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Development of Regional Joint Master Program in Maritime Environmental Protection and Management
- MEP&M -

Environmental risk assessment and sediment quality guidelines

WP3. Capacity Building through staff training and equipment purchase .
Dev 3.4.2 KNOW-HOW TRANSFER TO TEACHING STAFF RELATED TO THE MEP&M

Miriam Hampel, Department of Physical Chemistry (University of Cádiz)

20.12.2021

Virtual meeting via Google-meet application

This project has been funded with support from the European Commission. This presentation reflects the views only of the author, and the Commission cannot be held responsible for any use which may be made of the information contained therein.

Project no. 619239-EPP-1-2020-1-ME-EPPKA2-CBHE-JP

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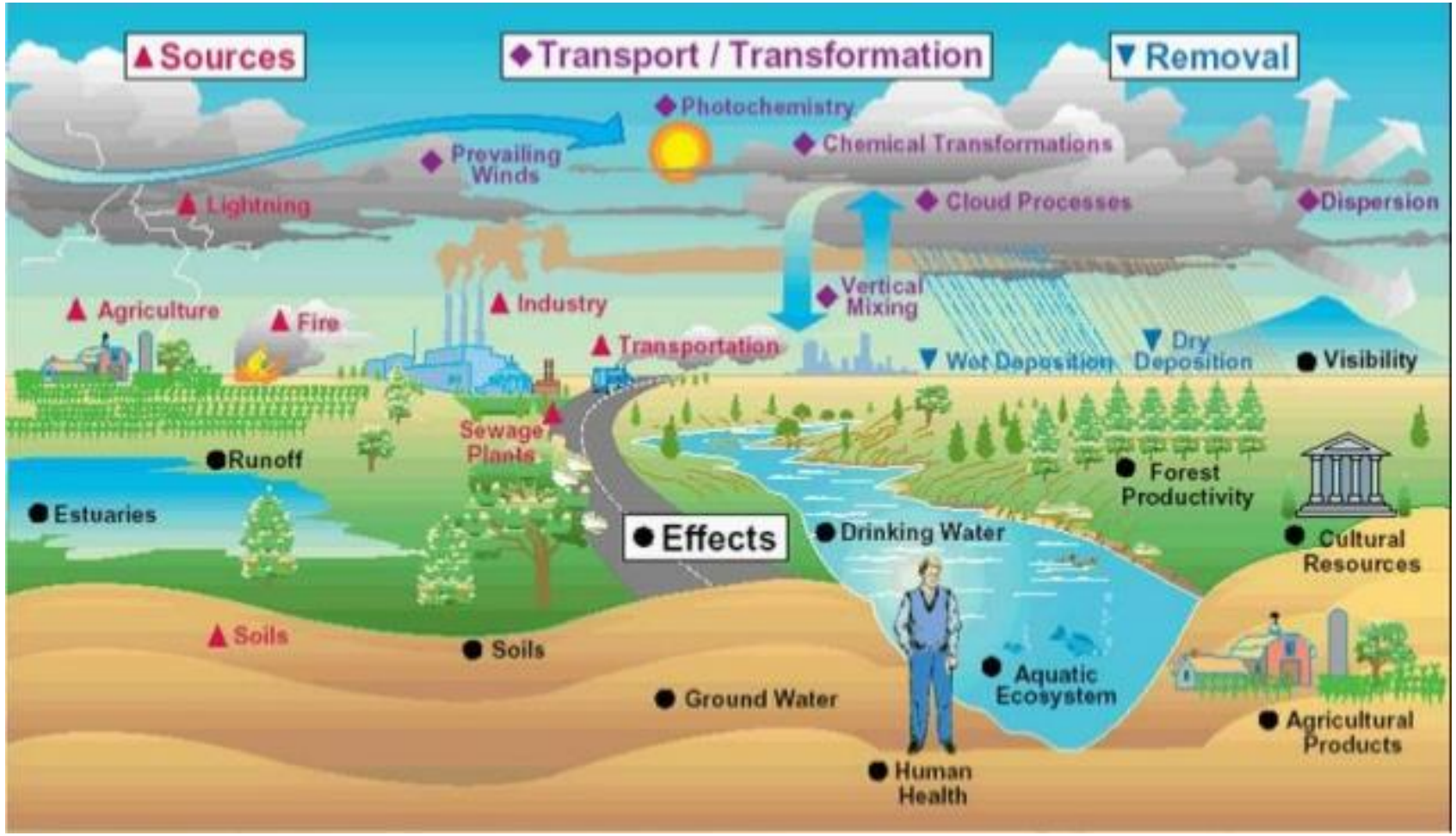
Toxicity tests for environmental risk assessment - ecotoxicology

Aquatic environment: negatively affected by almost all human activities (European Environment Agency, 2015).



Industry, Transport, Agriculture Urbanization

⬇

Nutrients
Metals
Minerals,
Oil
Synth. chemicals.

Presence of synthetical compunds/chemicals

The CAS number is a unique number applied to a specific chemical by the Chemical Abstracts Service (CAS).

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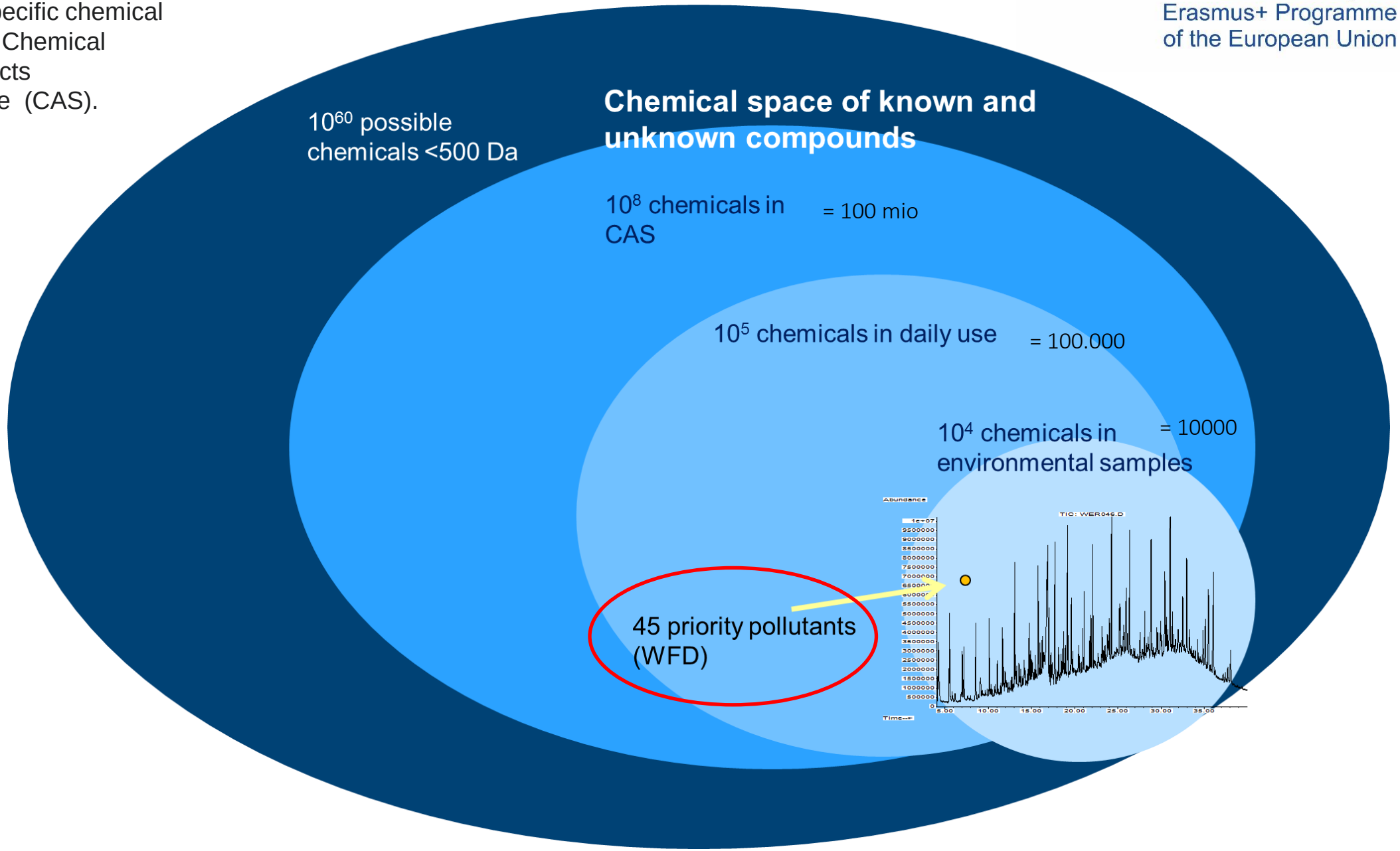

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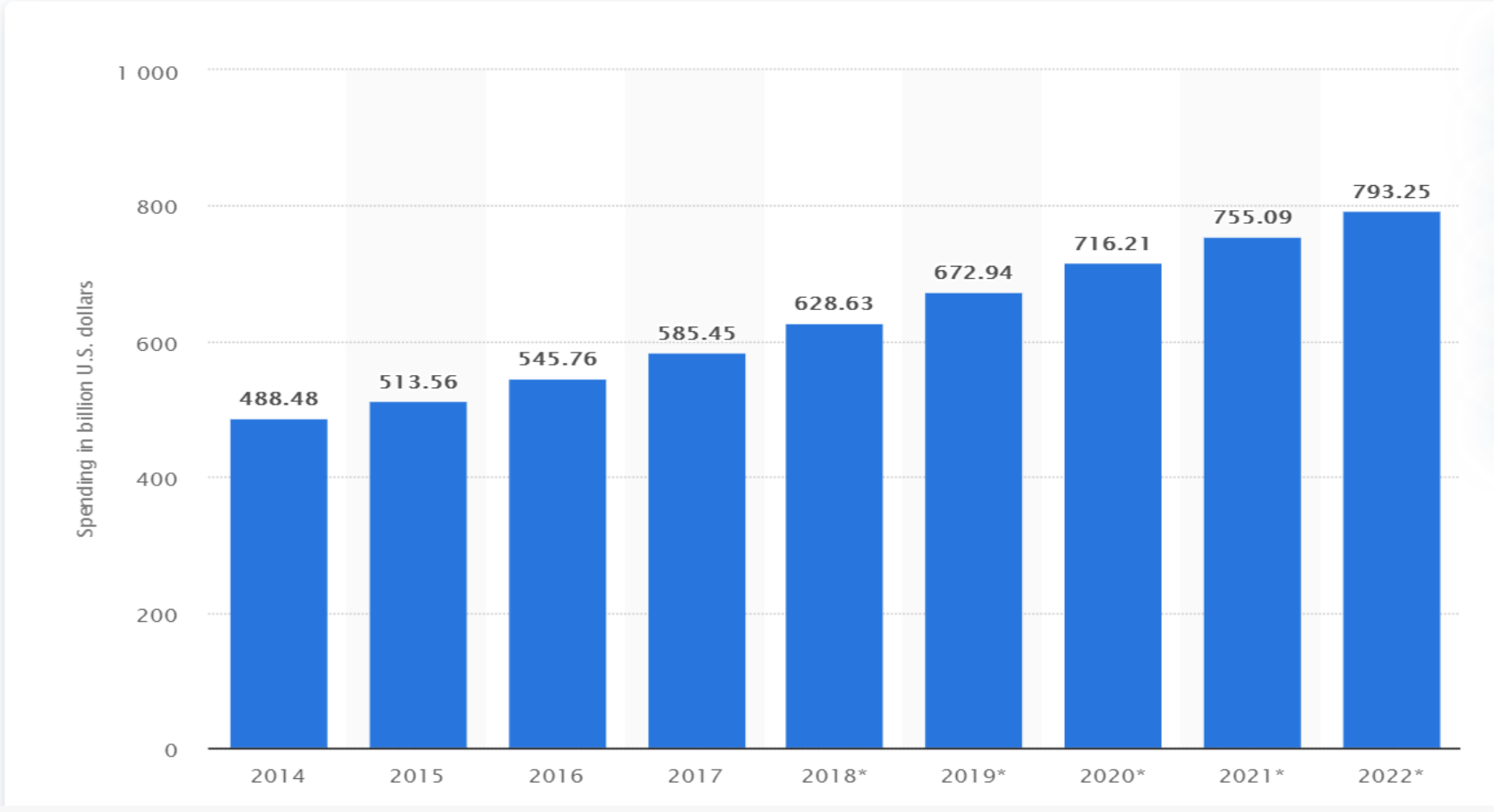
Global advertising spending from 2014 to 2022

(in billion U.S. dollars)



A. Guttmann, 2019

It is calculated that between 2018 and 2022 global advertising spending will increase by more than 160 billion U.S dollars, reaching close to 790 billion by the end of that period.

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Contaminant classification:

Known knowns:	*Traditional, regulated contaminants:* Persistent Organic Compounds (POPs): e.g. Dioxins, DDT. etc
Unknown knowns:	*Traditional, regulated contaminants with new unknown effects:* Surfactants: e.g. Linear Alkylbenzene Sulphonate (LAS), Nonylphenol
Unknown unknowns:	*New, unregulated contaminants with new unknown effects:* Emerging contaminants: e.g. Bisphenol A, Fragrances,…

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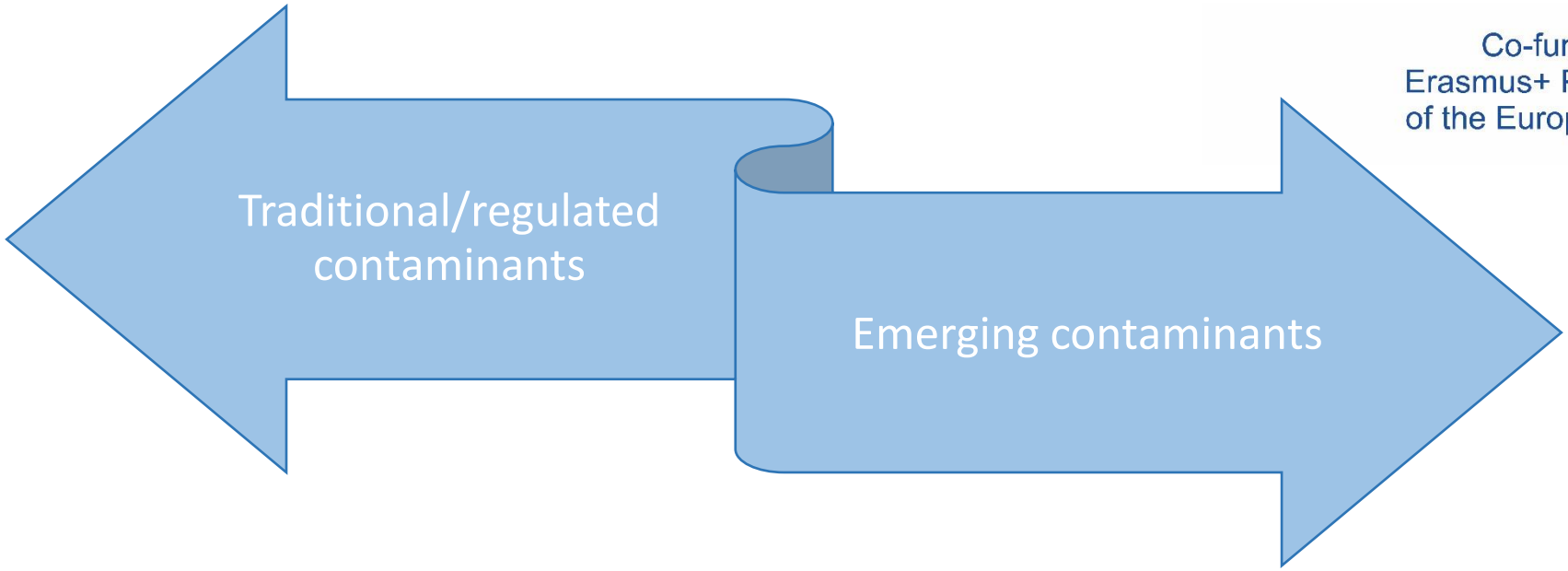

Regulated contaminants:

Their potential to cause damage in the environment is well known ➔ production and use are **legally regulated**.

Until 60ies: intense scientific and technical development ➔ release of multiple substances into the environment without previous evaluation of possible environmental and toxicological consecuences.

From 60ies: growing public and scientific concern ➔ environmental conscience

First environmental analyses ➔ detection of harmful substances for human health and environment

Emerging contaminants:

Newly developed/detected synthetic chemicals.

Improved technology for environmental analyses ➔ detection of unknown/unexpected substances in the environment.

Their potential to cause damage in the environment is unknown ➔ production and use are **not legally regulated**.

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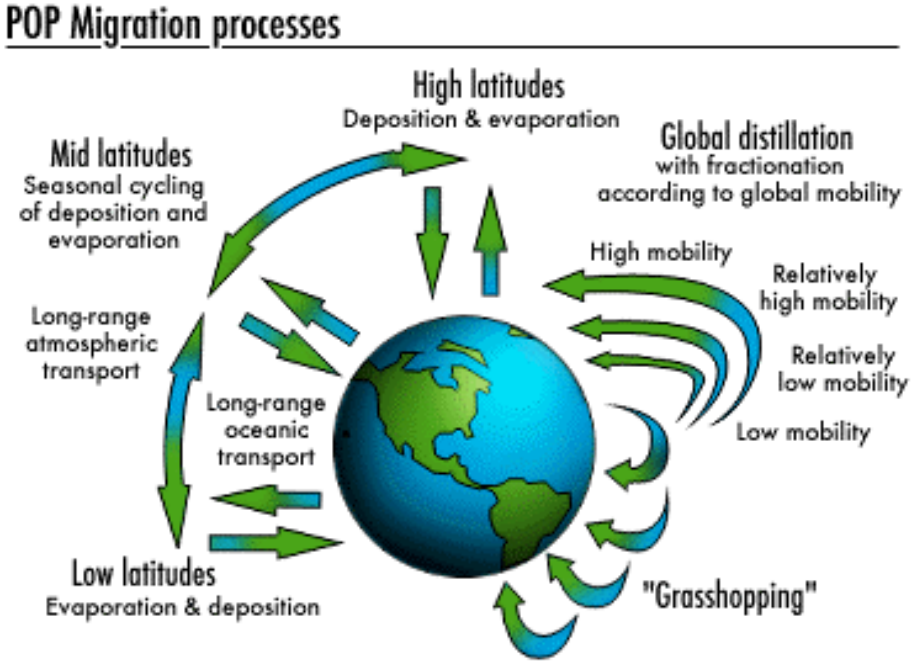
Regulated contaminants: e.g. Priority Organic Pollutants (POPs):

Characteristics:

- High stability (resistant to degradation) ➔ decennia or centuries to be degraded
- Mobility (Transported by draughts of air or water at great distances from their sources)
- Toxicity (produce adverse effects)
- Bioaccumulation (lipophilic ➔ accumulate in organisms over time) and biomagnification (move from one species to another through the food chain) capacity



Stockholm Convention (2001): Signed by 90 countries ➔ Regulation to reduce or eliminate the production, use and discharge of the 12 most dangerous POPs = Dirty Dozen.

Reduce exposure ➔ reduce risk of harmful effects.

Dirty dozen

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Compuesto	Año de inicio	Producción mundial (Tm)	Uso
Aldrin	1949	240,000	insecticida
Clordano	1945	70,000	insecticida
DDT	1942	3 million	insecticida
Dieldrin	1948	240,000	insecticida
Endrin	1951	4,000	rodenticida/insecticida
Heptacloro	1948	~1,000	insecticida
Hexaclorobenceno	1945	1 - 2 million	funguicida
Mirex	1959	No data	insecticida
Toxafeno	1948	1.3 million	insecticida
PCBs	1929	1 - 2 million	químico industrial
Dioxinas	?	?	-
Furanos	?	?	-

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Emerging contaminants: Unknown knowns; unknown unknowns

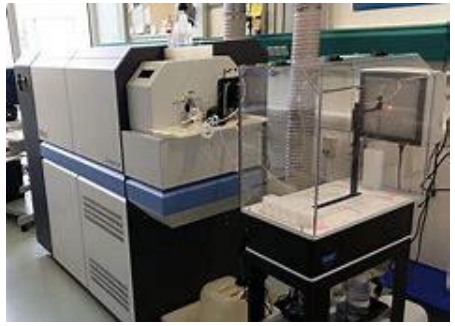
Synthetic compounds newly introduced and recently detected in the environment.

Concept of Emerging Pollutant ➔ Provisional character ➔depends on time and perspective:

- Advances in analytical techniques (LC-MS)

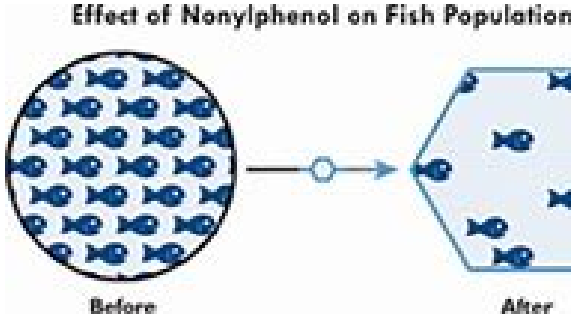
➔ detection of contaminants at trace levels, non-targeted screening

Chromatography (LC/GC)
coupled with mass spectrometry

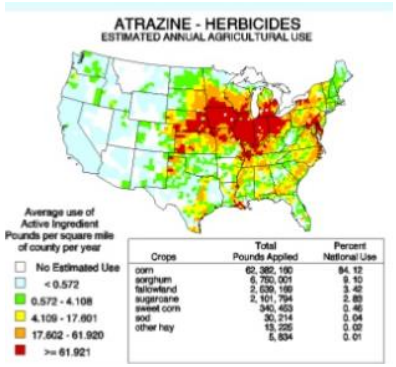


Plasma mass spectrometry
inductively coupled ICPMS

- New information on unknown effects by known contaminants



➔ e.g. surfactants (NP)
➔ endocrine disruptor



➔ e.g. herbicide Antrazine
➔ endocrine disruptor

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Emerging contaminants: further regulations for chemicals.

