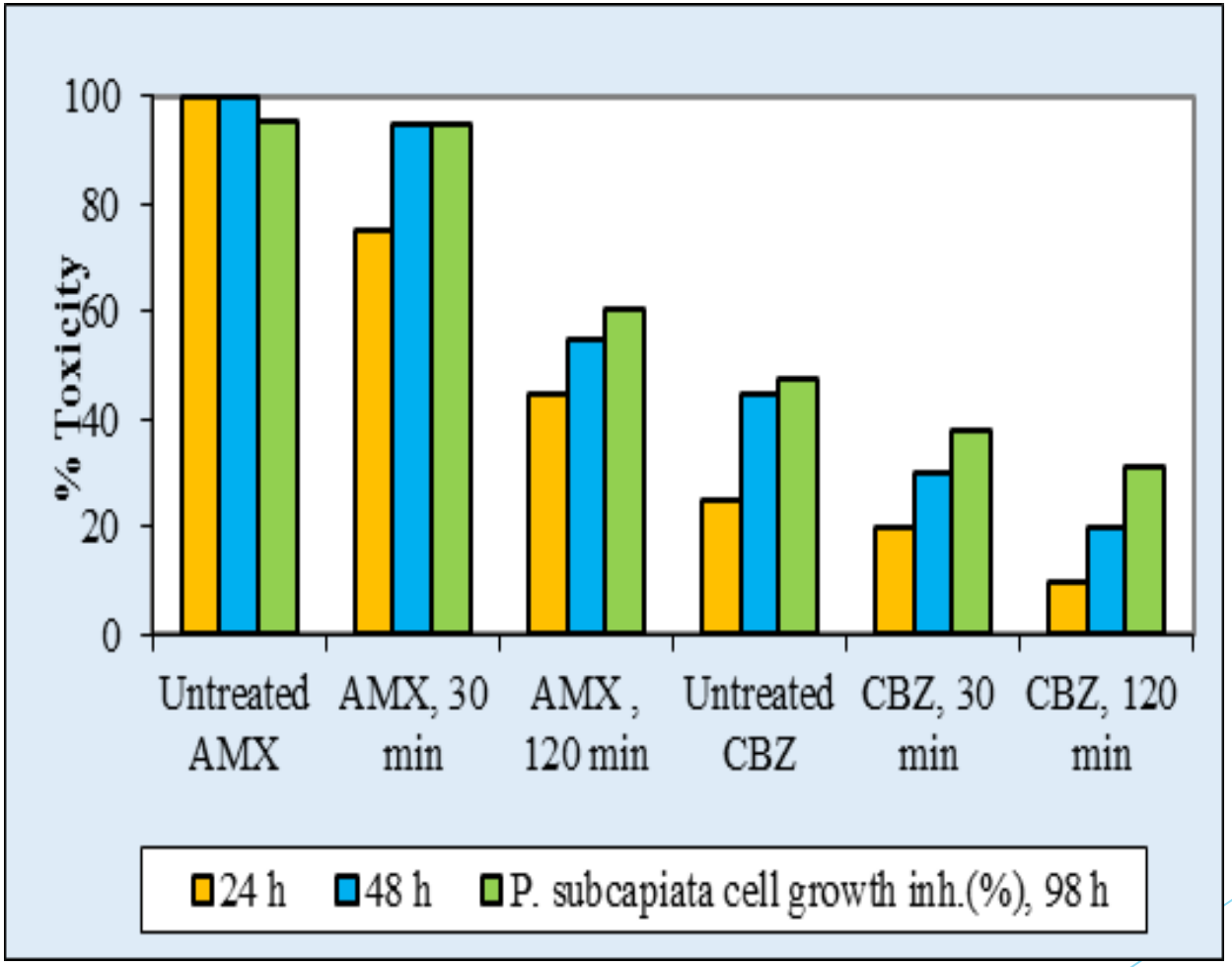
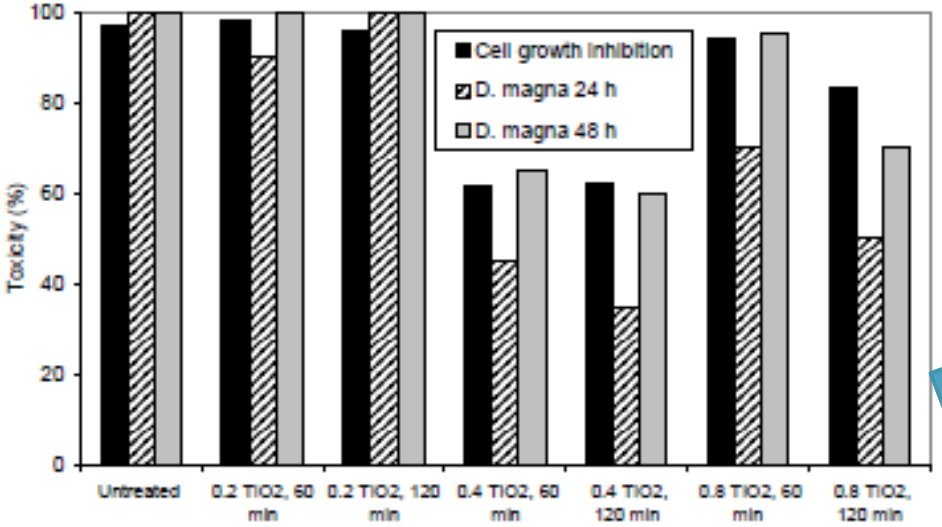