The European Water Framework Directive (EU WFD, 2000/60/EC):
Assessment of risk for another 500 priority chemical substances in the European rivers.
Decision based on monitoring and identification of chemical pollutants in the river basins of Elbe, Scheldt, Danube and Llobregat.
➔ 45 substances or groups of substances are on the list of priority substances for which environmental quality standards were set in 2008.

Selected chemicals:
Anthracene, Atrazine, Benzene, Brominated diphenyles, Cadmium and compounds, Chloroalkanes, Chloroalkanes, Chlorfevinphos, Chrorpyrifos-ethyl, 1,2-Dichlroethane, Dichloromethane, Di(2- ethylhexyl)phthalate (DEHP), Diuron, Endosulfan, Hexachlorobenzene, Hexachlorocyclohexane, Lead and compounds, Mercury and compounds, Naphthalene, Nickel and compounds, Nonyphenols, Pentachlorobenzene, Pentachlorophenol , Polyaromatic hydrocarbons, Benzo(a)pyrene and other PAHs, Simazine, Tributyltin compounds, Trichlorobenzene, Chloroform or Trichloromethane.

REACH: Registration, Evaluation and Authorization of Chemical Substances (1907/2006).
It is the companies and industries that manufacture or import chemicals that have to provide information on the properties and safety measures for their use in a database of the European chemical Agency (ECHA).
http://ec.europa.eu/environment/chemicals/reach/reach_intro.htm

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Emerging contaminants:

Most of new emerging pollutants belong to one or more of these classes:

1. Non-chlorinated halogenated compounds: chemical structure similar to that of persistent organic pollutants, but with F or Br instead of Cl. Flame retardants

2. Personal care Products (PCPs): Substances that are part of the formulation of products such as shampoos, toothpastes, lotions, etc.

3. Drugs: Illegal substances like cocaine, heroin, anabolic steroids, LSD,...

4. Food additives: Substances added to food products to preserve or increase their taste and appearance, etc.

5. Pharmaceutically active compounds (PhACs): pharmaceuticals and their pharmaceutically active metabolites.

6. Endocrine disruptors (EDCs): both natural and synthetic origin. Dangerous because they can interfere with endocrine functions, hormones and hormone target tissues.

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Emerging pollutants pose a big challenge to water management.

- Long term low level effects of these compounds are not known
- Can have unexpected effects on ecosystems, environment, biodiversity and finally human health.
- Interdisciplinary field of ecotoxicology ➔ biology, ecology and toxicology, mathematics, chemistry, statistica, informatics

Ecotoxicology:

- study of the effects of toxic chemicals and environmental pollutants on biological organisms, especially at the **population, community and ecosystem level**.

- integrates the effects of stressors across all levels of biological organisation from the molecular to whole communities and ecosystem.

⇔ Environmental toxicology: focuses upon effects of environmental contaminants at the **individual level**.

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OECD
BETTER POLICIES FOR BETTER LIVES

Organization for Economic Cooperation and Development:

- represents 34 industrialized countries in North and South America, Europe and the Asia and Pacific region, as well as the European Commission.

- co-ordinates and harmonizes policies, discuss issues of mutual concern to respond to international problems on **trade, environment, consumer and workers' safety**.

In 1981: > 60000 organo-synthetic compounds in use.
Each year: manufacture and release of 1000 – 1500 new products

They include daily-use products such as detergents, drugs, personal care and hygiene products, plastics or fireproof compounds.

Need to promote research to identify whether the emerging pollutant is dangerous to the environment and human health. → ECOTOXICITY TESTING

If so ➔ legislation and pass to be regulated pollutants: limit /ban on production volumes

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OECD Test Guidelines

= collection of internationally agreed test methods used by government, industry and independent laboratories.

Used to determine the safety of chemicals and chemical preparations (mixtures), including pesticides and industrial chemicals.

Internationally accepted as standard methods.
Updated to keep pace with progress in science, and to address animal welfare concerns.

The Environment, Health and Safety Division publishes documents in 11 different series:

1. Testing and Assessment;
2. Good Laboratory Practice and Compliance Monitoring;
3. Pesticides;
4. Biocides;
5. Risk Management;
6. Harmonisation of Regulatory Oversight in Biotechnology;
7. Safety of Novel Foods and Feeds;
8. Chemical Accidents;
9. Pollutant Release and Transfer Registers;
10. Emission Scenario Documents;
11. Safety of Manufactured Nanomaterials.

Most OECD publications are available for free in the internet site of the organization

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OECD GUIDELINES FOR THE TESTING OF CHEMICALS

Fish Embryo Acute Toxicity (FET) Test

INTRODUCTION

1. This Test Guideline (TG) 236 describes a Fish Embryo Acute Toxicity (FET) test with the zebrafish (*Danio rerio*). This test is designed to determine acute toxicity of chemicals on embryonic stages of fish. The FET-test is based on studies and validation activities performed on zebrafish (1)(2)(3)(4)(5)(6)(7)(8)(9)(10)(11)(12)(13)(14). The FET-test has been successfully applied to a wide range of substances exhibiting diverse modes of action, solubilities, volatilities, and hydrophobicities (reviewed in 15 and 16).

2. Definitions used in this Test Guideline are given in Annex 1.

PRINCIPLE OF THE TEST

3. Newly fertilised zebrafish eggs are exposed to the test chemical for a period of 96 hrs. Every 24 hrs, up to four apical observations are recorded as indicators of lethality (6): (i) coagulation of fertilised eggs, (ii) lack of somite formation, (iii) lack of detachment of the tail-bud from the yolk sac, and (iv) lack of heartbeat. At the end of the exposure period, acute toxicity is determined based on a positive outcome in any of the four apical observations recorded, and the LC_{50} is calculated.

INITIAL CONSIDERATIONS

4. Useful information about substance-specific properties include the structural formula, molecular weight, purity, stability in water and light, pK_a and K_{ow}, water solubility and vapour pressure as well as results of a test for ready biodegradability (OECD TG 301 (17) or TG 310 (18)). Solubility and vapour pressure can be used to calculate Henry's law constant, which will indicate whether losses due to evaporation of the test chemical may occur. A reliable analytical method for the quantification of the substance in the test solutions with known and reported accuracy and limit of detection should be available.

5. If the Test Guideline is used for the testing of a mixture, its composition should, as far as possible, be characterised, e.g., by the chemical identity of its constituents, their quantitative occurrence and their substance-specific properties (see paragraph 4). Before use of the Test Guideline for regulatory

OECD Toxicity Test Guidelines:

➔ cover safety testing of chemicals in its broadest sense:

- physical-chemical properties

- effects on biotic systems (ecotoxicity)

- environmental fate (degradation/accumulation)

- health effects (toxicity)

- pesticide residue chemistry

- efficacy testing of biocides

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Toxicity tests

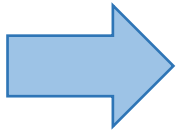
- exposure of test organisms to polluted environmental medium (air, water, sediment, soil) ➔ evaluation of the effects of pollution on survival, growth, reproduction, behavior on these organisms in comparison to a control.

- determine whether the pollutant concentrations are lethal (= acute effect, endpoint = death) or sublethal (non lethal, chronic effects).

> *Sublethal effects:*
> reduced growth, impaired reproduction, behavioural changes, reduction of communities, disruption of community functions among its species and ecosystem-level functions.

- can demonstrate whether chemical pollutants are bioavailable ➔ potential to cause biochemical damage to the biological tissues and organs of organisms.

-can be used to **monitor** at different positions and at different time
➔ characterization of the distribution of toxicity at an environmental site and time trends.

Environmental risk assessment  Development of remedial goals
➔ acceptable levels of contaminant with no adverse effects

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Basic principles of toxicity tests

Exposure in duplicate/triplicate of representative test species from the trophic levels in the ecosystem under controlled laboratory conditions to increasing concentrations (4-5 + control) of a selected contaminant (or mixture) or effluent during a certain time.

Observation of previously selected endpoints/responses: death, growth, reproduction.

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Model species

Three specific properties are evaluated:

Aquatic toxicity: The hazard of a substance to living organisms, based on toxicity tests to aquatic animals and plants.

Degradability: The persistence of the substance in the environment, based on molecular structure or analytical testing

Bioaccumulation/bioconcentration: The accumulation of a substance in living organisms (from water sources for bioconcentration), which may or may not lead to a toxic effect; based on calculations or bioconcentration factor (BCF) studies using fish

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No. 201 Freshwater Alga and Cyanobacteria, Growth Inhibition Test , 12/5/1981, 7/6/1984, 23/3/
No. 202 Daphnia sp. Acute Immobilisation Test ,12/5/1981 (adopted as Daphnia sp.14-day
Reproduction Test including an Acute Immobilisation Test) 4/4/1984, 13/4/2004
No. 203 Fish, Acute Toxicity Test , 12/5/1981, 4/4/1984, 17/7/1992
No. 204 Fish, Prolonged Toxicity Test: 14-Day Study, 4/4/1984
No. 205 Avian Dietary Toxicity Test, 4/4/1984
No. 206 Avian Reproduction Test, 4/4/1984
No. 207. Earthworm, Acute Toxicity Tests, 4/4/1984
No. 208 Terrestrial Plants, Growth Test 4/4/1984, 19/7/ 2006
No. 209 Activated Sludge, Respiration Inhibition Test (Carbon and Ammonium Oxidation) 4/4/1984, 22/7/2010
No. 210 Fish, Early-Life Stage Toxicity Test , 17/7/1992, 26/7/2013
No. 211 Daphnia magna Reproduction Test, 21/9/1998, 3/10/ 2008, 2/10/ 2012
No. 212 Fish, Short- term Toxicity Test on Embryo and Sacfry Stages, 21/9/1998
No.213 Honeybees, Acute Oral Toxicity Test, 21/9/1998
No.214 Fish, Juvenile Growth Test, 21/1/2000
No.215 Soil Microorganisms: Carbon Transformation Test, 21/1/2000
No.216 Soil Microorganisms: Nitrogen Transformation Test, 21/1/2000
No.217 Soil Microorganisms: Carbon Transformation Test, 21/1/2000
No.218 Sediment-Water Chironomid Toxicity Using Spiked Sediment, 23/11/2004
No.219 Sediment-Water Chironomid Toxicity Using Spiked Water, 23/11/2004
No.220 Enchytraeid Reproduction Test, 23/11/2004
No. 229. Fish short term reproduction assay, 8/9/2009
No. 230. 21-day Fish assay, 8/9/2009
No. 231. Amphibian metamorphosis assay, 8/9/2009.
No. 232. Collembolan reproduction test in soil, 8/9/2009
No. 233. Sediment-Water Chironomid Life-Cycle Toxicity Test Using Spiked Water or Spiked Sediment, 23/7/2010
No. 234. Fish Sexual Development Test, 26/7/2011
No. 235 .Chironomus sp., Acute Immobilisation Test, 26/7/2011
No. 236. Fish Embryo Acute Toxicity (FET) Test, 26/7/2013
No. 238 Sediment-free Myriophyllum spicatum Toxicity Test, 26/9/2014
No. 239 Water-Sediment Myriophyllum spicatum Toxicity Test, 26/9/ 2014 21-day Fish assay, 8/9/2009
No. 240. Amphibian metamorphosis assay, 8/9/2009.
No. 241. Collembolan reproduction test in soil, 8/9/2009
No. 242. Sediment-Water Chironomid Life-Cycle Toxicity Test Using Spiked Water or Spiked Sediment, 23/7/2010
No. 243. Fish Sexual Development Test, 26/7/2011

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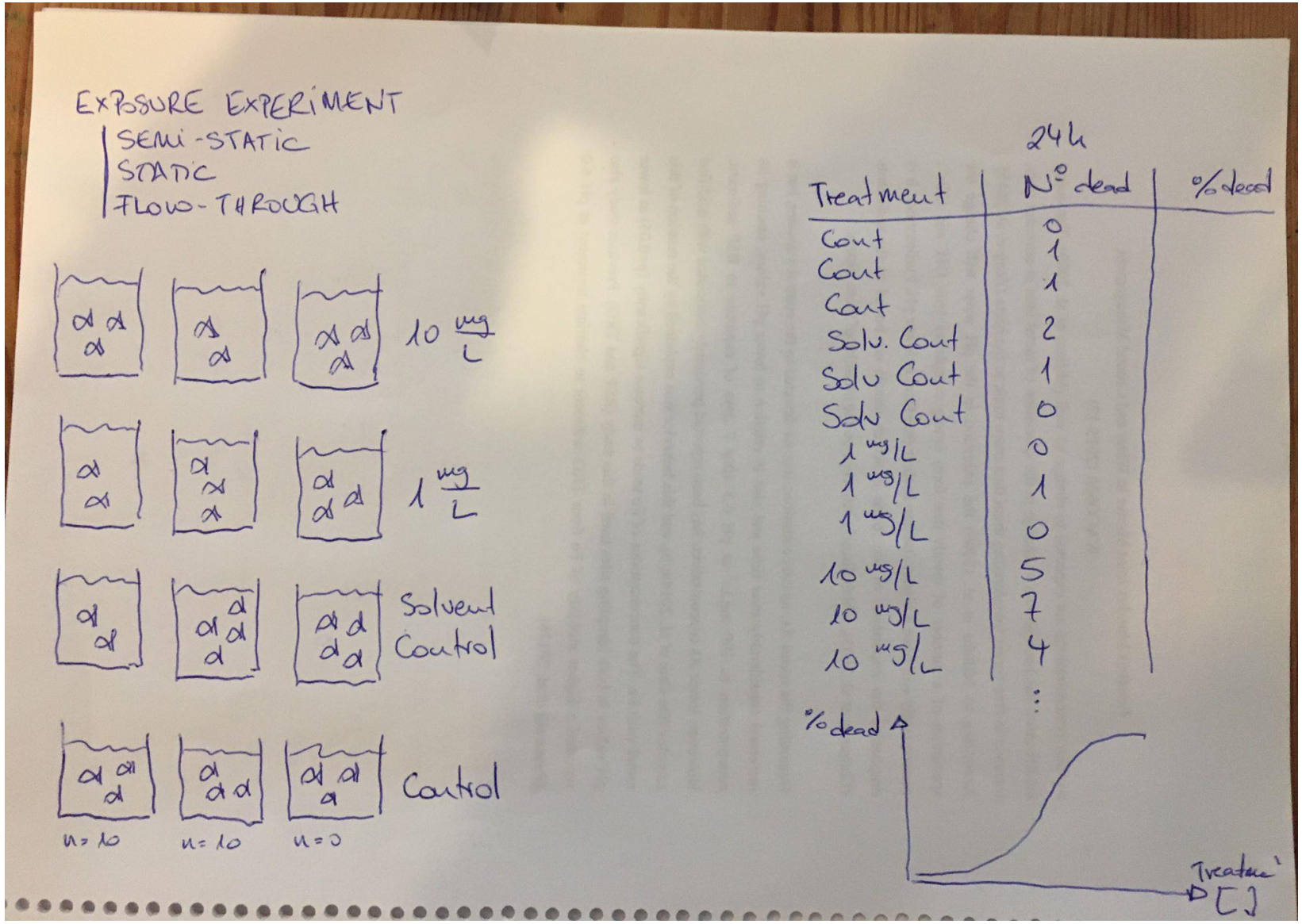

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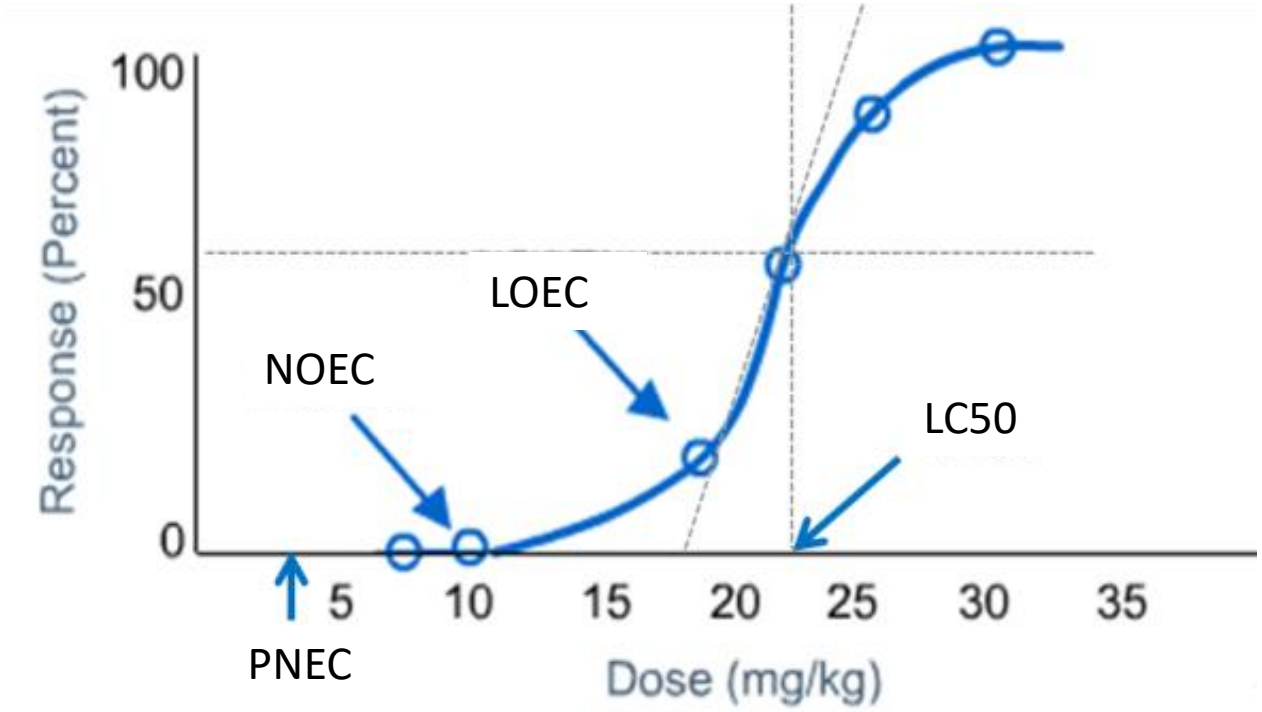

No Observed Effect Concentration (NOEC) :
highest exposure level at which there are no biologically significant increases in the frequency or severity of adverse effect between the exposed population and its appropriate control.
NOEC are typically obtained from chronic studies and reproductive toxicity studies

LC50 (Lethal Concentration 50%): statistically derived concentration at which 50% of the animals die.
LC50 are typically obtained from acute toxicity studies.

EC50 (Effect Concentration 50%): statistically derived concentration at which 50% of the animals show a defined response.
EC50 are typically obtained from sublethal toxicity studies.

Lowest Observed Effect Concentration (LOEC):
lowest exposure level at which there are biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control group.

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TEST NO. 201. Growth Inhibition Test in Alga and Cyanobacteria.

Purpose: determine the effects of a substance on the growth of freshwater microalgae and/or cyanobacteria.

Algae
= are primary producers in freshwater and marine ecosystems.
= provide the basis of the aquatic food chain.
Herbivorous organisms ➔ depend directly on algae as nutrition. Consumers ➔ trophic cascade.

➔Algae are very sensitive to xenobiotics and standardized tests systems are established since many years.
➔Tests generally conducted with microalgae

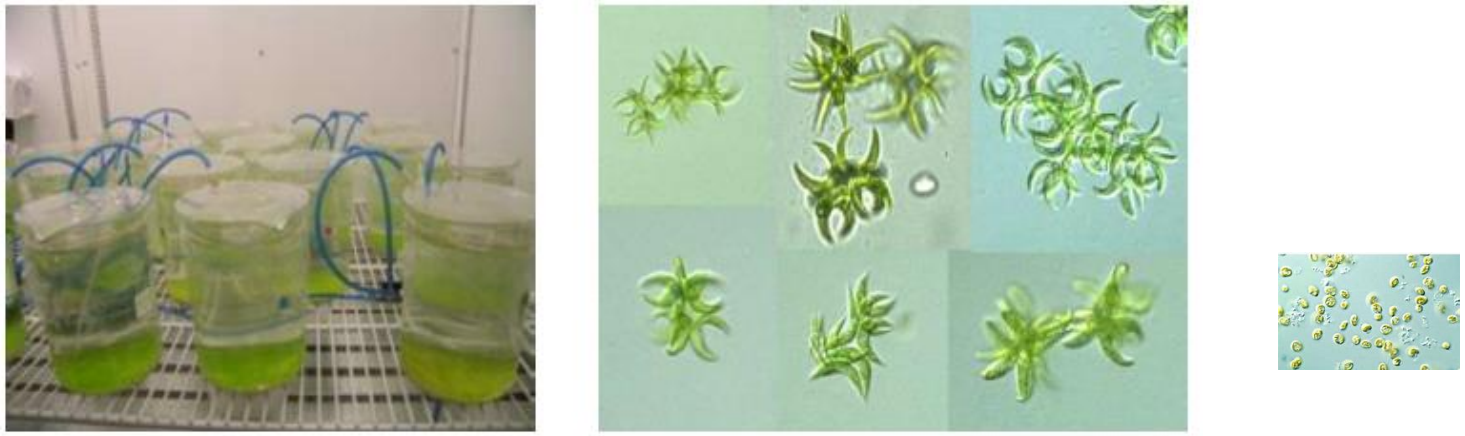

Figure 4. The test for the inhibition of growth on microalgae or cyanobacteria is the quantified measurement of toxic effects of chemical substances (pollutants) on primary producers in freshwater or marine ecosystems.

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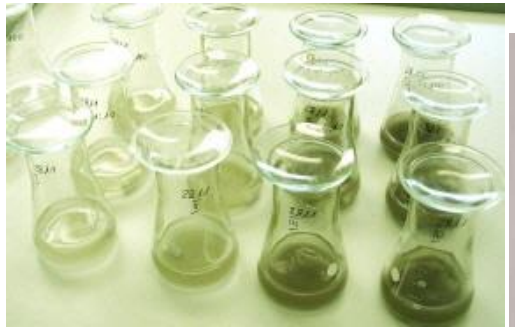
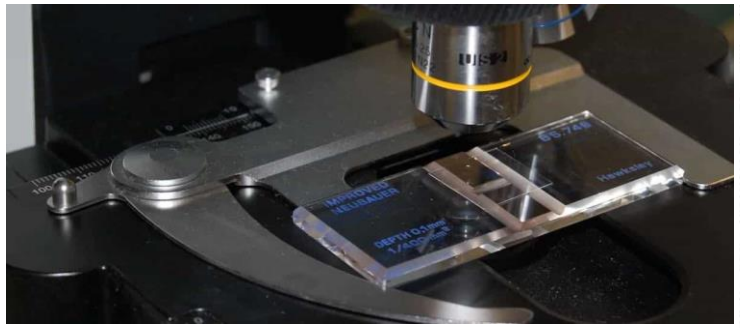
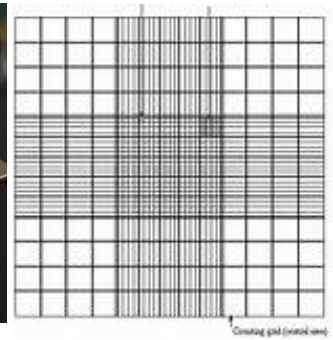
Exponentially growing test organisms are exposed to the test substance in batch cultures over a period of normally 72 hours.

➔ counting in Neubauer chamber, microscope







Algal cultures exposed to ≥ five concentrations of a test substance.

Cultures: unrestricted exponential growth, unlimited nutrients and continuous fluorescent illumination.

Three replicates at each test concentration

Response: reduction of growth in comparison with the average growth of control cultures along the time.

TEST No. 202. Daphnia sp. Acute Immobilisation Test.

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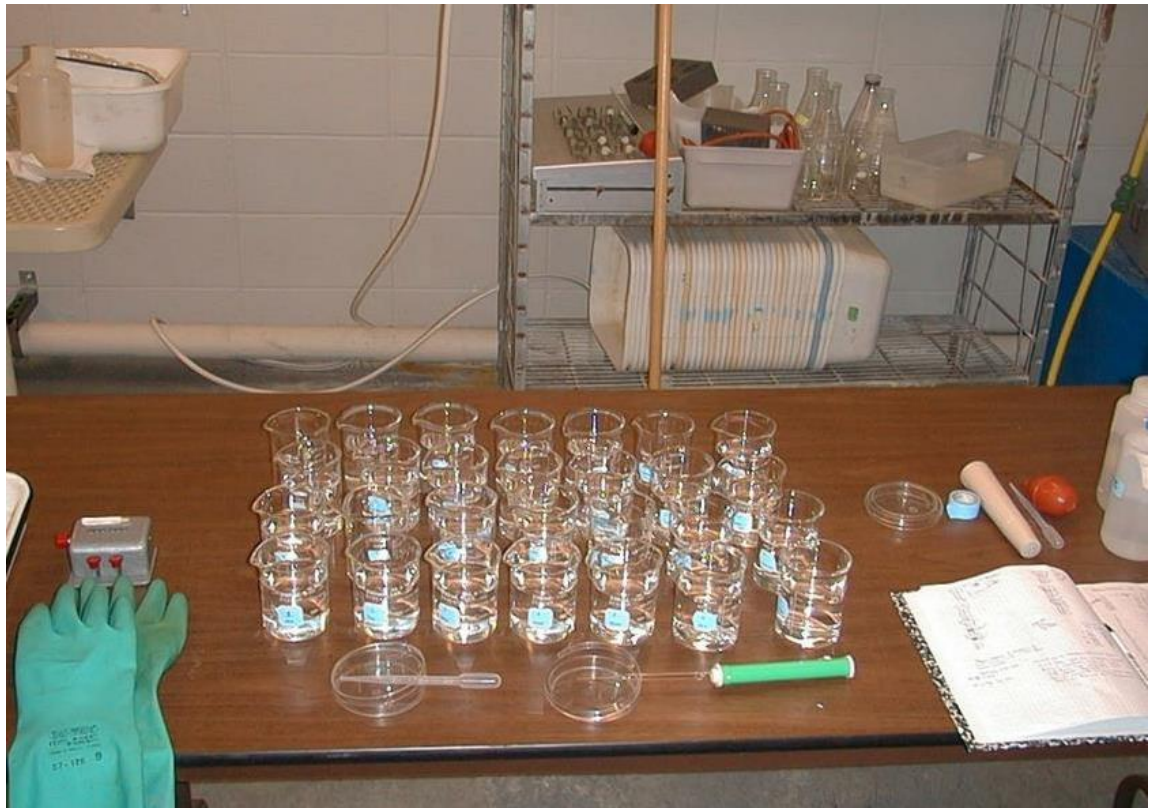
Daphnids = zooplankton; <24h at the start of the test.

Daphnia (magna) is commonly used in aquatic toxicity testing
➔ easy and economical to culture in the laboratory (small size, short life cycle, high fecundity, and parthenogenetic reproduction).



Exposed to test substance at ≥ five concentrations for 48h

3 replicates.

Response: immobilization after 24 and 48h hours compared with control.

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Figure 5. *Daphnia magna* (zooplankton) immobilization test is the basic aquatic test of OECD for toxic substances, drugs and other pollutants.

Objective: Calculation of EC50 after 48h. Determination of the EC50 at 24h is optional.

At least 20 animals (4 replicates of 5 individuals) at each test concentration and controls.
Volume required: at least 2 ml of test solution / animal (i.e. a 10 ml for 5 daphnids per test vessel).
The limit test corresponds to one dose level of 100 mg/L.

Report: observation for immobilized daphnids at 24 and 48h, measures of dissolved oxygen, pH, concentration of the test substance, at the beginning and end of the test (nominal vs measured).

TEST No. 203. Fish, Acute Toxicity Test.

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- Exposure for 96h.
- Mortalities: 24, 48, 72 and 96h
- Determination of LC50 where possible.
- At least seven fishes must be used at each test concentration and in the controls.
- At least, five concentrations in a geometric series with a factor preferably not exceeding 2.2.
- The limit test corresponds to one dose level of 100 mg/L.
- Cumulative % mortality for each exposure period is plotted against concentration.

Leponis mactochirus

Pimephales promedas (fathead minnow)

Oryzias latipes

Brachydanio rerio

Cyprinus caprio

Oncorhynchus mykiss (rainbow trout)

Figure 6. The acute toxicity test in fish is normally for the duration of 4 days (96 hrs) with the toxic substance administered in at least 5 concentrations. The cumulative % mortality is plotted against log concentration and the LD_{50} or LC_{50} is estimated from the concentration-response curve.

TEST No. 231. Amphibian metamorphosis assay.

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Amphibians: recommended species: *Xenopus laevis (African clawed frog)*
➔metamorphosis assay

Metamorphosis = most dramatic example of extensive morphological, biochemical and celular changes ocurring during postembryonic development

- Hypothalamus-Pituitary-Thyroid (HPT) axis
  - controls metabolic processes in the body
    - thermo-regulation
    - generation of energy
    - growth
    - development of the central nervous system
    - control of the cardio-vascular system (heart beat)
    - reproduction
  - in fish
    - smoltification
  - in amphibians
    - larval development & metamorphosis

In amphibians: thyroid-dependent, responds to substances active within the hypothalamic-piyuitary-thyroid axis.
➔ to screen substances which may interfere with the normal functioning of the hypothalamic-pituitary-thyroid axis

- Three test chemical concentrations and controls, carrier control if necessary.
- 4 replicates.
- Start: tadpoles at the development stage 51 on the Nieuwkoop and Faber scale
- Duration: 21d.
- Day 7: sub-set of tadpoles from each treatment ➔ measurement of the length of the hindlimb.
- Day 21: measurement of developmental stage, snout-to-vent length and hind limb length.
- Sub-set of tadpoles from each treatment level is fixed (whole-body or dissected) for histopathology of the thyroid gland.

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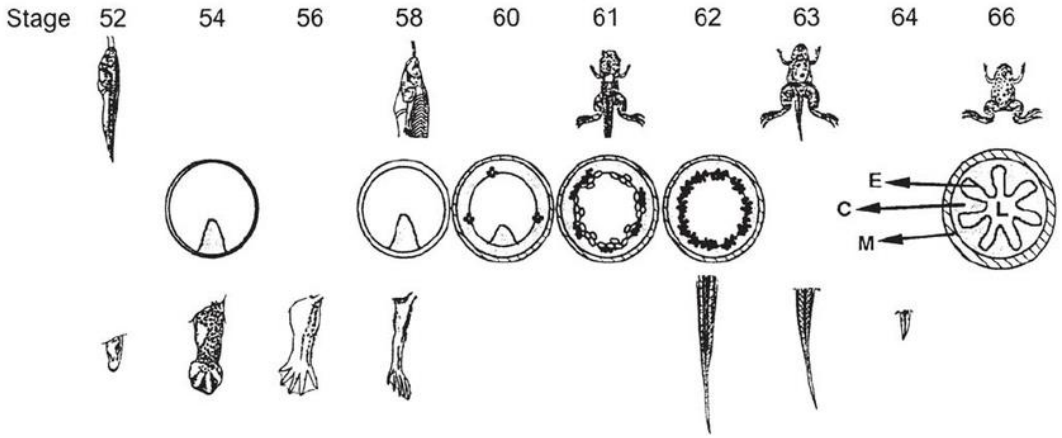
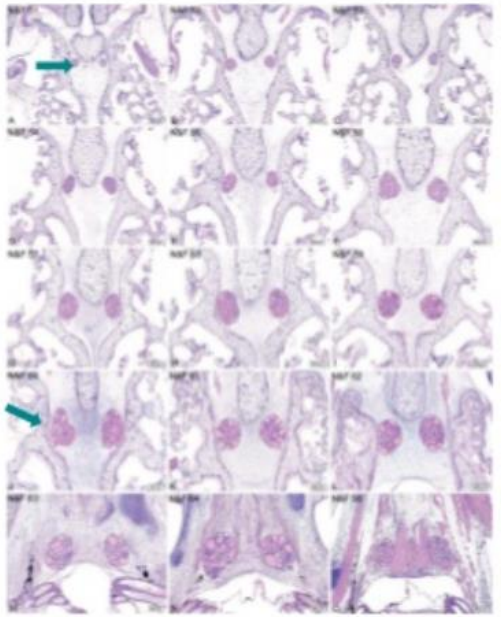
- Metamorphosis
  - → before stage 46 = no need for thyroid hormones = tadpole
  - → stage 46 to 53 (pre-metamorphosis) = hind limb visible
  - → stage 57/58 (post-metamorphosis) = front limbs visible
  - → stage 66 (climax) = tail and gills absorbed = froglet

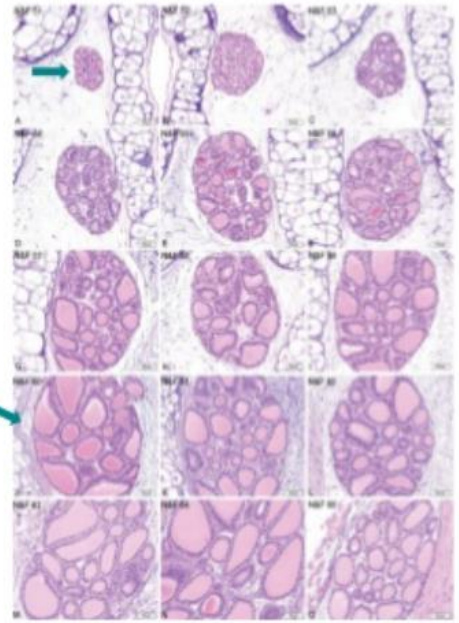


Nieuwkoop PD and Faber J (1994) Normal table of Xenopus laevis. Garland publishing. New York.

development of thyroid gland



thyroid gland



Toxicologic Pathology, 37: 415-424, 2009; K. Christiana Grim *et al*

TEST No. 236. Fish Embryo Acute Toxicity (FET) Test.

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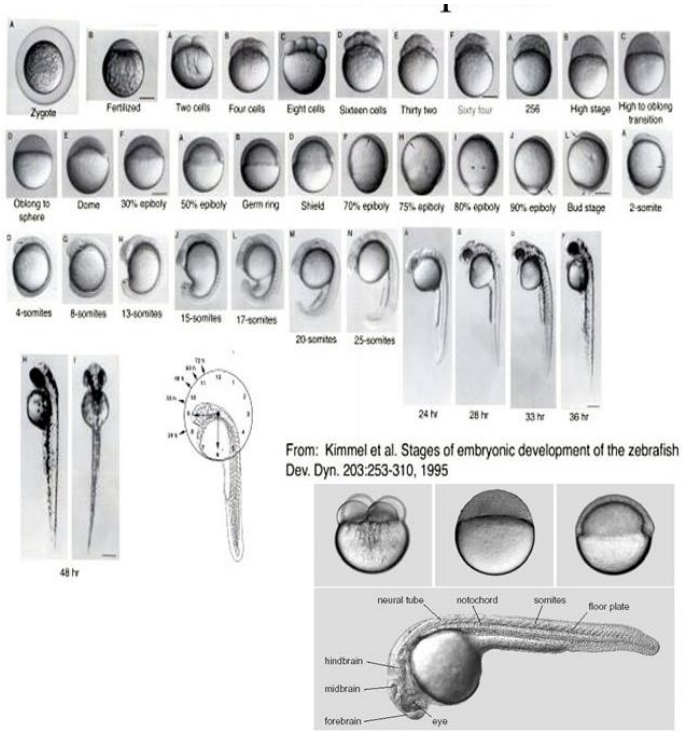
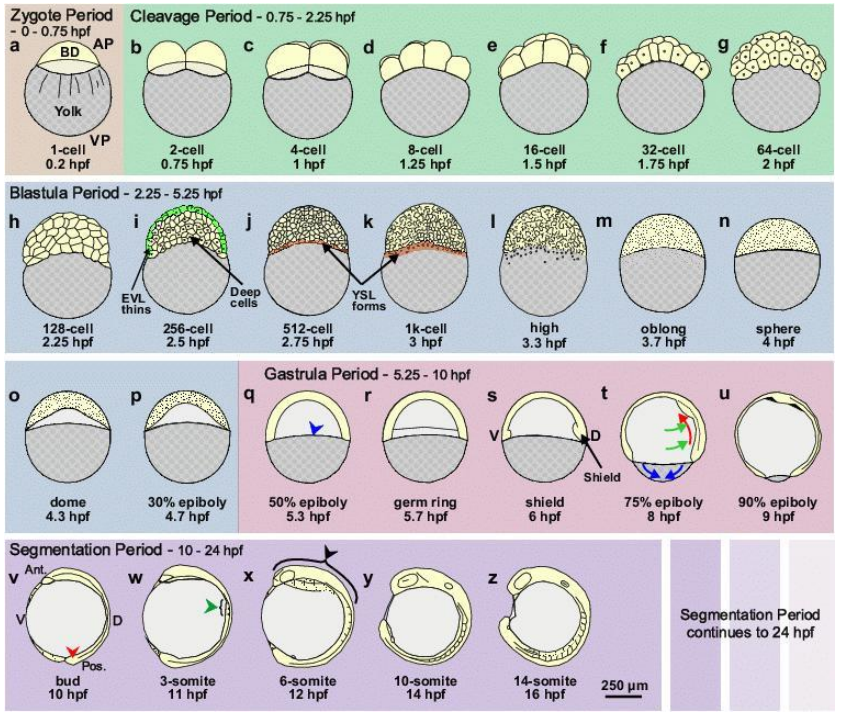
Zebrafish (Danio rerio): freshwater tropical fish, easy to grow, short growth period of high fecundity.
Important model for environment and human health risk assessment of chemicals.

Test to determine the acute or lethal toxicity of chemicals on embryonic stages.
Exposure of newly fertilized zebrafish eggs to a chemical for a period of 96 hrs.
5 increasing concentrations of the chemical tested and a control and carrier if required.





All developmental stages perfectly known and derivations from normal development standardized

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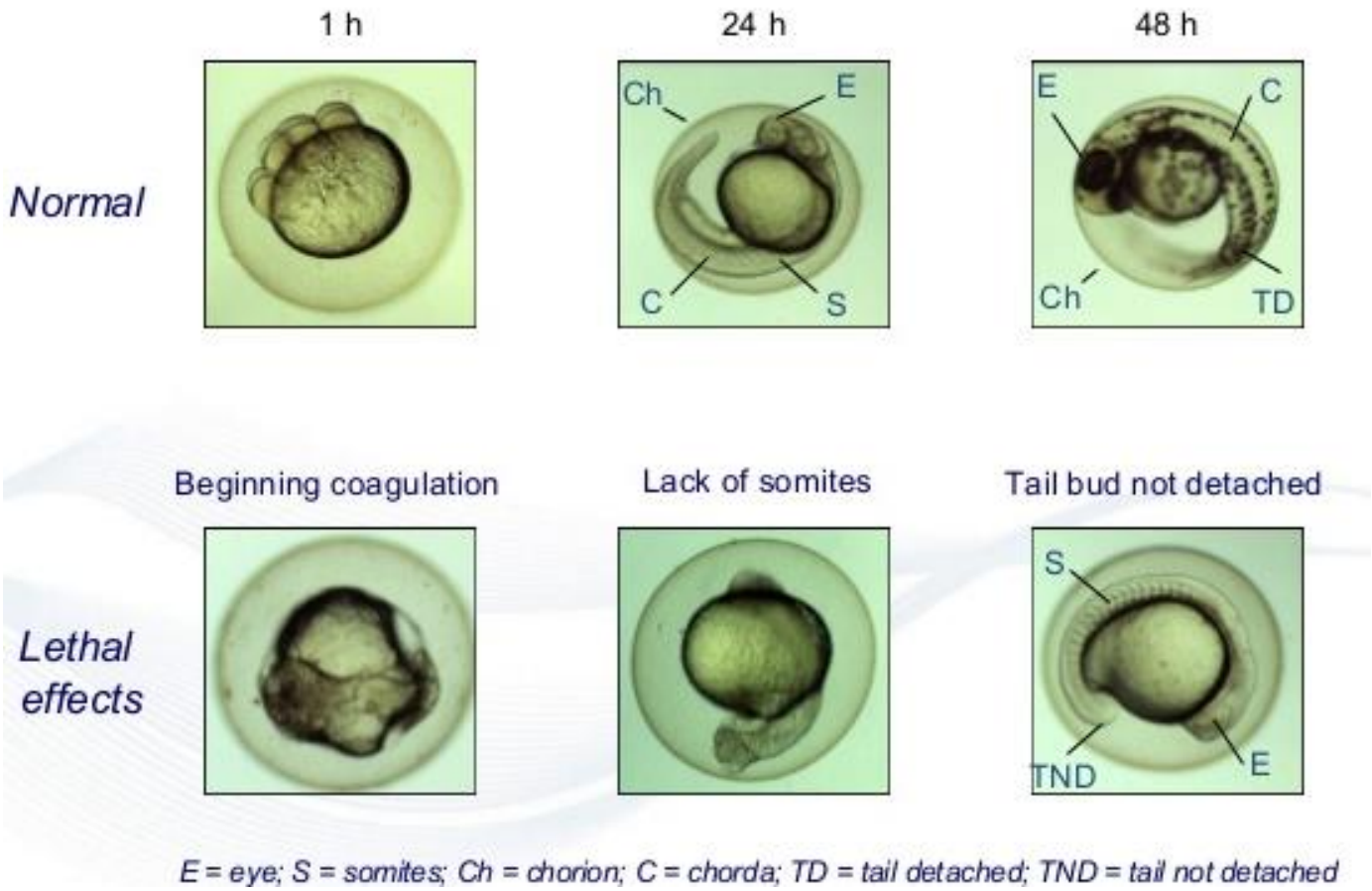
Responses (➔ indicators of lethality):
evaluated every 24h
optical observation:
- (i) coagulation of fertilised eggs,
- (ii) lack of somite formation,
- (iii) lack of detachment of the tail-bud from the yolk sac,
- (iv) lack of heartbeat.
- (v) End of exposure: determination of acute toxicity ➔ LC50.



Also in test report:
- dissolved oxygen,
- pH,
- total hardness,
- temperature,
- conductivity of solutions,
- measured concentrations of the chemical tested,
- whether the validity criteria of the test were met (e.g.: mortality in control < 20%).