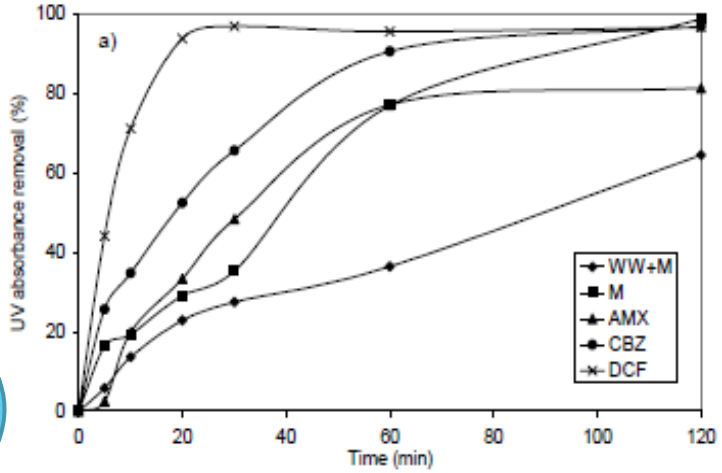
Changes in toxicity to *D. magna* and *P. subcapitata in* photocatalytıc treated 10 mg L^{-1} Amoxıcıllın (AMX) and 5 mg L^{-1} and Carbamapezın (CBZ) solutıons using 0.8 g TiO2 L^{-1}