Advanced biomicrofluid technology for integrated high-performance analysis of multi-level biological responses in ecotoxicological research, CHIP4ECO (FEDER-UCA18-108163)

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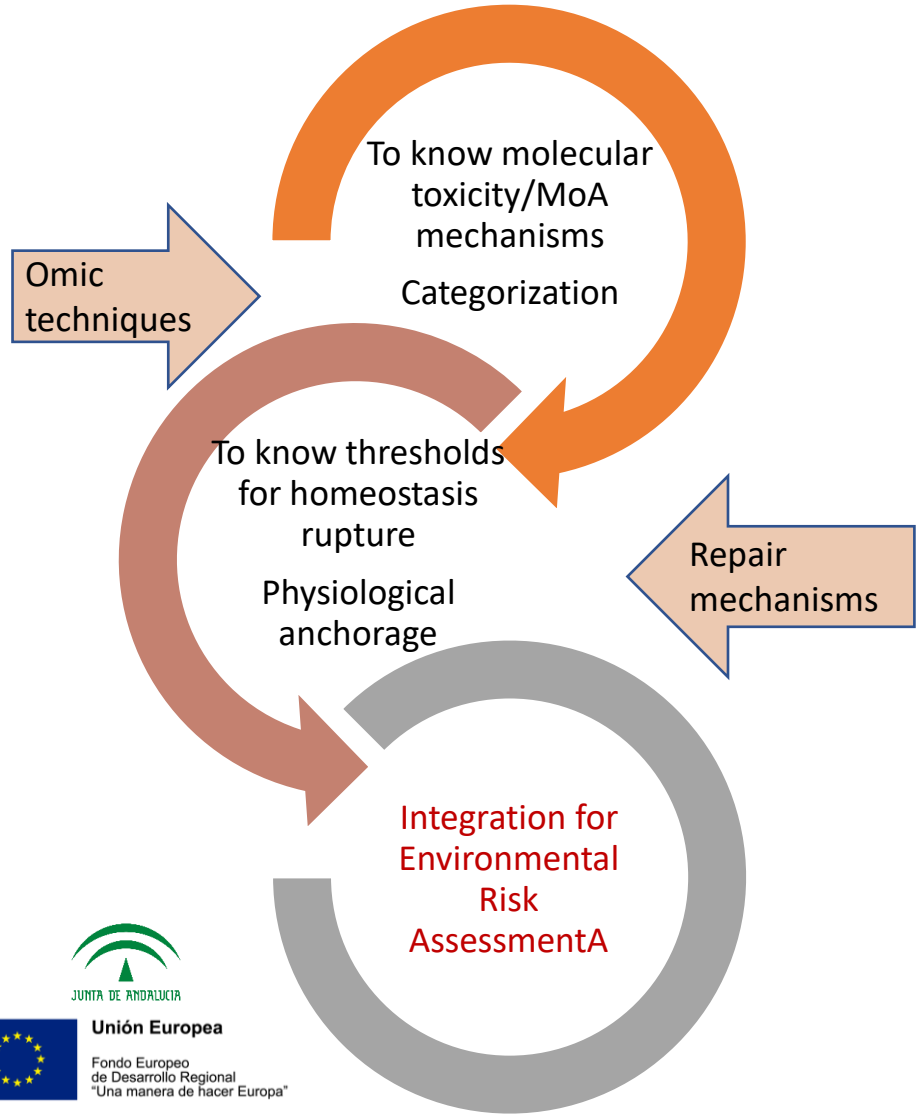


JUNTA DE ANDALUCIA
Unión Europea
Fondo Europeo de Desarrollo Regional
"Una manera de hacer Europa"

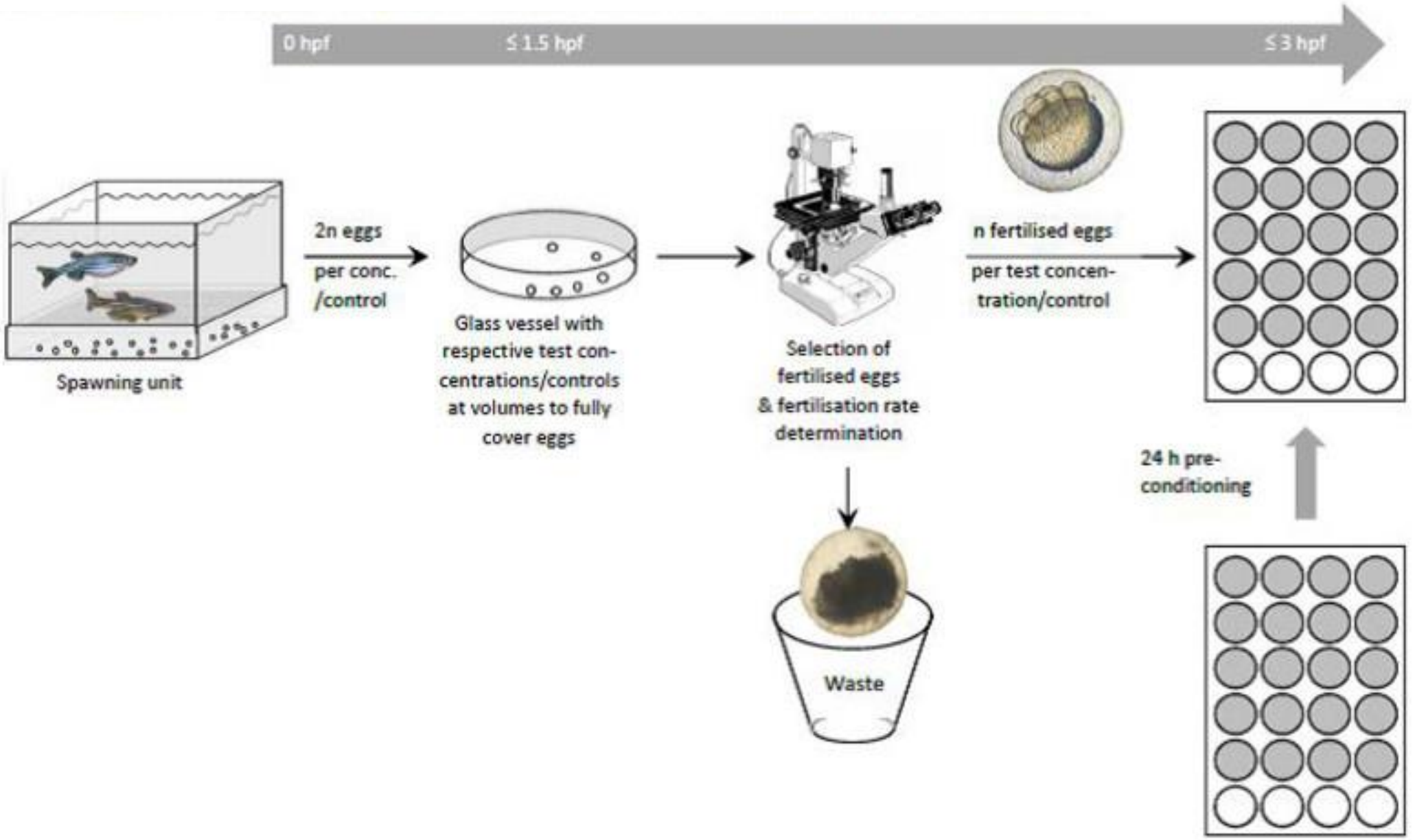
Adaptation of the OECD Fish embryo toxicity (FET) test for a marine species, Sparus aurata

Simultaneous exposition

- Multi well
- Biomicrofluidic device
- Emerging and conventional contaminants (pharmaceutical compounds, metals)
- Lethal and subletal toxicity (development, behavior, omics)

➔Analysis of physiological anchoring to relate induced alterations in the proteomic profile to behavioral phenotype/development

- Multi well approach - conventional



- Biomicrofluidic device ➔ automatization

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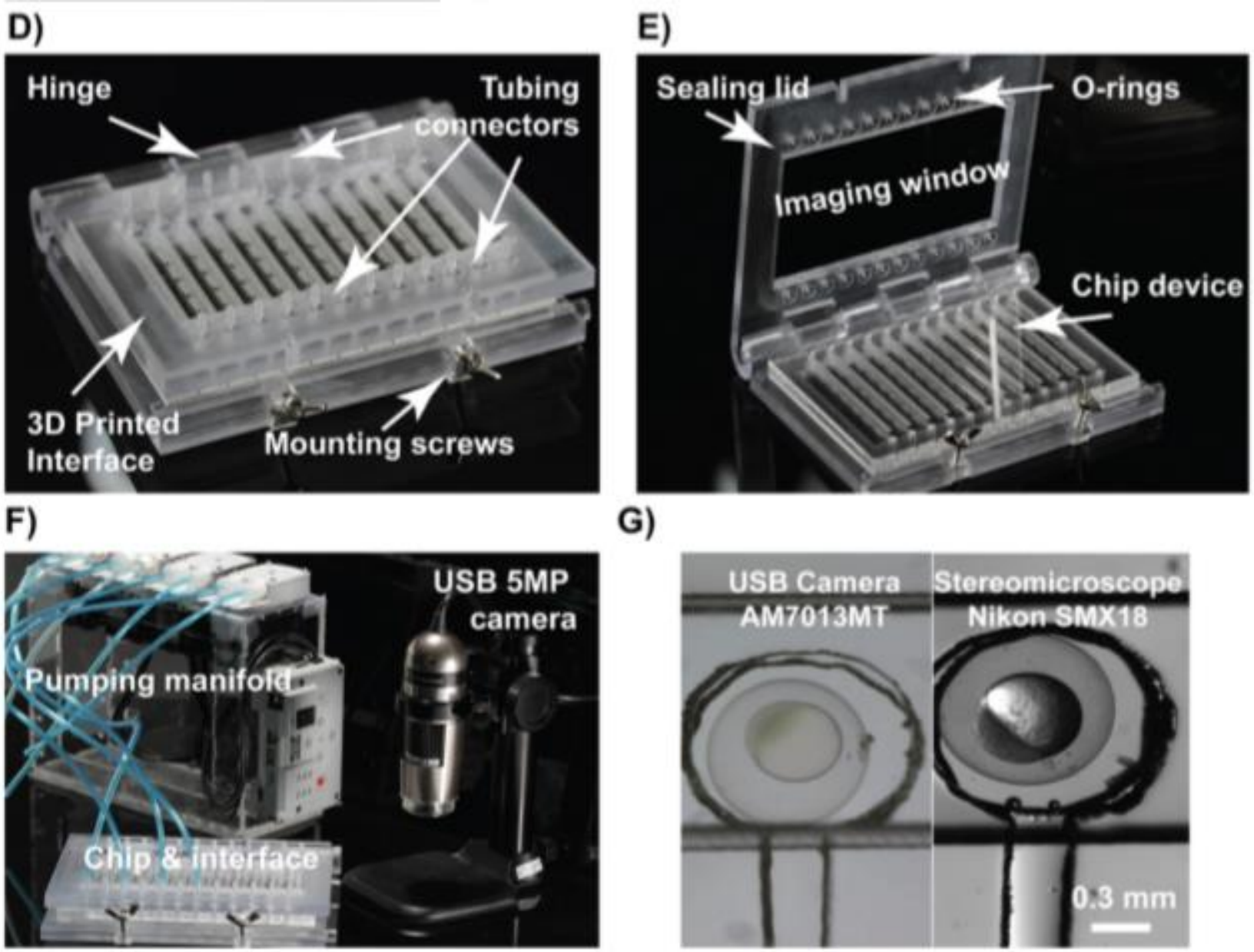

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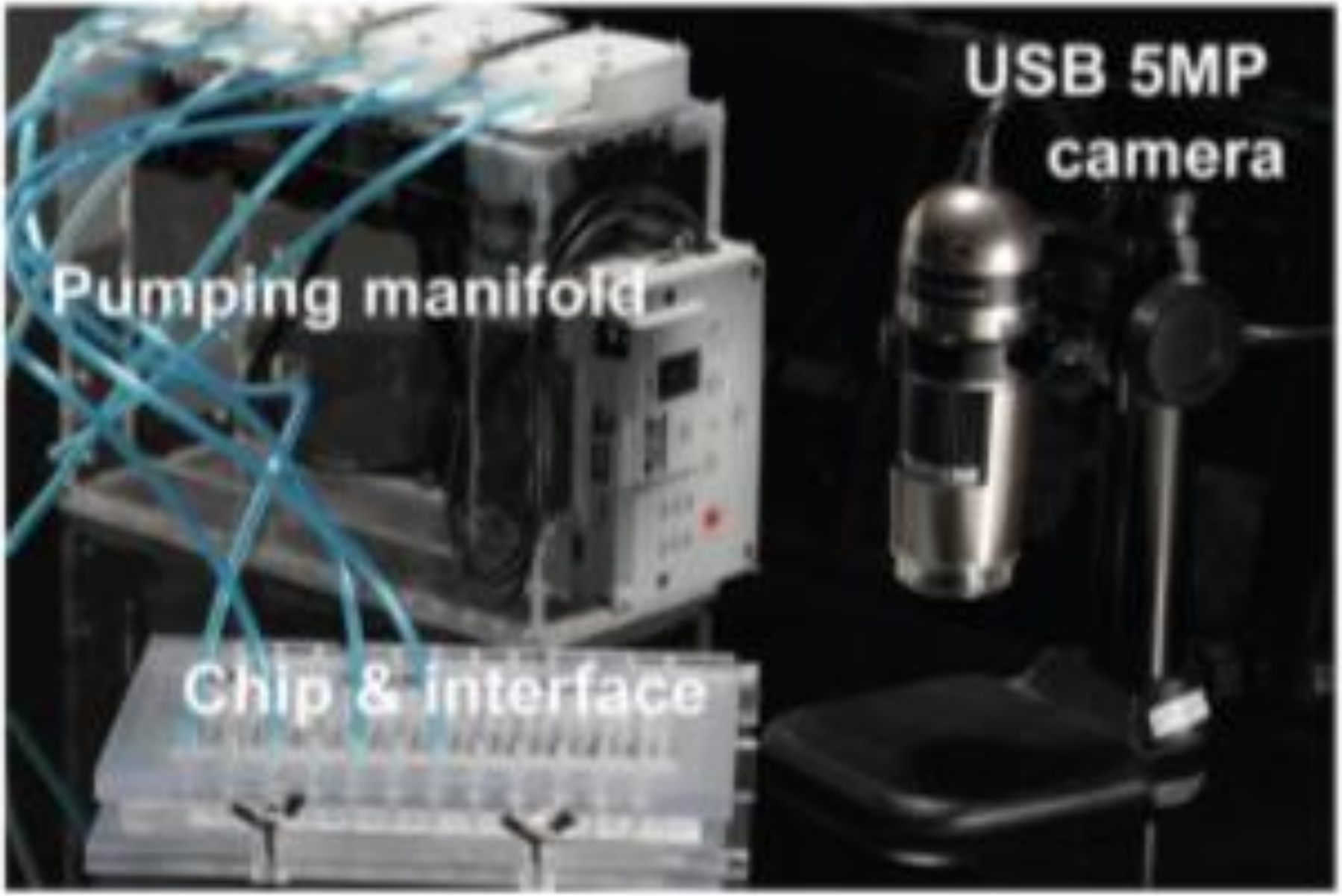

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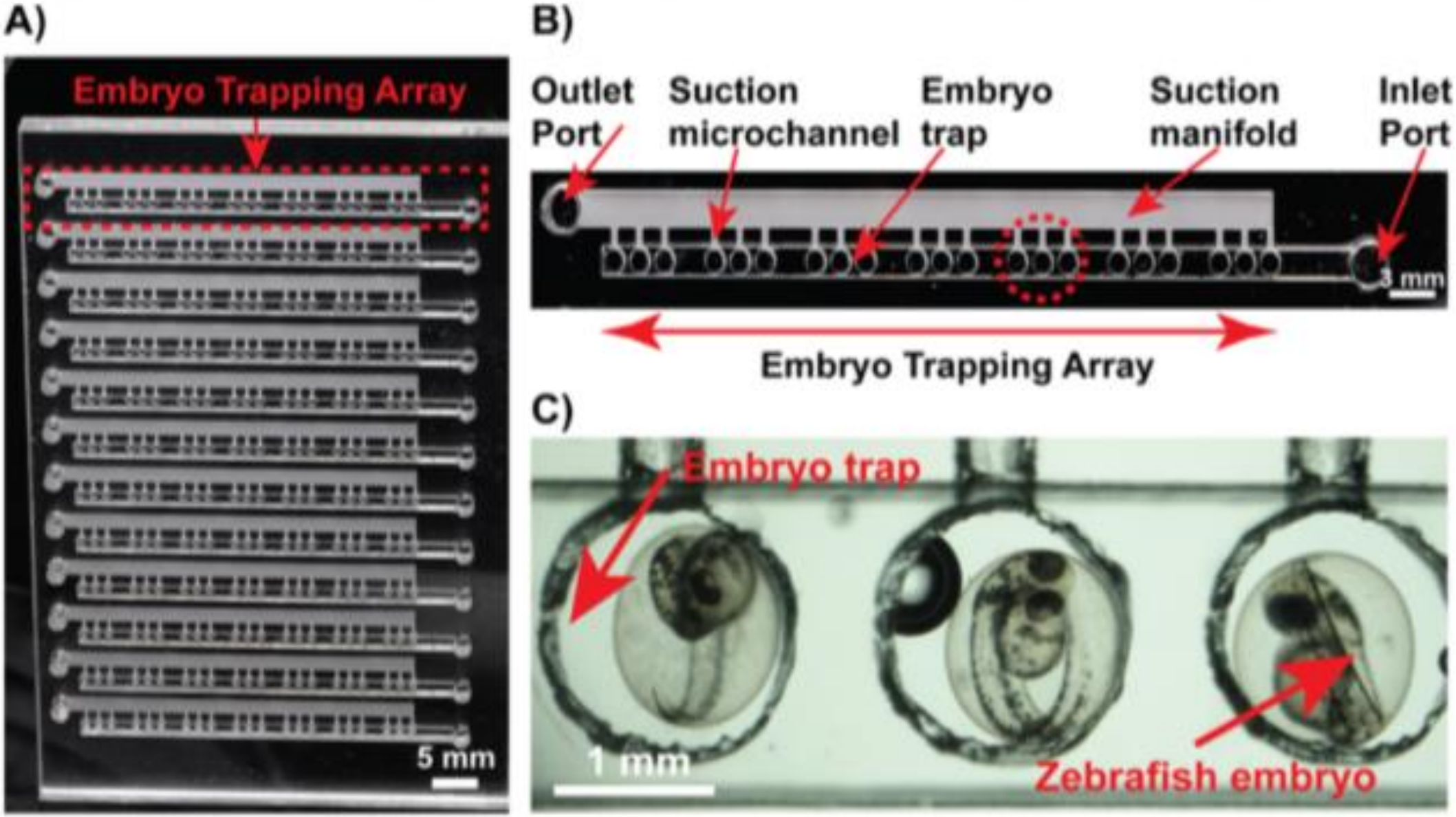

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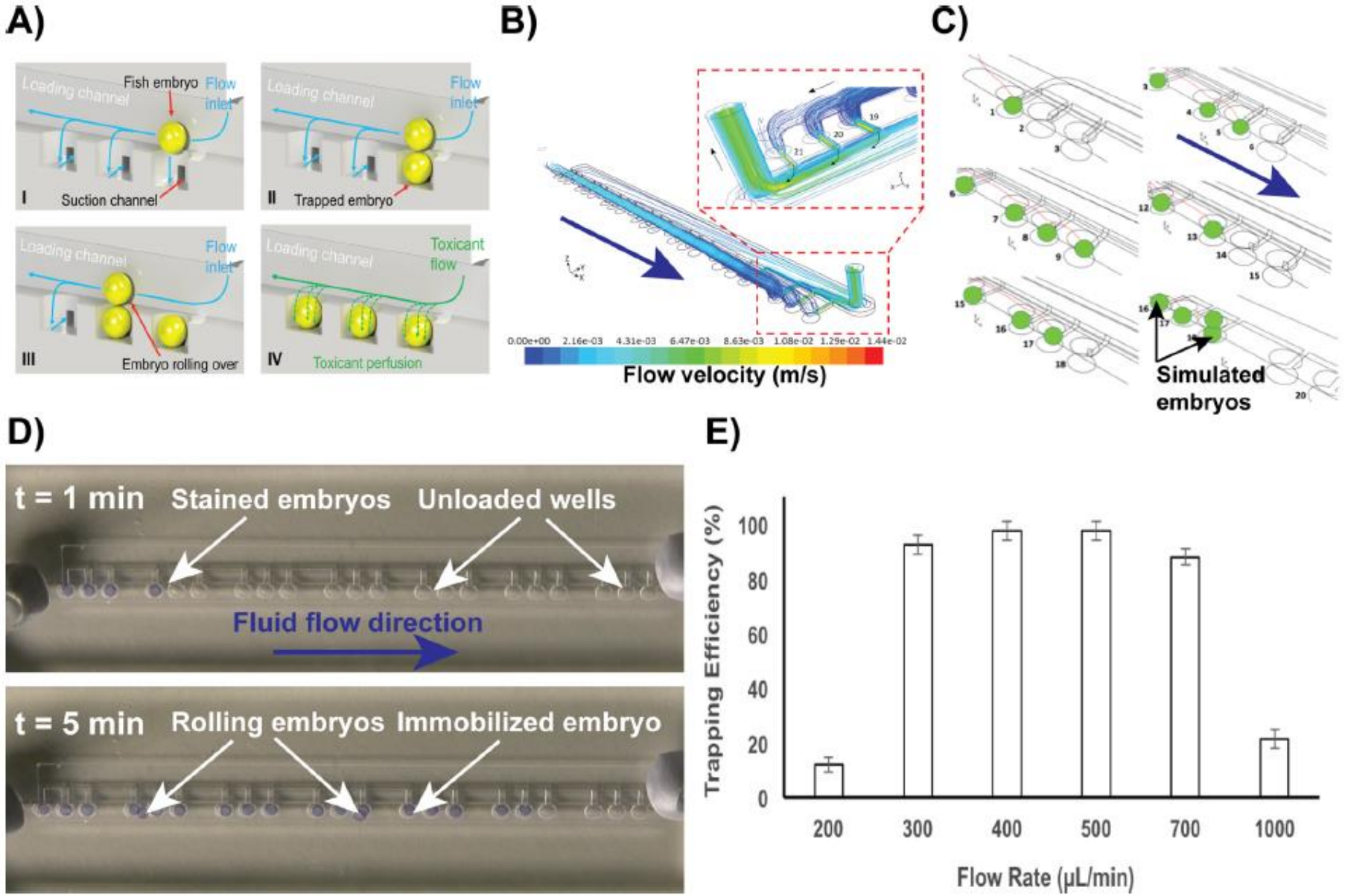

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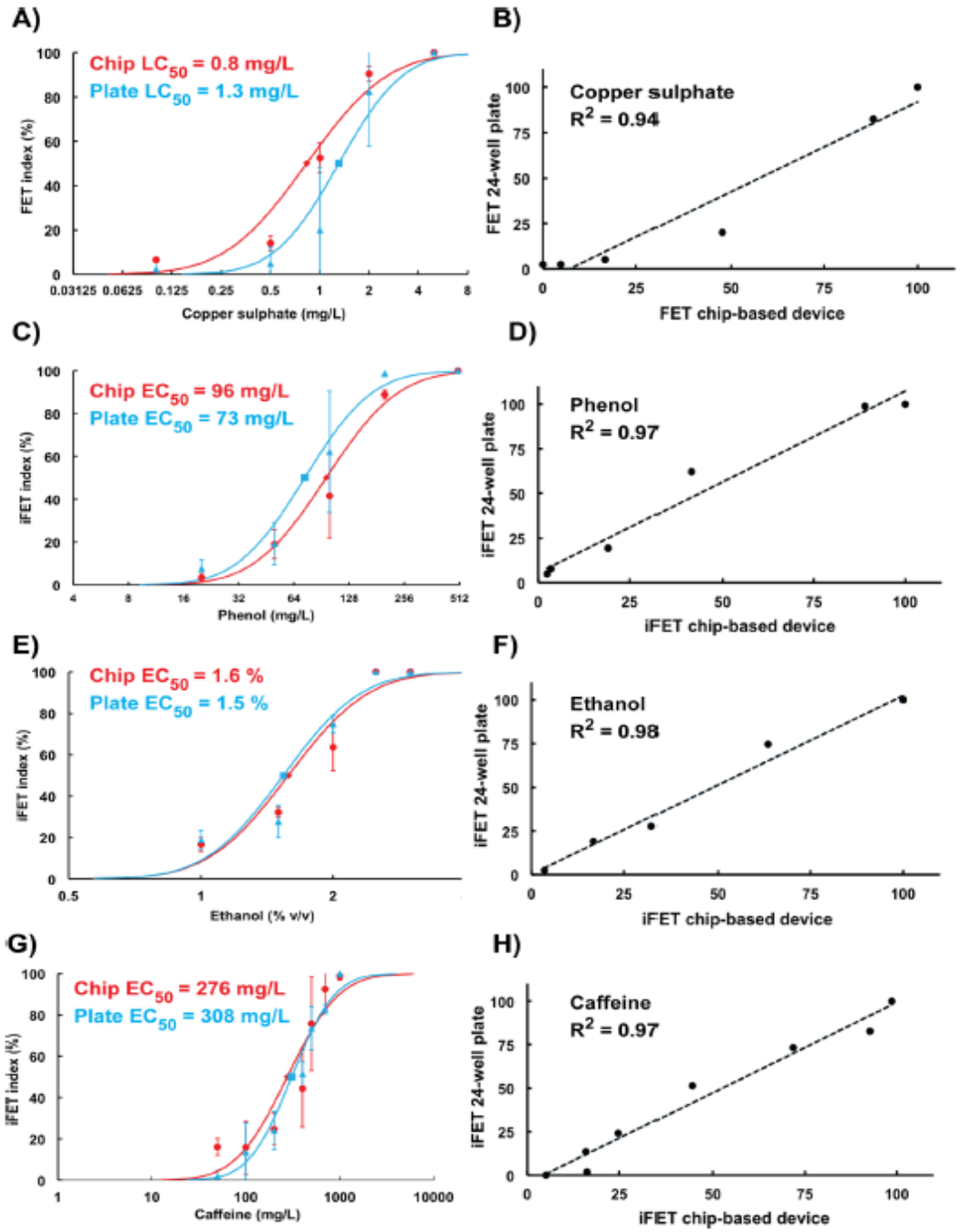

Automated experiments ➔ controlled, simultaneous

<u>Acute tests</u>
➔LC50

<u>Sublethal exposures</u>
➔ Material for Omic Techniques
Mechanism of action, initial molecular event

➔ <u>Developmental, behavioral endpoints</u>

Physiological anchoring
Homeostasis

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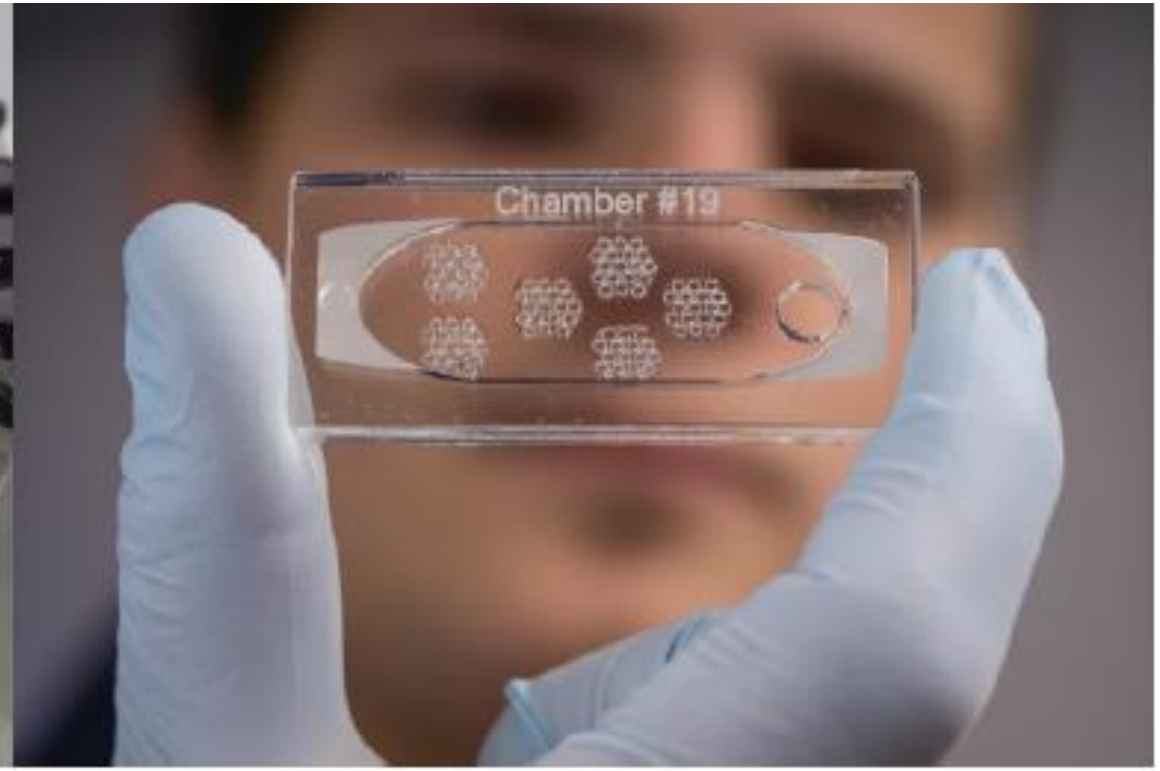

Space
Residues

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Toxicology and Ecotoxicology Databases for Hazardous Chemicals

Databases ➔ to reduce unnecessary multiple animal testing
➔ existing information on (eco-)toxicology studies is gathered in public databases

There are numerous ecotoxicology databases kept by national organizations in various countries and by international organizations like OECD, WHO, UNEP, etc.

ECETOC Aquatic Toxicity database. EAT (http://www.ecetoc.org)
Ecotoxdatabase of Environmental Protection Agency (http://www.epa.gov/ecotox/).
ESIS (European Chemical Substances Information System) (http://esis.jrc.ec.europa.eu/).
HERA (Human and Environmental Risk Assessment) (http://www.heraproject.com)
HSDB (Hazardous Substances Data Bank) (http://toxnet.nlm.nih.gov).
OECD Integrated HPV database (http://webnet.oecd.org/hpv/ui/Default.aspx).
OHMTADS The Oil and Hazardous Materials/Technical Assistance Data System (http://www.nisc.com/cis/details/ohm-tads.htm).
Riskline, Swedish Chemical Inspectorate (http://apps.kemi.se/riskline/).
Japanese Ministry of the Environment (http://www.env.go.jp/en/chemi/)

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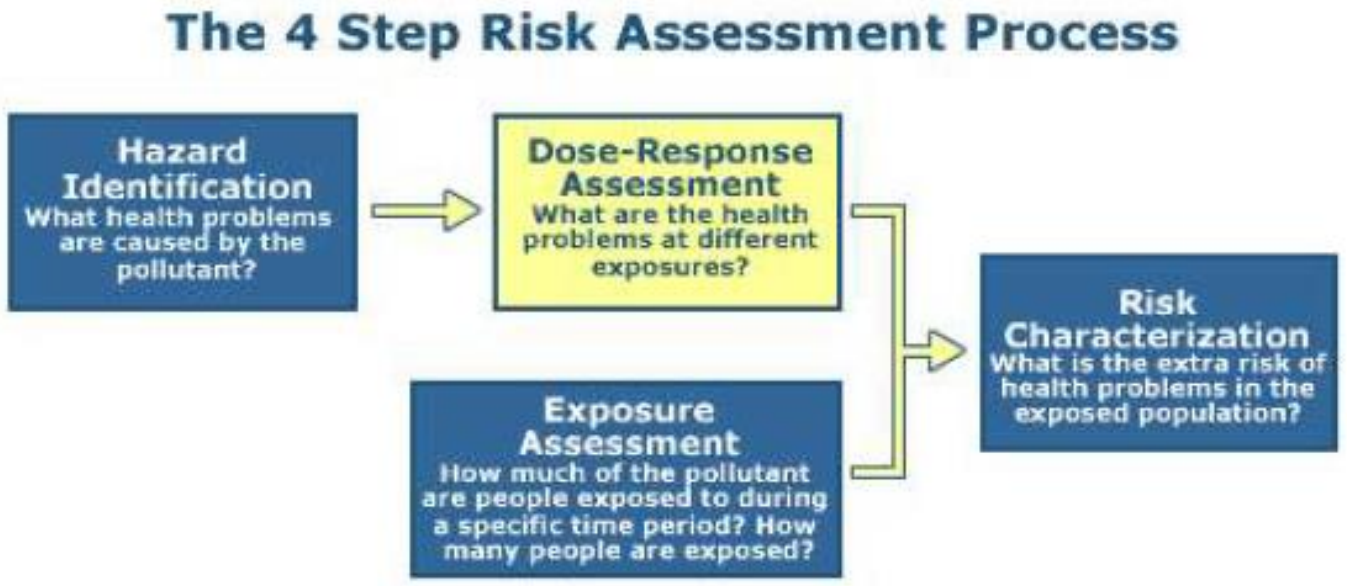
Risk Assessment Process from Toxicological Studies

U.S. EPA in 1992: detailed framework for the Ecological Risk Assessment Process by pollutants and environmental stressors

Stressor = any physical, chemical, or biological entity that can induce an adverse ecological response.
Adverse responses = sublethal chronic and acute effects in individual up to organisms to a loss of ecosystem function.
Contains the steps needed for evaluating on scientific terms the adverse effects of pollutants (stressors) on ecosystems and components of ecosystems.



4 fundamental phases:

1. Hazard identification
2. Dose-response assessment
3. Exposure assessment
4. Risk characterization.

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The Ecological Risk Assessment under the **European Environment Agency**
➔ developed from that already established for human health.

General principles = widely agreed upon **but**:

- Human risk assessment deals only with **one target organism = human** and concerned with individuals and morbidity and mortality
- Environmental risk assessment = concerned with biological **populations and communities** and the effects of substances on **mortality and fecundity**, multitude of organisms, all with varying sensitivities to chemicals and **various groups** have **distinct exposure scenarios**, such as free swimmers and sediment dwellers.

➔ difficulty in obtaining toxicity data on all organisms in an ecosystem

➔ recognized practice = test selected representatives of major taxonomic groups and use these as surrogates for the whole system.

➔ questionable as it may not protect the most sensitive species exposed in the environment.
➔ Failure to identify the effects of an agent on a potential receptor can result in widespread damage to organisms and ecosystems.

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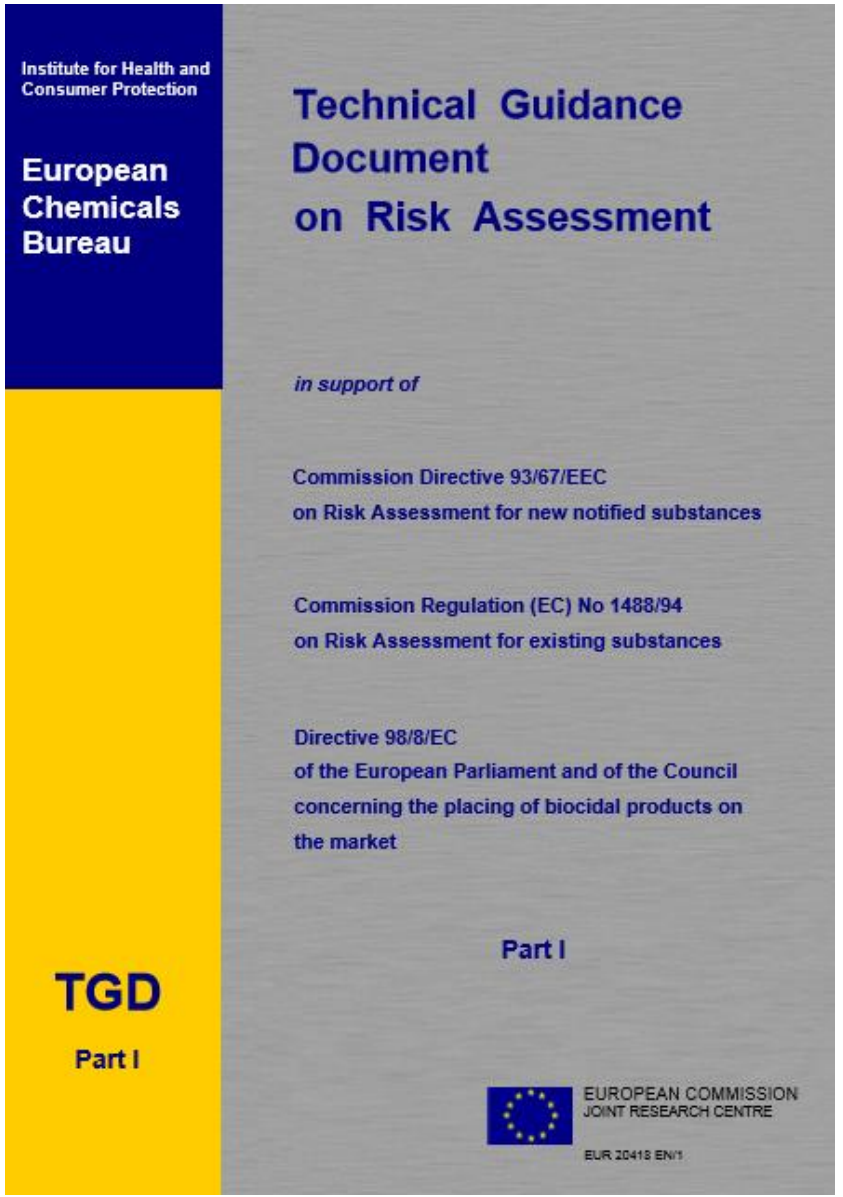
European Union: Technical Guidance Document (EU TGD)

Environmental compartments considered for the inland environment :
- Aquatic
- Terrestrial ecosystem
- Top predators
- Microbial activity in STP
- Atmosphere.

A new chapter on Marine risk assessment was added.

➔ for each of these compartments a PNEC has to be derived for the chemical studied.

PNEC = Predicted No Effect Concentration
➔concentration below which an unacceptable effect will most likely not occur.



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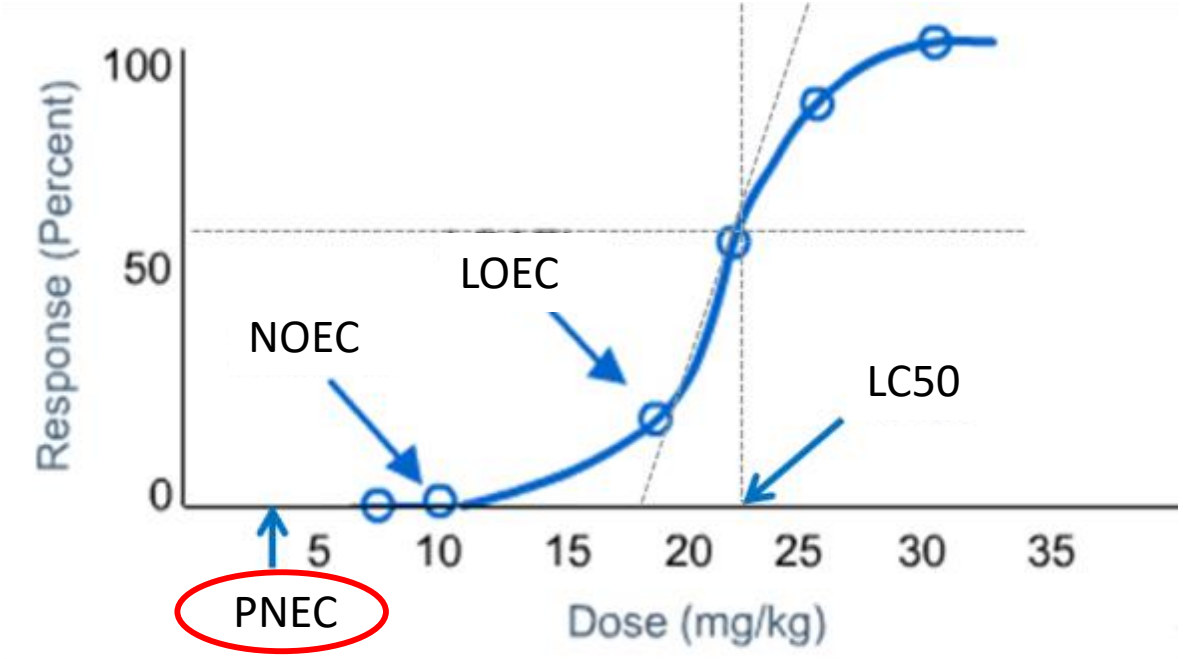

➔ PNEC is calculated by dividing the lowest short-term L(E)C50 or long-term NOEC value by an appropriate **assessment factor.**

Assessment factors: reflect degree of uncertainty in extrapolation from laboratory toxicity test data for a limited number of species to the 'real' environment.

Assessment factors applied for long-term tests are **smaller** as the uncertainty of the extrapolation from laboratory data to the natural environment is reduced.

➡ Long-term data are preferred to short-term data.

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Derivation of PNEC from toxicity data

PNEC = concentration that, if not exceeded, ensures an overall protection of the environment.

Assumptions taken to extrapolate from single-species (short-term) toxicity data to ecosystem effects:

- ecosystem sensitivity depends on the most sensitive species,
- protecting ecosystem structure protects community function.

➔ By establishing which species is the most sensitive ➔ extrapolation can subsequently be based on the data from that species.

➔ The functioning of any ecosystem in which that species exists is protected.

➔ It is generally accepted that protection of the most sensitive species should protect structure, and hence function.

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Assessment factors

➔ For most substances: pool of data from which to predict ecosystem effects is very limited
➔ in general, only short-term toxicity data are available.
➔ empirically derived assessment factors must be used to extrapolate from LC50/EC50/NOEC data to PNECs.
➔ the intention is to predict a concentration below which an unacceptable effect will most likely not occur.

Size of these assessment factors ➔ depends on the confidence with which a PNEC can be derived from the available data.

Uncertainties must be addressed to extrapolate from single-species laboratory data to a multi-species ecosystem.

- intra- and inter-laboratory variation of toxicity data;
- intra- and inter-species variations (biological variance);
- short-term to long-term toxicity extrapolation;
- laboratory data to field impact extrapolation (additive, synergistic and antagonistic effects from the presence of other substances may also play a role here).

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➔ confidence increases if more data are available for a number of trophic levels, taxonomic groups and with lifestyles representing various feeding strategies

➔ Lower assessment factors can be used with larger and more relevant datasets than the base-set data, e.g. if a large data set from long-term tests for different taxonomic groups is available

Cases

1) Only short-term toxicity data are available ➔ **AF = 1000** applied on the lowest L(E)C_{50} irrespective of whether or not the species tested is a standard test organism**.**
2) Long-term tests with a relevant test organism ➔ **Lower AF will** be applied on the lowest NOEC

If large number of validated short-term L(E)C_{50} is available for the same species and end-point

Calculation of geometric mean if more than one L(E)C_{50} value is available.

Prior to calculating the geometric mean an analysis of test conditions must be carried out in order to find out why differences in response were present

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Available data	Assessment factor
At least one short-term L(E)C50 from each of three trophic levels of the base-set (fish, Daphnia and algae)	1000 [a]
One long-term NOEC (either fish or Daphnia)	100 [b]
Two long-term NOECs from species representing two trophic levels (fish and/or Daphnia and/or algae)	50 [c]
Long-term NOECs from at least three species (normally fish, Daphnia and algae) representing three trophic levels	10 [d]
Species sensitivity distribution (SSD) method	5-1 (to be fully justified case by case) [e]
Field data or model ecosystems	Reviewed on a case by case basis [f]

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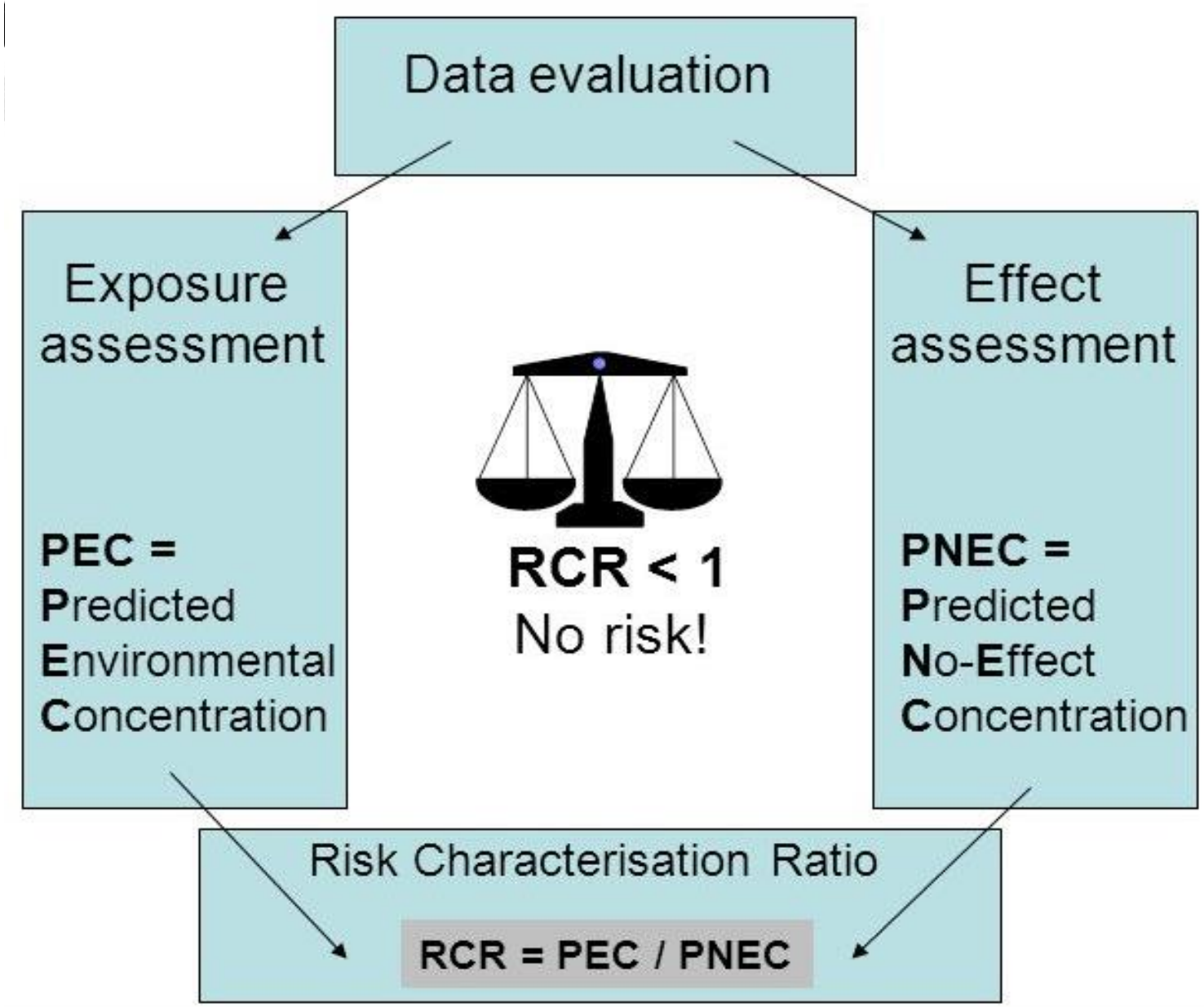

Data availability in environmental compartments

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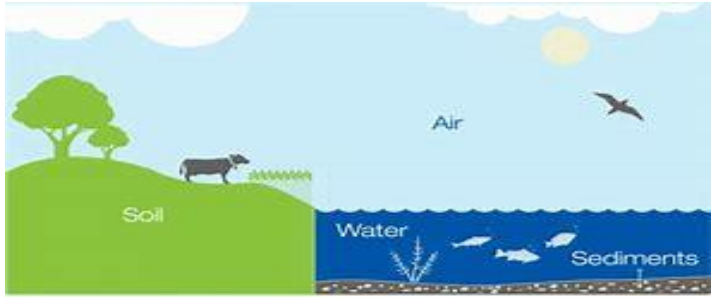
<u>Aquatic compartment:</u> most toxicity data available ➔ detailed assessment of the environmental risk

- New substances: base-set of toxicity testing consists of effect data for **aquatic organisms**
- Existing substances: most of the available data will be for aquatic organisms.