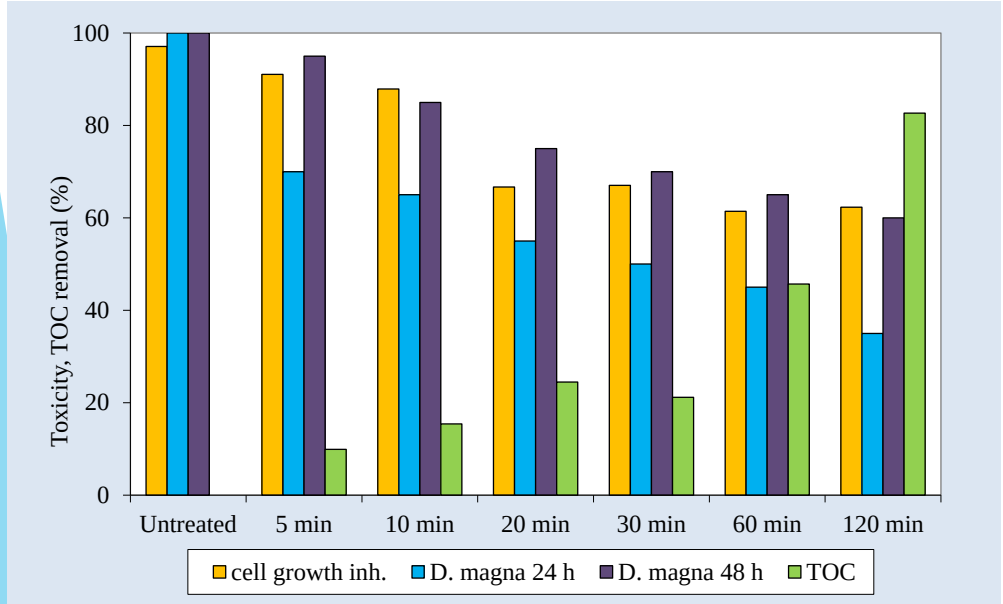


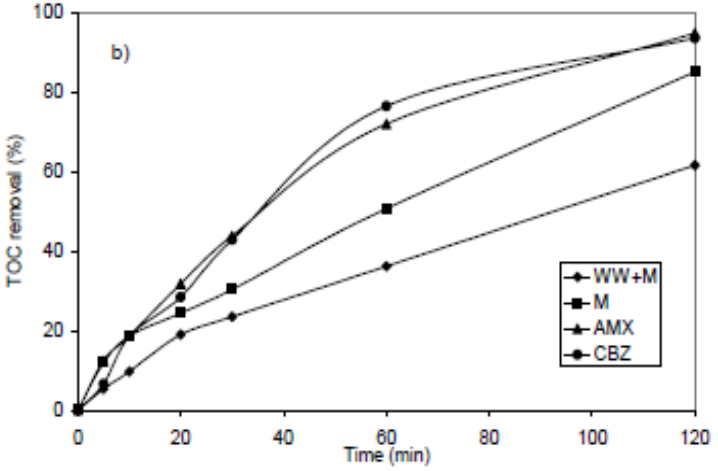
Rizzo et al. (2009). *Heterogenous photocatalytic degradation kinetics and detoxification of an urban wastewater treatment plant effluent contaminated with pharmaceuticals.* Water Research, 43, 4070–4078.)

Changes in TOC and toxicity to *D. magna* and *P. subscapitata* exposed to the mixture (M) of 10 mg L^{-1} AMX, 5 mg L^{-1}, CBZ and 2,5 mg L^{-1} Diclofenac spiked to urban wastewater (UWW) during photocatalytıc treatment using 0.4 g TıO2 L^{-1}