<u>Sediment compartment:</u> for most compounds no data available for sediment-dwelling organisms.

- Appropriate test systems and standardized guidelines are still under development (spiking protocols, flow through, contaminated water,…)
- The **equilibrium partitioning method** is proposed as a screening method for derivation of a PNECsed to compensate for this lack of toxicity data.
- If sediment test results are available ➔ the PNECsed is derived from these data by applying assessment factors.

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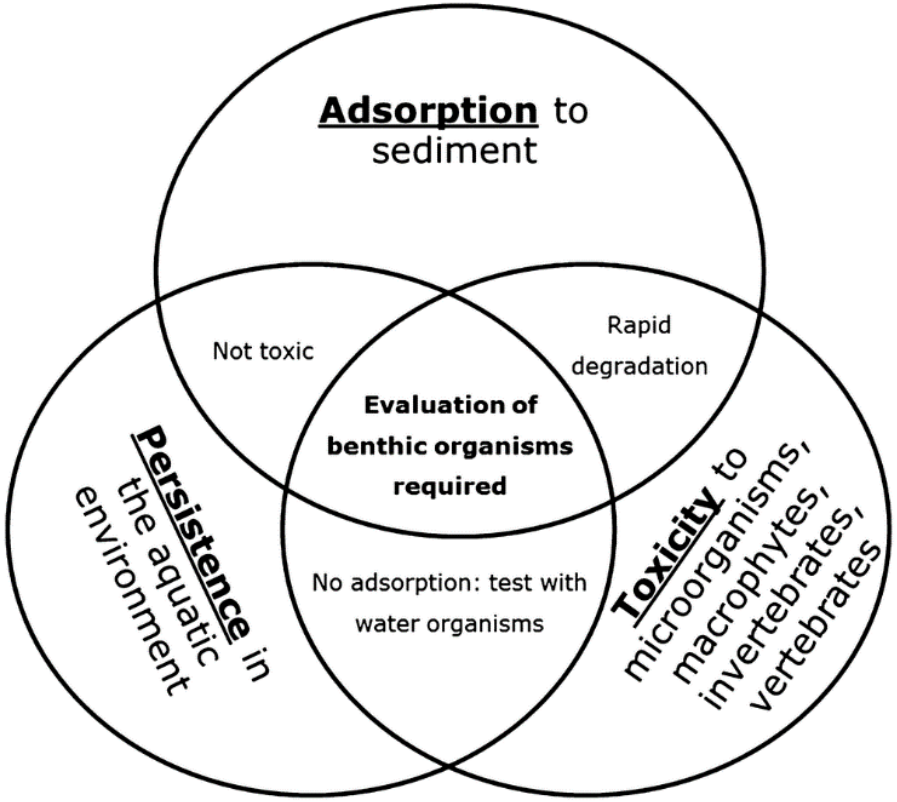
Effect assessment for sediment organisms

Substances that are potentially capable of depositing on or sorbing to sediments to a significant extent have to be assessed for toxicity to sediment-dwelling organisms.



Generally:
substances with a log Koc or **log Kow of < 3**
➔ not likely sorbed to sediment (SETAC, 1993).

➔To avoid extensive testing of chemicals:
a log Koc or **log Kow of ≥ 3** is used as a trigger value for sediment effects assessment.

Equilibrium partitioning method

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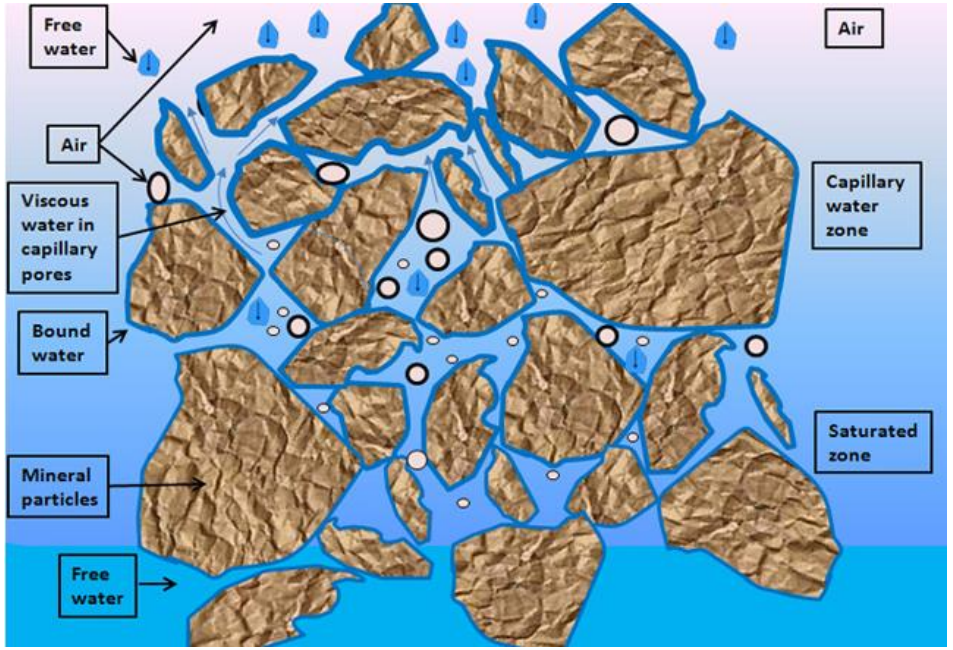
Most chemicals

➔ absence of any ecotoxicological data for sediment-dwelling organisms

➔ PNECsed may be provisionally calculated using the equilibrium partitioning method = screening approach.



Uses PNECwater for aquatic organisms and the sediment/water partitioning coefficient as inputs (OECD, 1992b; Di Toro et al., 1991).

Assumptions:

- Sediment-dwelling organisms and water column organisms are equally sensitive to the chemical;
- Concentration of the substance in sediment, interstitial water and benthic organisms are at thermodynamic equilibrium: the concentration in any of these phases can be predicted using the **appropriate partition coefficients;**
- **Sediment/water partition coefficients** can either be measured or derived on the basis of a generic partition method from separately measurable characteristics of the sediment and the properties of the chemical.

Kow = proxi

Equilibrium partitioning method

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$$PNEC_{sed} = \frac{K_{susp-water}}{RHO_{susp}} \cdot PNEC_{water} \cdot 1000$$

$$K_{susp-water} = Fwater_{susp} + Fsolid_{susp} \cdot \frac{Foc_{susp} \cdot Koc}{1000} \cdot RHO_{solid}$$

Parameter	Description	Default value
$K_{susp-water}$	Suspended matter-water partition coefficient	Calculated
RHO_{susp}	Bulk density of suspended matter	1150kg/m3
$Fwater_{susp}$	Volume fraction water in suspended matter	0.9
$Fsolid_{susp}$	Volume fraction solid in suspended matter	0.1
Foc_{susp}	Weight fraction organic carbon in suspended matter	0.1
Koc	Organic carbon water partition coefficient, measured or estimated from log Kow	Key input
RHO_{solid}	Density of solid phase	2500kg/m3

➔ Results from this screening ➔ decision on whether whole-sediment tests with benthic organisms should be conducted.

➔ Tests with benthic organisms using spiked sediment are necessary if, using the equilibrium partitioning method, a PEC/PNEC ratio > 1 is derived.

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Three situations for deriving a PNECsed:

1. **No toxicity test results are available** for sediment organisms

➔ Equilibrium partitioning method for identification of potential risk to sediment organisms = "screening approach".

➡ BUT: Considers exposition only through interstitial water, not the sediment bound contaminant. Hydrophobic chemicals (e.g. PAHs) tend to be bound to OC in sediment.

2. Only **acute toxicity test results for benthic organisms** are available

➔ risk assessment is performed both on the basis of the test result of the most sensitive species using an **assessment factor of 1000** and on the basis of the **equilibrium partitioning method**.

➔ The lowest PNECsed is used for the risk characterisation;

3. **Long-term toxicity test data are available for benthic organisms**

➔ PNECsed is calculated using assessment factors for long-term tests

➔ this result should prevail in the risk assessment.

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The $PNEC_{sediment}$ is derived from the lowest available NOEC/EC10 obtained in long-term tests by application of the following assessment factors (**Table 19**):

Table 19 Assessment factors for derivation of $PNEC_{sed}$

Available test result	**Assessment factor**
One long-term test (NOEC or EC10)	100
Two long-term tests (NOEC or EC10) with species representing different living and feeding conditions	50
Three long-term tests (NOEC or EC10) with species representing different living and feeding conditions	10

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However: Toxicity testing ➔ time and cost intensive:

<u>One new chemical:</u>
➔ ideally tested on different organisms, at different developmental stages, in different environments.

<u>Chemical mixtures ➔ different effects:</u>
➔ ideally tested on different organisms, at different developmental stages, in different environments.

<u>Each year:</u>
➔ manufacture and release of 1000 – 1500 new products

➔ Exponential numbers of tests required

➔ Traditional toxicity testing not feasible

➔ Huge amount of money and work load to test all chemicals

➔ Impossible to continue with conventional laboratory toxicity evaluation for ERA purposes

➔ Need for identifying and developing novel, rapid approaches for assessing the hazards of substances

Mechanistic toxicity ➔ molecular approaches

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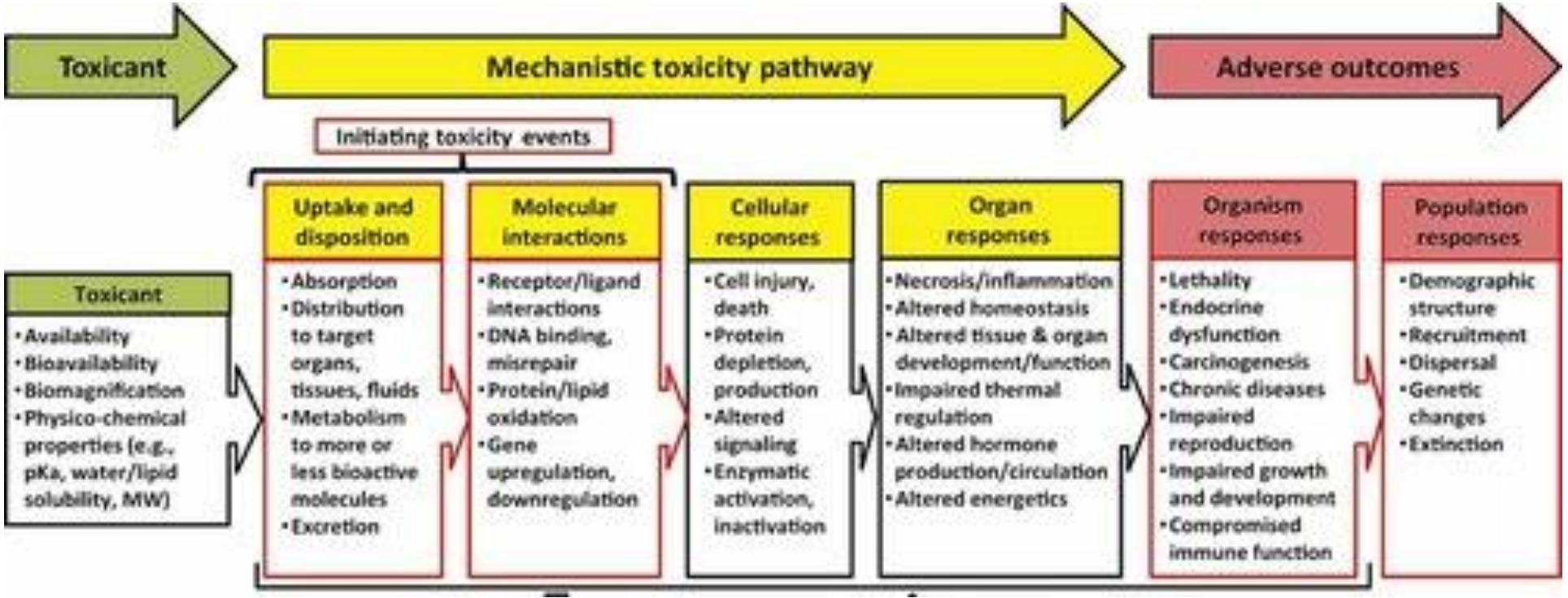
Identification and understanding molecular, cellular and biochemical basis by which chemicals exert toxic effects.

Mechanistic studies ➔ essential for development of tests for risk prediction and facilitating search for safer chemicals

Molecular structure:
➔Determines Molecular Initiating Event
➔Prediction of effects (Q)SAR
➔ Categorisation
➔3Rs: Reducing number of toxicity tests ($$ y time)
➔Early warning



B2_M1: Ecotoxicity tests in risk assessment. Erasmus Mundus Master in Water and Coastal Management.

Summary

During this course we will learn how to assess the risk that the presence of a certain contaminant poses to site specific environments. We will see how to obtain different toxicity parameters from laboratory toxicity tests with different test organisms and how to use this information to determine the hazard of this compound at certain environmental concentrations taking into account different environmental matrixes and situations.

In order to evaluate this session, I will ask you to perform an environmental risk assessment of a contaminant and environment/area of your choice and to represent it in a 10 minutes Power Point presentation. For this, you will be asked to:

- Select a regional/typical/exceptional economic activity in your country
- Identify the major contaminant this activity would generate considering its fate
- Design adequate test battery to evaluate the risk of the major compounds (compartments/organisms)
- Use publicly available data (literature) for toxicity parameters of the contaminant (LC50; EC50 values) if possible for your selected test organism.
- Search databases for environmental concentrations of the selected compound in your area.
- Carry out environmental risk assessment

Power point presentations (10 mins): to be sent to me

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WACOMA

Erasmus Mundus Joint Master Degree in WAter and COastal MAnagement
2-years Master Degree/Second Cycle Degree

Guide for practical session

TOXICITY TESTS IN INITIAL STAGES OF GILTLE DEVELOPMENT, SPARUS AURATA.

Marine ecosystems are receptors of a multitude of anthropogenic substances of inorganic and organic origin that are discharged directly or through riverbeds. Substances of anthropogenic origin can interact, at different levels, with the organisms present and may ultimately lead to variations in ecosystem productivity or a loss of ecosystem biodiversity.

Among the organic substances that are discharged into aquatic ecosystems, surfactants have a high importance derived from the high production and consumption volumes worldwide. The term surfactant is used for a wide group of substances whose solution behaviour makes them have characteristic properties such as moisturizer, dispersant, detergency and solubilising. These superficially active substances modify the structure of interfaces and affect mass and energy transfer processes. The surface activity of these substances is related to the asymmetrical structure of their molecule, which has a hydrophilic and a hydrophobic part.

Although there are many surfactants, Linear Alkylbenzene Sulfonate (LAS) is the most commonly used surfactant in the formulation of personal hygiene and household cleaning products. Globally, LAS production was estimated at about 4 million tonnes in 2000. The interest of these substances from an environmental point of view is evident from this data.

The exposure of aquatic organisms to LAS results mainly from wastewater treatment plants. Even if they operate with high efficiency, they introduce a certain quantity of these substances through the effluents of their facilities into the surface waters of the receiving waters where the present organisms are exposed to them. Hence, the need to know the risk these substances may pose to individuals, populations and ultimately ecosystems.

The objective of the environmental risk assessment is therefore to estimate the risk or likelihood of adverse effects in communities of species that are potentially exposed to pollutants.

The gilthead, *Sparus aurata* (Linnaeus, 1758) is a teleost fish belonging to the family Actinopteri (Fig. 1.). It is distributed throughout the Mediterranean and the eastern shores of the Atlantic Ocean from Great Britain to the Cape Verde Islands, with the Gulf of Cadiz being an important fry-producing area. In 1997 alone ten million fry were produced. It is a typically coastal and eurihaline species that performs reproductive migrations occupying coastal waters and estuaries. Thus, its habitat can easily coincide with sewage discharge zones, potentially getting into contact with all kinds of chemical compounds.

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Practical laboratory session:

Exposure of eggs of the seabream, Sparus aurata to **commercially used surfactants** for 24 h.

Evaluation of mortality and LC50 derivation

Risk assessment for the Bay of Cadiz (known surfactant concentrations)

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SEDIMENT TOXICITY

INTEGRATIVE TOOLS TO DETERMINE ENVIRONMENTAL QUALITY

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Sediments:

Detrital, inorganic, or organic particles eventually settling on the bottom of a body of water (Power and Chapman 1992).

Deposited by:

- natural forces of currents (a constant flow of water in a predominant direction)
- gravity (attraction between two masses)
- flows of incoming streams and rivers

Composed of:

- clastic/mechanical materials: inorganic accumulations of flakes, grains, or pieces of weathered rock such as silt, sand, and gravel.
  (➔ erosion)
- chemical materials: natural precipitates such as rock salt and gypsum.
- organic materials: organic remains
  (➔ decomposition of natural elements, animals, plants, coal, shells)
- water: interstitial pore water

Very complex and dynamic nature, particularly when considered on watershed scale.

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Why is sediment important?

Sediment = stored in wetlands, floodplains, streams, lakes, and the banks of the shorelines.

- Important part of many ecosystem processes
- Important for many species (life cycles, reproductive and nursing habitat, feeding)
- Very productive ecosystems

Changes in deposition rate:

The amount of sediment reaching these areas is primarily altered by

- draining or filling wetlands,
- changes in shoreline,
- channelization of streams,
- dams
- dredging

Aquatic toxicity vs sediment toxicity

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Toxicity of most contaminants ± consistent among different WATER bodies

➔ same concentration of a contaminant that produces a toxic effect in one water body will produce a similar effect in other water bodies.

Toxicity depends upon state/ form of the contaminant and the characteristics of the environment in which it is dissolved.

Sediment = complex material ➔ more complicated effect on the toxicity of contaminants than water.

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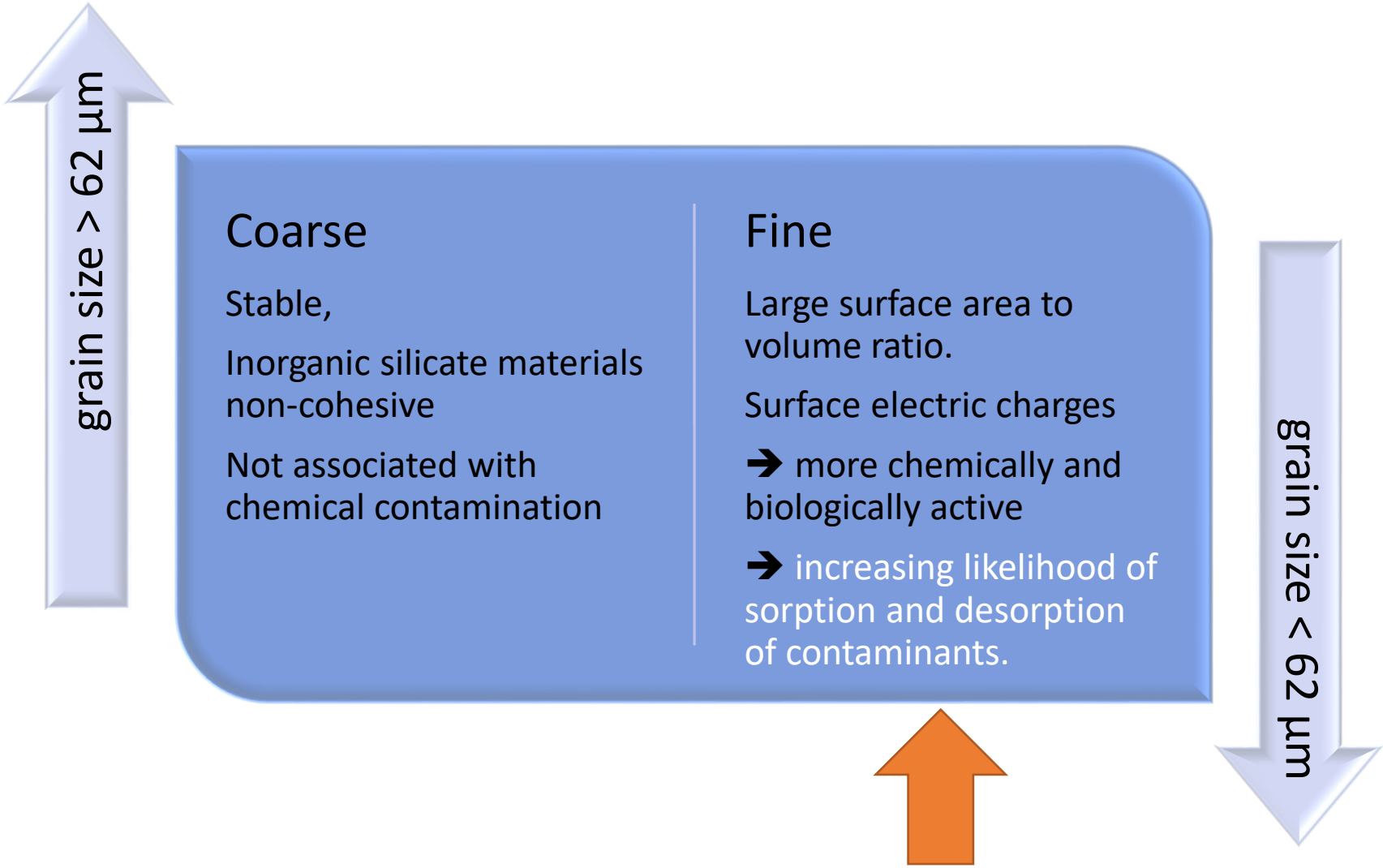
Classification into two groups - Power and Chapman (1992)



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Dynamic character:

Sedimentation ➔ contaminants/toxic microorganisms in the water carried to bottom sediments ➔ accumulation

SOURCE

Change in existing conditions

➔ RESUSPENSION

Severe weather: storm, high flows, ice scour

Changes in discharge

Human activities:

- dredging
- trawling, …

(Ad-)sorption onto particulate matter

Sedimentation

➔ Bottom sediments

ACCUMULATION

SINK

Resuspended contaminants ➔ risk to aquatic life. Toxicity of contaminants can be altered under different conditions.

Must be taken into consideration when evaluating risks from contaminated sediment.

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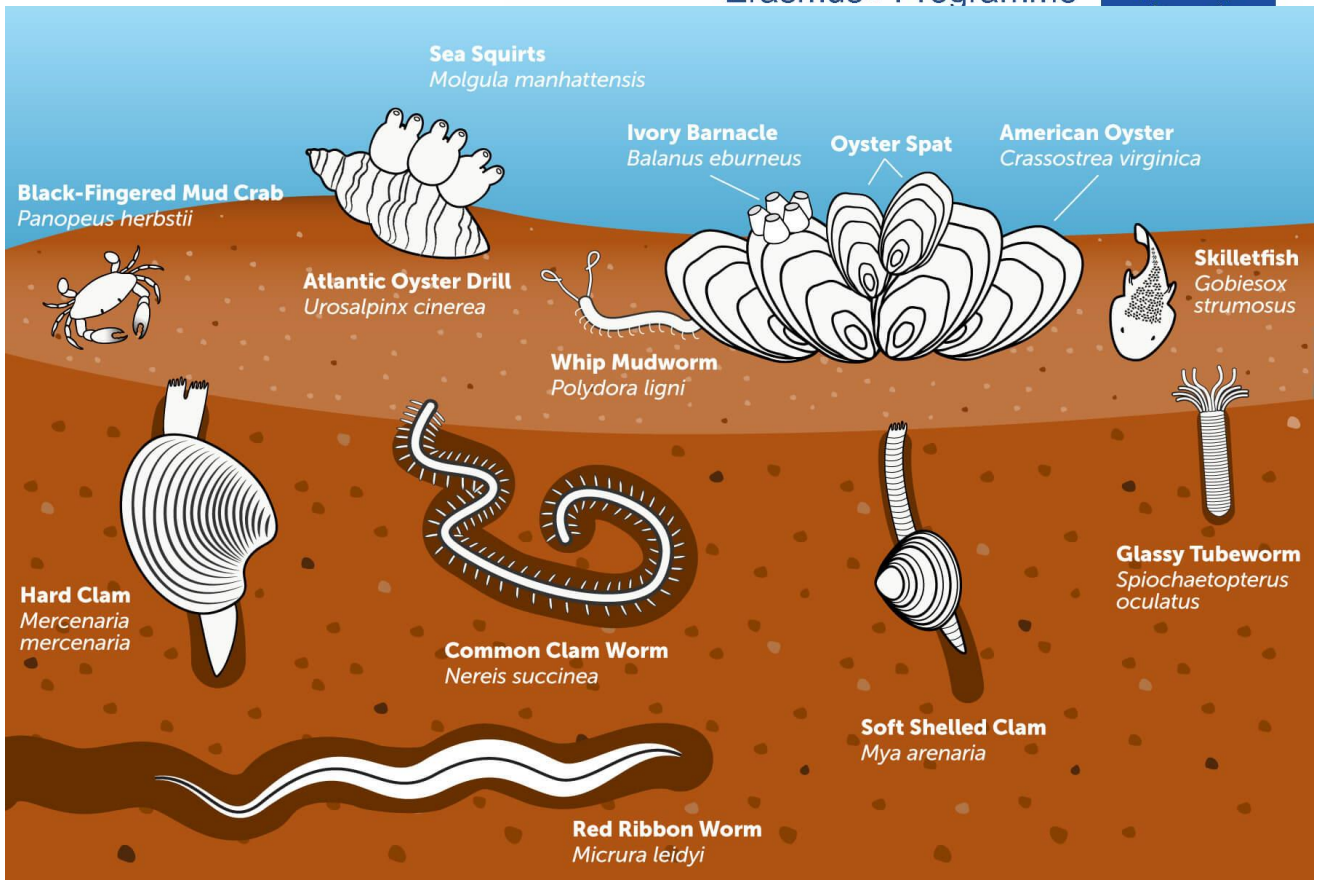
Bioavailability

➔ relationships between the concentration of a contaminant in sediment and the portion of that concentration an organism incorporates.



Sediment characteristics:

- pH,
- cation exchange capacity (CEC),
- redox potential,
- oxic state,
- composition of sediment (e.g., sand, clay, silt),
- amount and type of clay present,
- grain size,
- pore size,
- nature and volume of organic carbon present,
- presence of sulfides, nitrates, carbonates, and other organic and inorganic substances.

➔ Alteration of chemical and biological activity of contaminants
➔ Sediment characteristics: determine bioavailability of contaminants.
➔ Life styles

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Example:

Metal bound to a clay particle or present as a sulfide precipitate is not available for uptake from pore water through the gills, but that same metal fraction could be bioavailable as it passes through the digestive tract of an organism following ingestion.

Organisms:

exposed to **bioavailable fraction** of a contaminant in sediment available for uptake of organism ➔ causes toxicity.

Bioavailable fraction

➔ not a fixed quantity
➔ can be altered continuously by physical, chemical, and biological processes
➔ depends on exposure pathways.
➔ high degree of variability in the concentration of a contaminant that is bioavailable and likely to cause toxicity in different sediments

➔ no single concentration of a contaminant in sediment can accurately represent a threshold toxicity for benthic organisms in all sediments

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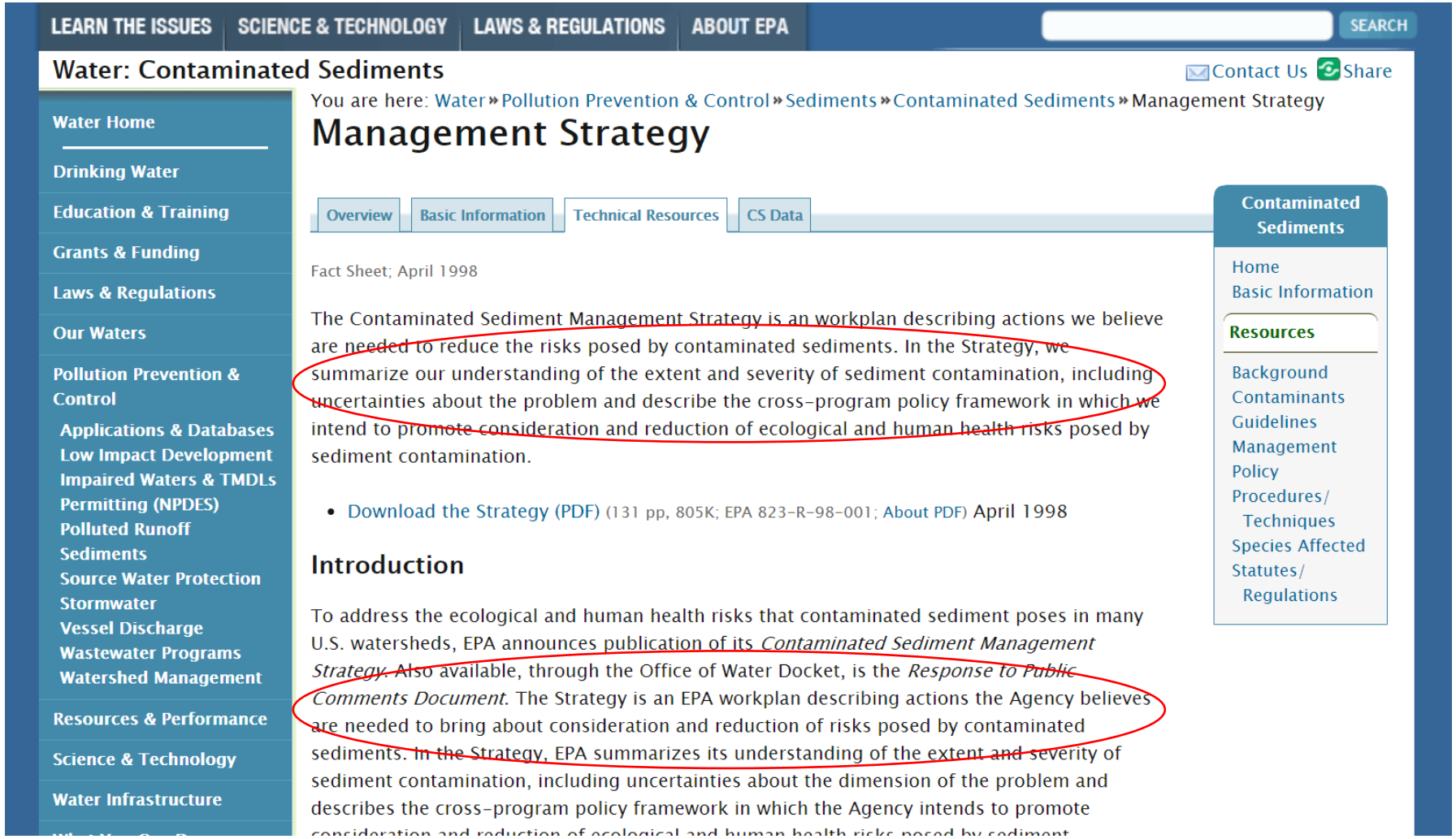
Problem of sediment contamination: Increasing in many areas throughout the world

EPA survey 1998

- ➔ Hundreds of contaminated sites
- ➔ Many coastal áreas ➔ rich hábitats for animals and plants
- ➔ Every major harbour in USA has some degree of contamination in local sediment



https://archive.epa.gov/water/archive/polwaste/web/html/stratndx.html

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Concerns about sediment contamination

Recent studies of the quality of the nation's lakes, rivers, and bays, and concerns about the economic impacts associated with contaminated fish and disposal of contaminated dredged material make sediment contamination an important issue.

- EPA estimates that 10 percent of the nation's lakes, rivers, and bays have sediment contaminated with toxic chemicals that can kill fish living in those waters or impair the health of people and wildlife who eat contaminated fish (*Listing of Fish and Wildlife Consumption Advisories*, EPA 823-C-97-004, 1997; *The Incidence and Severity of Sediment Contamination in Surface Water of the United States*, EPA 823-R-97-006, 007, 008, 1998).

- Fifteen percent of the nation's lake acreage and 5 percent of the nation's river miles are under state-issued fish consumption advisories. All of the Great Lakes and a large portion of the nation's coastal waters are also under advisory (*Listing of Fish and Wildlife Consumption Advisories*).

- Billions of dollars of economic activity are potentially affected by contaminated sediment because of the loss of recreational and commercial fishing and the increased cost of disposing of contaminated material dredged to aid navigation.

Find Contamination

Why does EPA need a Contaminated Sediment Management Strategy?

EPA needs an Agency-wide Contaminated Sediment Management Strategy because cooperation among many EPA offices is necessary to address the problem of contaminated sediment.

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Sediment Quality Guidelines

<1980s: contamination level of sediments was determined by comparing the concentration of a chemical in sampled sediments to **"background" or reference values**.

But: does not account for
- types of biological resources in an aquatic environment
- concentration at which an adverse response would be observed in these organisms.

➔ Development of sediment quality guidelines (SQGs)
➔ assessing sediment quality = contaminant concentrations that cause adverse effects (SETAC, 2002).

SEDIMENT QUALITY GUIDELINES (Pellston Workshop):
Numerical chemical concentrations intended to be either protective of biological resources, or predictive of adverse effects to those resources, or both.
All SQGs can be used to asses individual chemicals by comparing the chemical concentration with the limit concentrations or to estimate the probability of acute sediment toxicity and to determine the possible biological effect of combined toxicants

Background concentration levels

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Contaminants = chemical compounds that
- generally, do not occur naturally in sediment
- have the potential to harm aquatic life

➔ Sediment is considered contaminated if it contains a concentration of a compound that is not produced naturally or is present in a concentration other than what would be expected to result from natural processes, and that has the potential to be harmful to aquatic life.

Some compounds: can also occur naturally.
- Metals = natural components of minerals that originated from weathered rock.
- Organic compounds: e.g. polycyclic aromatic hydrocarbons (PAHs): also naturally produced during forest fires, ammonia or acetone: result of microbial metabolism.

Prior to evaluating risks of contamination ➔ one must decide which substances qualify as contaminants.

Background concentrations: concentrations of naturally occurring "contaminants"

"The concentration that is the result of natural processes, including weathering and subsequent erosion of local soil and bedrock, and atmospheric deposition unaffected by anthropogenic activity." (Rice, 1999)

Synthetic organic compounds
➔ not produced naturally
➔ Background = concentration of the same compound in sediments of a "clean site"

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Chemical based Sediment Quality Guidelines

SQV

Response to society's increasing demands for *greater environmental protection of aquatic resources* and maintenance of dredged rivers, estuaries and ports

Development of methodologies for evaluating the degree to which sediment associated chemicals might adversely affect aquatic organisms

Better protection of benthic organisms

Maintenance of designated uses of freshwater, estuarine and marine environments

Assistance sediment assessors and managers for the interpretation of sediment quality

Assessment of potential risks to aquatic life from contaminant concentrations in sediment regardless of their possible source.

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Empirical SQG

Dose-response relationships

Database of sediment chemistry and observed biological effects (toxicity tests and benthonic community alteration)

Metals: much available information

Theoretical SQG

Equilibrium partitioning relationships,
➔bioavailability

Theoretical understanding of the factors that govern bioavailability.

Organic compounds: less information available

(Burton, Jr., 2002)

Predict adverse ecological effects from sediment contamination by the response of benthic organisms.
Classification of a contaminant in a sediment sample into one of three categories (A, B, C) of sediment contamination, relative to its potential risk.

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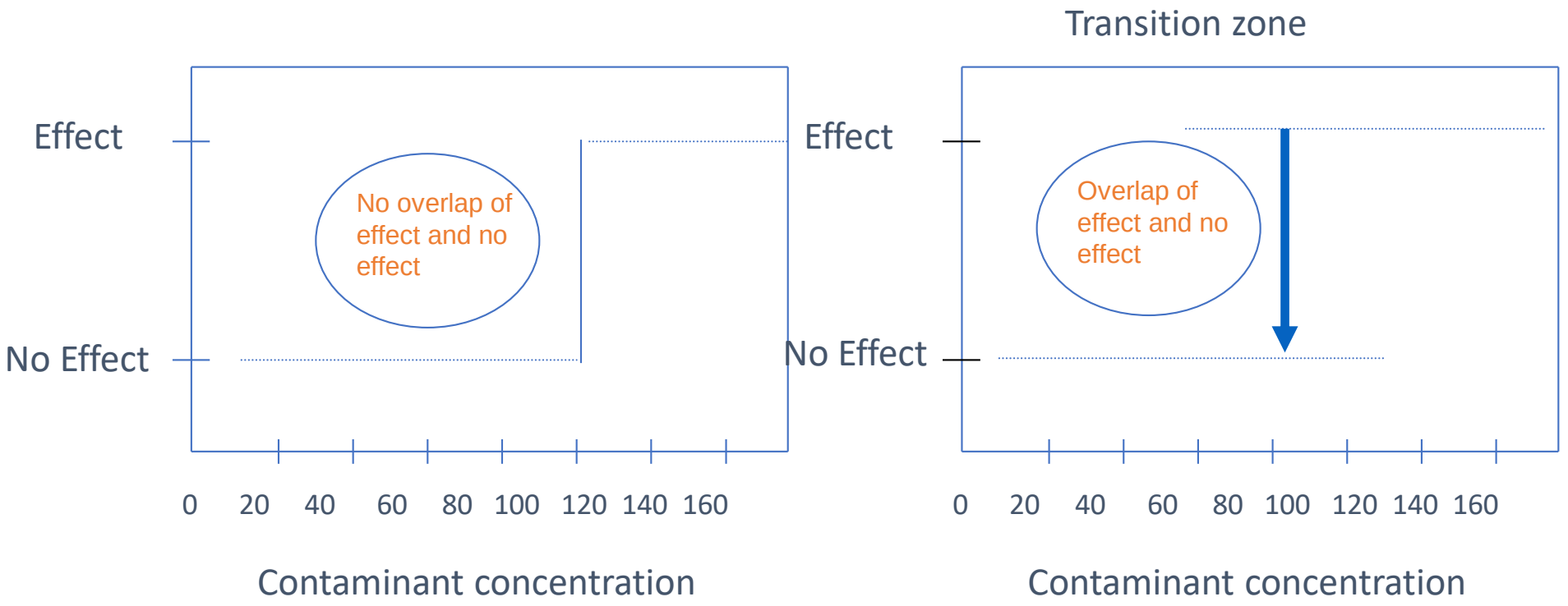
Limitations of SQGs



Typical pattern across a contaminant concentration gradient

- Low concentrations: toxicity does not occur,
- High concentrations: toxicity consistently occurs.
- Intermediate concentrations: concentration and toxicity results are mixed

➔ a given contaminant concentration might be toxic in one sediment sample but not in another.

➔ Toxicity within this range cannot be predicted reliably from the contaminant concentration in sediment.

High variability in concentration of contaminants in sediment that cause toxicity.

No guideline can unequivocally separate all sediments showing effects from those that not

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➔ To address this characteristic pattern of sediment toxicity ➔ two threshold concentrations are needed

- concentration of a contaminant below which toxicity is not expected to occur: C1
- concentration of a contaminant above which toxicity is expected to occur frequently: C2

➔ contaminants in a sediment sample can then be segregated into one of three different categories; Class A, B or C.

Class A – [contaminant] < C1 ➔ contaminant presents little or no potential for risk to aquatic life.

Class B – C1 (class A) < [contaminant] < C2 (class C)
➔ additional information is needed to determine the potential risk to aquatic life.
➔ The potential for risk to aquatic life cannot be ascertained from contaminant concentration data alone.

Class C – [contaminant] > C2 ➔ high potential for the sediments to be toxic to aquatic life.

Limited predictive capabilities in the “grey” region of contaminant concentrations between the 2 thresholds.

➔ Site-specific analysis: observation of health and behavior of benthic organisms.

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Chemical based Sediment Quality Guidelines
SQV

ADVANTAGES

- Predict sediments to be either toxic or non toxic in laboratory tests (acute toxicity) or in benthic community assessment
- Interpretation of sediment chemistry data
- Interpret or design environmental monitoring programs

DISAVANTAGES

- Difficult to predict the presence or absence of chronic toxicity in laboratory and field collected sediments
- They do not predict effects resulting from bioaccumulation of sediment-associated contaminants ➔ HUMAN RISK
- SQGs are site-specific
- Limitations of SQGs scientific underpinnings
- They are developed taking into consideration a group of contaminants that do not include emerging pollutants: EUROPEAN WATER FRAMEWORK

Useful tool that provides a first guess at the nature of a sediment contamination problem.
Combined with appropriate field and laboratory sampling and testing, SQGs are an important tool in practice for sediment contamination, remediation, and risk assessments.