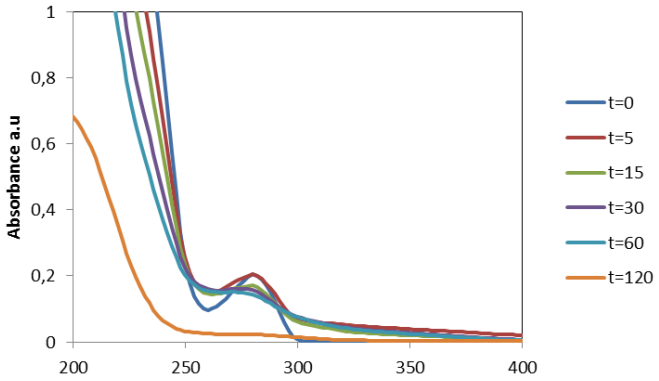




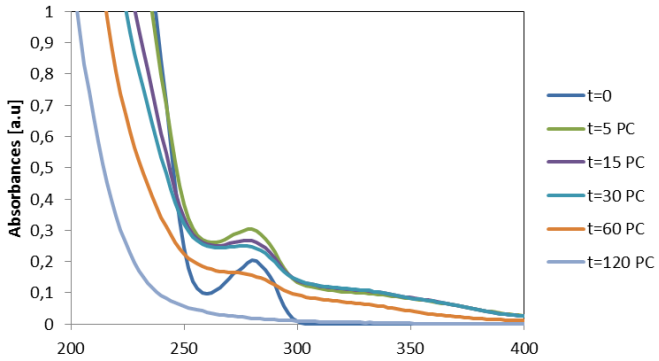


Vancomycın B Removal

05 gTıO2/L, 60min



05 gZnO/L, 60min

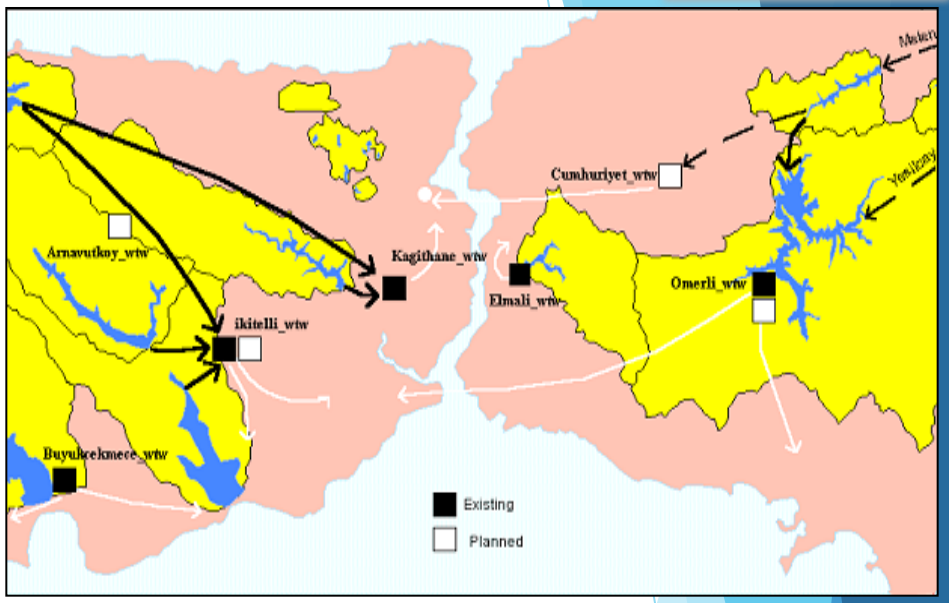


VAN 20 mg.L studies	% Immobility (24 h)		% Immobility (48 h)	
$TiO_{2\ loading}$ ($g.L^{-1}$)	0,1	0,5	0,1	0,,5
5 min	30	25	35	30
15 min	35	30	45	35
30 min	65	40	80	50
60 min	70	50	95	65
120min	80	55	100	75
VAN 50 $mg.L^{-1}$	0.5 $g.L^{-1}$ TiO_2	0.5 $g.L^{-1}$ ZnO	0.5 $g.L^{-1}$ TiO_2	0.5 $g.L^{-1}$ ZnO
5 min	35	100	50	100
15 min	35	100	60	100
30 min	55	100	75	100
60 min	65	100	85	100
120min	80	100	90	100

NATO GRANT (NATO Grant EST.CLG.980506): Evaluation of Alternative Water Treatment Systems For Obtaining Safe Water in Three Countries

. Water characteristics

	Buyukcekmece	Omerli
pH	7.65	**7.18**
Alkalinity, mg $CaCO_3$ L^{-1}	150	**70**
Bromide, µg L^{-1}	274	**95**
Chloride,□ mg L^{-1}	98	**45**
TOC, mg L^{-1}	3.61	**3.05**
UV_{254}, cm^{-1} in raw water	0.100	**0.097**
TTHMs raw (µg L^{-1})	159.4	**128.5**
THAAs raw (µg L^{-1})	89.9	**117.1**
$SUVA_{254}$ (m^{-1} L mg C^{-1})	2.77	**3.18**
Specific TTHMFP (µg mg C^{-1})	44.2	**42.1**
Specific THAAFP (µg mg C^{-1})	**24.9**	**38.4**



Kinetic parameters of photocatalytic degradation of raw water samples (TiO_2:0.1 $mgmL^{-1}$, 30 min).

	UV_{254}		UV_{280}		TOC	
	k, min^{-1},$x10^{-3}$	$t_{1/2}$ min	k, min^{-1},$x10^{-3}$	$t_{1/2}$ min	k min^{-1},$x10^{-3}$	**$t_{1/2}$ min**
Buyukcekmece	7.63	91	9.13	76	4.53	**153**
Omerli	**6.78**	**102**	**8.37**	**83**	**1.75**	**396**

M. BEKBOLET ET AL. (2005) *APPLICATION OF OXIDATIVE REMOVAL OF NOM TO DRINKING WATER AND FORMATION OF DISINFECTION BY-PRODUCTS*. *DESALINATION*, 176, 155-166.

Formation of Disinfection By-products

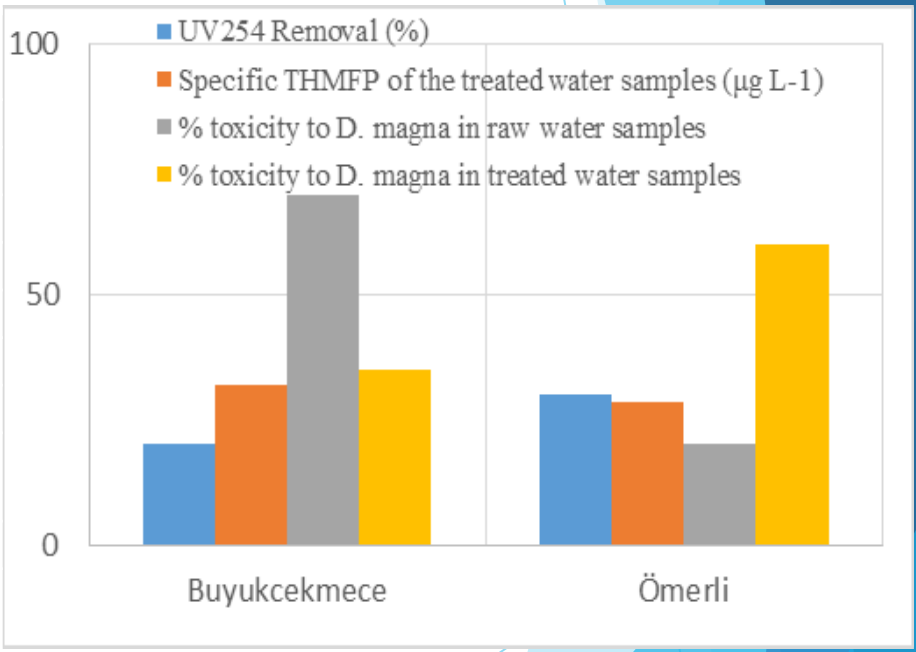
$HOCl + Br^- +$ Natural Organic Matter (NOM) $\rightarrow \rightarrow \rightarrow$ DBPs

Trihalomethanes (THMs) $\rightarrow CHCl_3$, $CHBrCl_2$, $CHBr_2Cl$, $CHBr_3$

Haloacetic acids (HAAs) $\rightarrow$ ClAA, Cl_2AA, Cl_3AA, BrAA, Br_2AA, Br_3AA, BrClAA, $BrCl_2AA$, Br_2ClAA

Other chloro-, bromo- and bromochloro species

DBPs (μgL^{-1})	Büyükçekmece		Ömerli	
	Raw	After treatment	Raw	**After treatment**
Monochloroacetnitrile	2.4	2.1	2.0	**1.5**
Dichloroacetonitrile	3.5	3.7	3.4	**3.6.**
Trichloroacetnitrile	n.d.	n.d.	n.d.	**n.d.**
Chloral hydrate	69.2	24.5	78.1	**14.9**
1,1-dichloropropane	0.6	1.1	0.4	**1.1**
1,3-dichloropropane	n.d.	n.d.	n.d.	**n.d.**
1,1,1-trichloropropane	0.7	0.7	0.7	**0.8**
Monobromoacetonitrile	1.6	0.8	0.6	**0.7**
Dibromoacetonitrile	1.1	1.0	0.9	**0.9.**
Bromochloroacetonitrile	n.d.	3.3	n.d.	**3.5.**
Chloropicrin	**n.d.**	**n.d.**	**n.d.**	**n.d.**



Degradation, toxicity and THM-formation potential (THMFP) evolution in raw and photocatalytic treated surface water samples (30 min oxidation using 0.1 g TiO_2 L^{-1})

Conclusion

- Which organisms and which endpoints are relevant for biological potency testing of the pharmaceuticals and other emerging contaminants as well as their Chemico/photo-transformed compounds **?**
- More studies are needed to optimize treatment conditions and biological effect methologies since the studies in the literature run in different conditions.
- Concentrations of emerging pollutants, etc. vary and often-contradictory results are obtained from the biological assays.

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Thank you very much for your attention.

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