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Further requirements of SQGs

Ability to predict presence/absence of chronic toxicity in laboratory and in field-collected sediments

Ability to predict effects resulting from bioaccumulation

Ability to establish cause and effects relationships

Ability to predict effects on organisms exposed in the field

SQGs in conjunction with other tools as sediment toxicity tests, bioaccumulation and benthic community surveys

WOE for assessing the hazards associated with contaminated sediments

(Ingersoll et al. 1997; Chapman et al. 2002)

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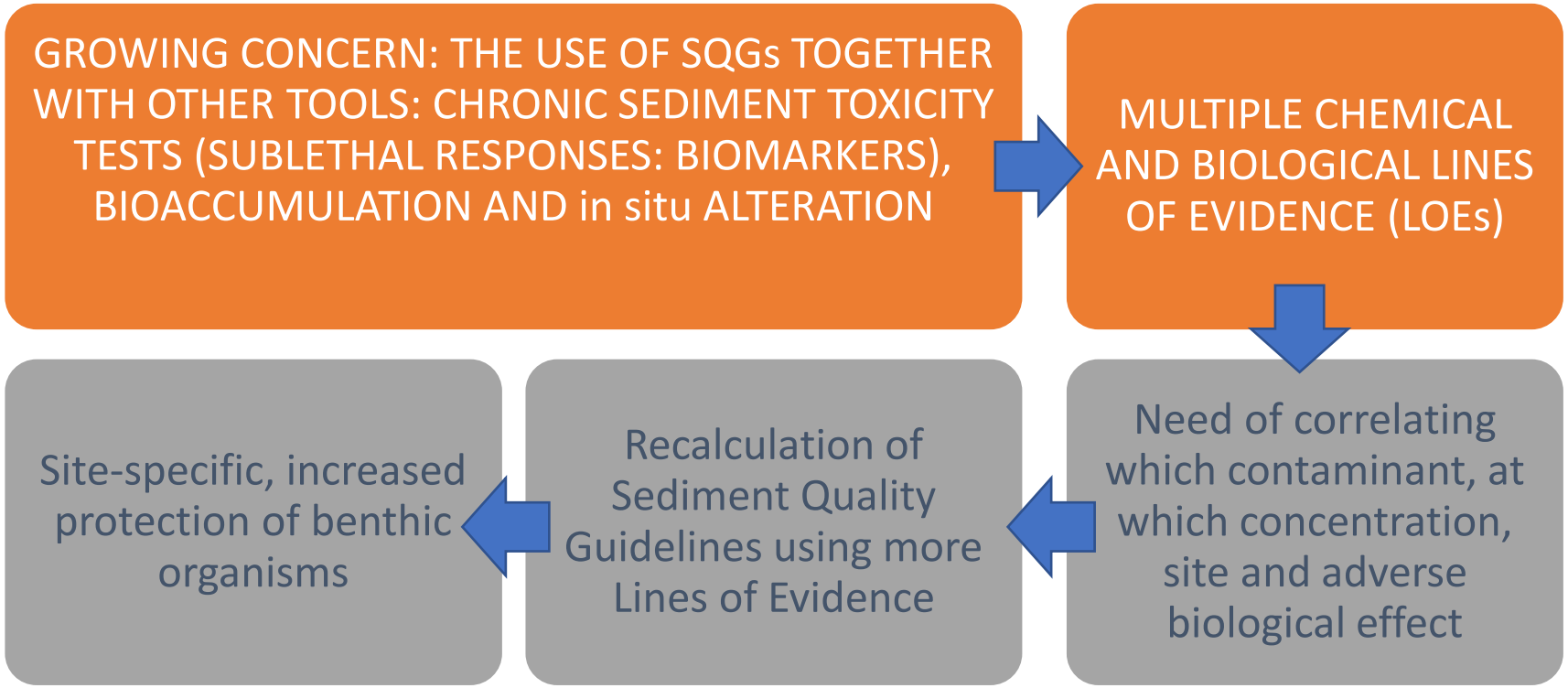
Weight of evidence approach

WOE



Assessment of potential risks to aquatic life from contaminant concentrations in sediment regardless of their possible source.

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Lines of Evidence (LOE)

- Strategic use of multiple approaches to address one question.
- Each approach has its own unrelated assumptions, strengths and weaknesses.
- Results that agree across different methodologies are less likely to be artefacts.

Weight of Evidence (WoE)

measure of amount of evidence on one side of an issue versus the evidence on the other side.

Weight of Evidence approach: inclusion of new LoEs

At least 4 key LoEs should be developed (Grapentine et al. 2002):

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Treatment of the data obtained synoptically ➔ global and realistic view of the state of the environment

➔ Identification of degraded zones and zones free of anthropogenic influence

➔ Determination of environmental quality criteria (contaminant concentrations associated and not associated to biological damage).

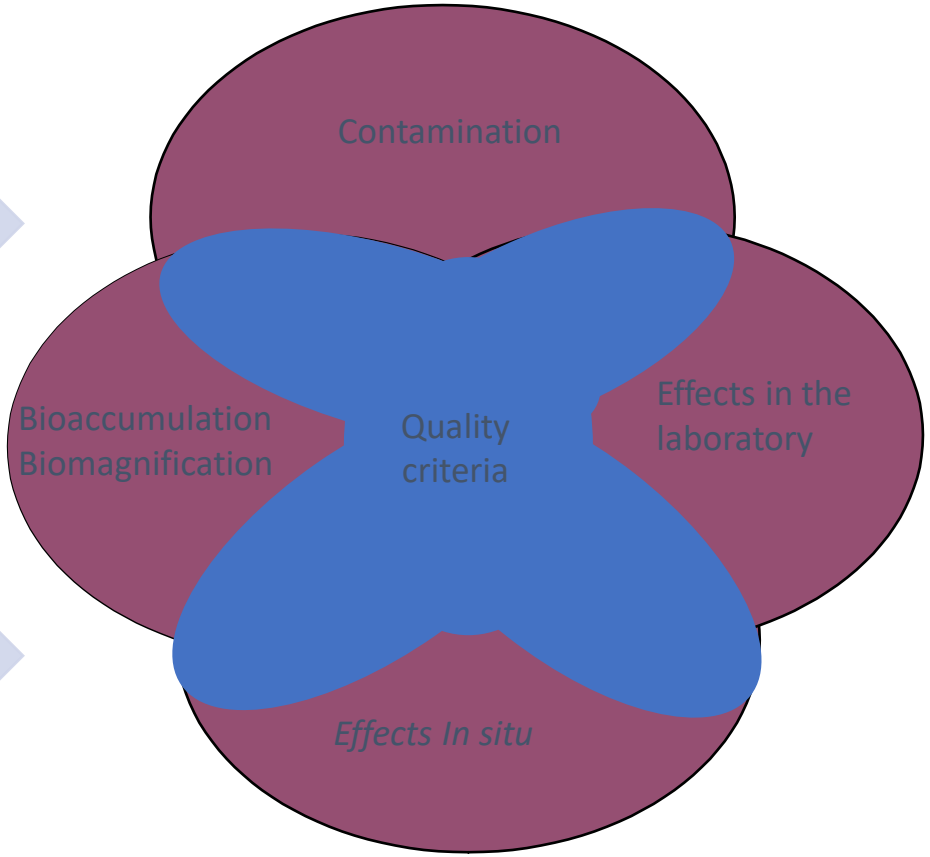
➔ By multivariate statistics.

ECOSYTEM HEALTH

- What contaminants?
- What levels?
- What biological effects?
- *Quantification of pollution*
- *Quality values*

HUMAN HEALTH

- Bioaccumulation
- Biomagnification
- *Tissue quality values*



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Different tools are proposed in order to obtain multiple LOEs in sediment quality assessment:

1) Sediment chemistry including numeric Sediment Quality Guidelines (SQGs);

2) Acute Toxicity tests;

3) Bioaccumulation tests;

4) Chronic Toxicity tests;

5) Resident aquatic community structure

These tools should provide the adequate estimation of the influence of the physical, chemical and biological factors in the level of exposure and bioavailability of the different xenobiotics in the sediment .

These tools expressing different lines of evidence are integrated in Environmental Risk Assessment methodologies and utilized in Sediment Monitoring and Assessment programs.

CONTAMINATION

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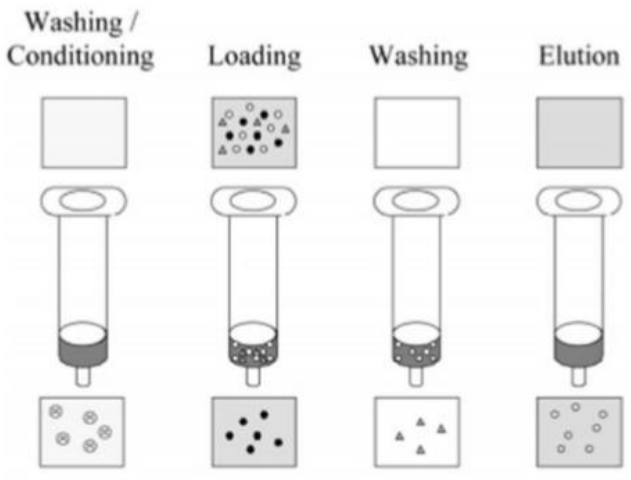
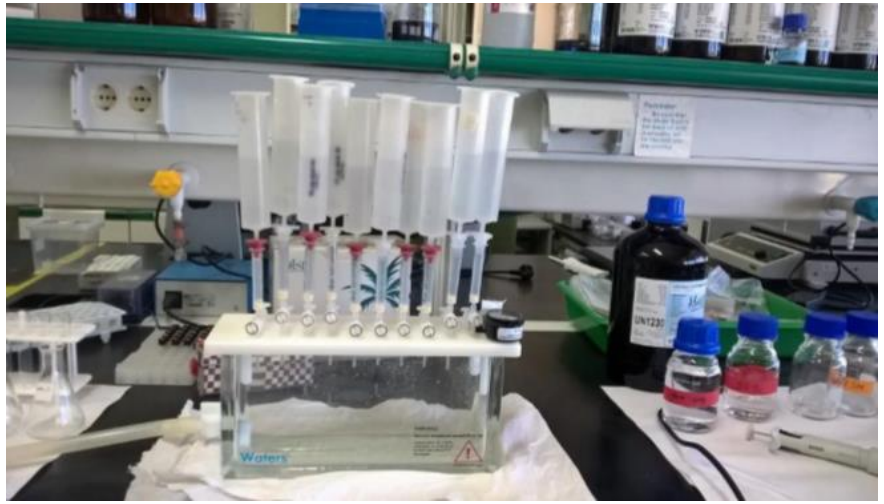
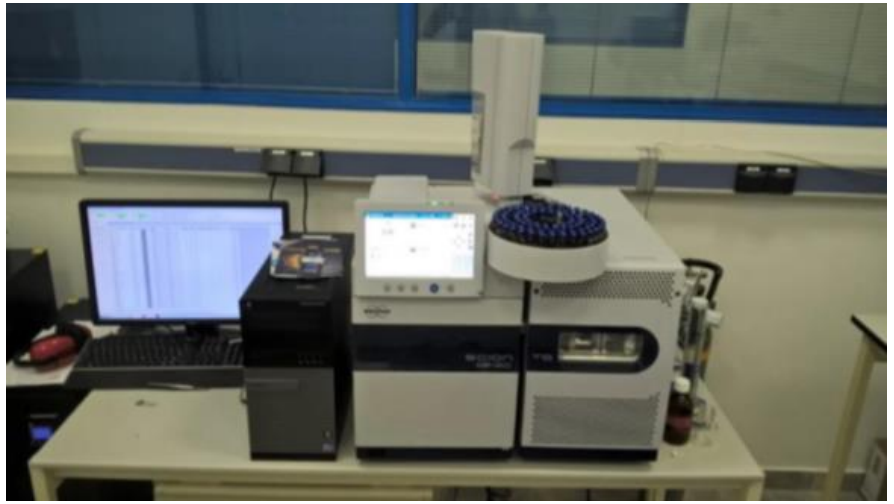
Sediment samples:

1) Sonication of sediments with MeOH
2) Distillation and resuspension in MilliQ
3) purificatión and preconcentration by Solid Phase Extraction (SPE)
4) Elution: 8 mL MeOH
5) Evaporation to dryness
6) Re-dissolution in MeOH-H2O (25:75), sonication, filtration
7) Ultraperformance liquid chromatography – tandem mass spectrometry (UPLC-MS/MS).

<u>Solid Phase Extraction</u>







Dissolved/suspended compounds: ➔ separated according to physical and chemical properties.

In situ effects

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- Chronic Toxicity
- Studies of benthic macrofauna

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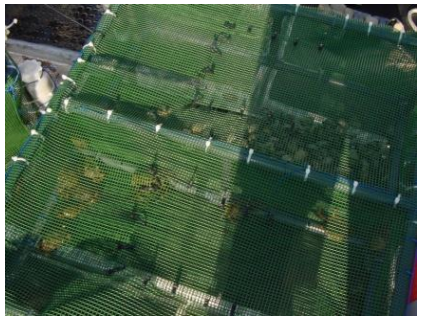
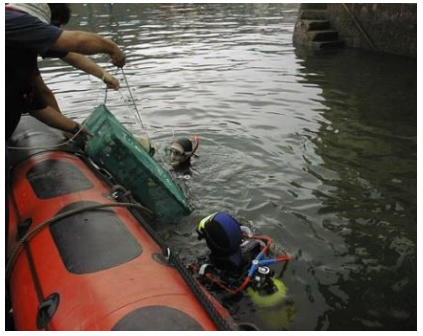
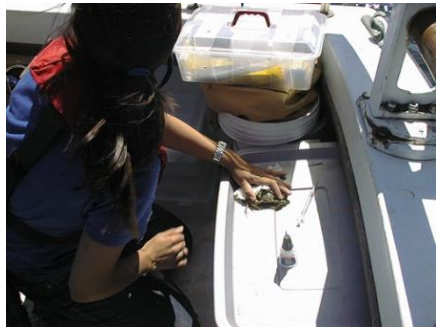
Chronic toxicity - Transplantation of organisms into the field





Organisms are shipped from the laboratory to the field.

Cages are divided in two different zones to maintain the crabs, *Carcinus maenas* (24 each cage) in one side and the clams, *Ruditapes philippinarum* (50 each cage) in the other side.

Cages are fixed to the bottom in duplicate each sampling zone.

Exposure during 30 days

Evaluation of mortality, growth, biomarkers,….

Study of benthic macrofauna

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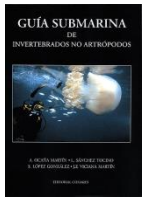
Sediment samples
Van Veen drag
0.025 m^2.

Sieveing

Preserve samples

Classification, identification and analysis

Stereoscopic microscope



Rio San Pedro Macrofauna
Abundance

	Samples - Punto muestreo/Réplica														
	SP1R1	SP1R2	SP1R3	SP2R1	SP2R2	SP2R3	SP3R1	SP3R2	SP3R3	SP4R1	SP4R2	SP4R3	SP5R1	SP5R2	SP5R3
Scrobicularia plana	0	0	0	0	2	0	0	0	1	28	10	10	41	43	32
Cerastoderma edul	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Columbella rustica	0	0	0	0	0	0	1	0	2	3	0	0	0	0	0
Hydrobia ulvae	0	0	0	16	7	4	12	6	4	7	17	19	38	21	28
Bittium reticulatum	0	0	0	0	0	0	4	1	2	2	0	1	2	1	1
Discus sp	0	0	0	0	0	0	2	1	1	0	0	0	0	0	0
Turritella sp	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Rissoa ventricosa	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Cerastoderma glau	0	0	0	0	0	0	0	0	0	0	0	0	1	2	1
Cyathura carinata	19	20	21	30	26	25	36	29	24	34	29	26	5	1	10
Pachygrapsus mar	0	0	0	0	0	1	0	0	1	0	0	0	0	0	1
Corophium volutat	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0
Copepoda	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Gammarus sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Nereis diversicolor	1	2	6	5	2	3	3	3	8	3	0	0	11	24	7
Paradoneis lyra	2	0	0	0	0	0	0	0	0	0	0	6	10	22	8
Pygospio elegans	0	0	0	0	1	0	0	0	3	3	10	12	7	7	9
Prionospio cirrifera	0	0	0	0	0	0	0	0	0	0	0	0	4	3	3
Capitela capitata	0	0	0	0	0	0	0	0	1	8	11	0	0	1	0
Oligochaeta sp1	0	0	0	0	0	0	0	0	0	2	0	14	0	1	0
Oligochaeta sp2	0	0	0	0	0	0	0	0	0	5	0	8	15	7	7
Oligochaeta sp3	0	0	0	0	0	0	0	0	0	1	0	6	3	9	6
Nematoda sp1	5	2	4	0	2	3	1	2	0	0	0	0	2	1	4
Nematoda sp2	0	0	0	0	0	0	0	0	0	9	8	12	3	1	4
Nematoda sp3	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0
Nematoda sp4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2

Univariate and multivariate analysis

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Bioaccumulation/Biomagnification

Endemic bioindicator species

Scrobicularia plana: mud clam

Bivalve mollusc

Sampling methodology

Captured by hand, low tide, intertidal zone.

40 organisms/sampling site

Depuration: 4 hrs in aquariums to remove traces of sediment.

Concentration analysis of selected contaminants and biomarkers

Effects in the laboratory – collected sediments

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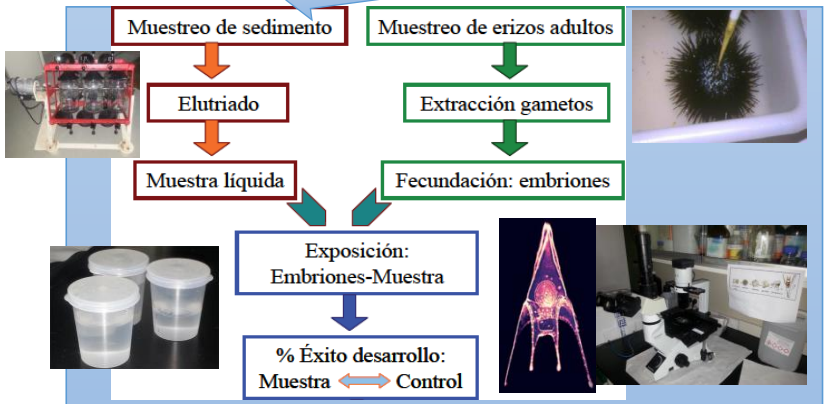
EC_{50} = concentración efectiva media, bioluminiscencia bacteria (*Vibrio fischeri*)



Éxito del desarrollo larval en erizos (*Paracentrotus lividus*) en el lixiviado de los sedimentos

Sobrevivencia anfípodo (*Ampelisca brevicornis*)

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estación	v	hg	cd	se	ni	cu	zn	as	cr	pb	graves	sand	fines	mo	asse	cdse
Ca1	61,1	25,62	2,18	9,9	0,19	99,77	0,04	1,07	3,42	0,92	0,1	6,68	4387	0,05	85,87	0,06
Ca1	74,06	32,2	3,09	15,27	0,19	99,77	0,04	1,07	3,42	0,92	0,1	6,68	4387	0,05	85,87	0,06
Ca1	48,14	19,05	1,28	4,52	0,19	99,77	0,04	1,07	3,42	0,92	0,1	6,68	4387	0,05	85,87	0,06
Ca2	61,92	27,44	4,47	32,61	0,05	40,42	59,53	13,75	30,77	1,32	14,94	202,8	26500	1,98	201,6	20,14
Ca2	64,23	30,68	4,96	32,92	0,05	40,42	59,53	13,75	30,77	1,32	14,94	202,8	26500	1,98	201,6	20,14
Ca2	59,6	24,19	3,99	32,31	0,05	40,42	59,53	13,75	30,77	1,32	14,94	202,8	26500	1,98	201,6	20,14
Ca3	65,44	30,94	6,97	61,91	0,3	17,8	81,9	20,3	16,61	1,23	8,43	46,76	19625	0,28	294,4	16,9
Ca3	70,06	31,24	10,88	86,54	0,3	17,8	81,9	20,3	16,61	1,23	8,43	46,76	19625	0,28	294,4	16,9
Ca3	60,83	30,65	3,06	37,28	0,3	17,8	81,9	20,3	16,61	1,23	8,43	46,76	19625	0,28	294,4	16,9
Ca4	75,63	26	4,18	20,21	0,03	0,38	99,59	24,33	7,81	1,25	14,22	32,07	23000	0,05	406,5	21,25
Ca4	92,81	28,25	5	25,09	0,03	0,38	99,59	24,33	7,81	1,25	14,22	32,07	23000	0,05	406,5	21,25
Ca4	58,45	23,76	3,37	15,32	0,03	0,38	99,59	24,33	7,81	1,25	14,22	32,07	23000	0,05	406,5	21,25
Hu1	62,75	35	2,18	86,01	0,07	9,71	90,22	20,27	839,95	4,35	32,89	1938,5	65750	2,38	383,3	34,57
Hu1	66,69	36,14	2,21	96,44	0,07	9,71	90,22	20,27	839,95	4,35	32,89	1938,5	65750	2,38	383,3	34,57
Hu1	58,82	33,86	2,15	75,58	0,07	9,71	90,22	20,27	839,95	4,35	32,89	1938,5	65750	2,38	383,3	34,57
Hu2	112	33	3,1	70	0,19	56,02	90,21	10,64	532,27	2,5	24,1	14,97	57125	1,99	303,6	7,1
Hu2	114,1	35,8	3,2	80	0,19	56,02	90,21	10,64	532,27	2,5	24,1	14,97	57125	1,99	303,6	7,1
Hu2	109,9	30,2	3	60	0,19	56,02	90,21	10,64	532,27	2,5	24,1	14,97	57125	1,99	303,6	7,1
Hu3	66,45	30,14	9,8	63,83	0,03	16,13	43,95	6,3	272,78	1,32	8,13	772,5	41250	1,2	354,45	128,55
Hu3	69,53	30,97	14,78	73,17	0,03	16,13	43,95	6,3	272,78	1,32	8,13	772,5	41250	1,2	354,45	128,55
Hu3	63,37	29,3	4,81	54,49	0,03	16,13	43,95	6,3	272,78	1,32	8,13	772,5	41250	1,2	354,45	128,55
Bi1	44,25	38	2,59	55,99	2,39	20,28	77,33	14,81	67,26	2	18,27	102,6	32200	0,74	109,05	26,39
Bi1	57,96	42,67	4,44	87,9	2,39	20,28	77,33	14,81	67,26	2	18,27	102,6	32200	0,74	109,05	26,39
Bi1	30,54	33,33	0,74	24,09	2,39	20,28	77,33	14,81	67,26	2	18,27	102,6	32200	0,74	109,05	26,39
Bi2	64,79	30,76	9,45	138,12	38,12	14,48	47,4	15,07	104,49	2	23,11	204,1	42000	1,43	396,6	32
Bi2	66,66	31,61	13,8	233,78	38,12	14,48	47,4	15,07	104,49	2	23,11	204,1	42000	1,43	396,6	32
Bi2	62,93	29,91	5,1	42,46	38,12	14,48	47,4	15,07	104,49	2	23,11	204,1	42000	1,43	396,6	32
Bi3	42,79	33	3,15	35,42	0,19	6,22	93,59	16,73	21,71	0,04	3,48	23,03	16980	0,18	191,35	15,72
Bi3	43,99	33,3	3,26	45,42	0,19	6,22	93,59	16,73	21,71	0,04	3,48	23,03	16980	0,18	191,35	15,72
Bi3	41,59	32,7	3,04	45,42	0,19	6,22	93,59	16,73	21,71	0,04	3,48	23,03	16980	0,18	191,35	15,72
Pa1	65,85	24,96	4,91	196,44	0,84	28,87	70,29	14,43	39,13	0,68	26,73	158,1	33400	1,07	140,05	33,49
Pa1	66,95	25,06	5,41	197,44	0,84	28,87	70,29	14,43	39,13	0,68	26,73	158,1	33400	1,07	140,05	33,49
Pa1	64,75	24,86	4,41	195,44	0,84	28,87	70,29	14,43	39,13	0,68	26,73	158,1	33400	1,07	140,05	33,49
Pa2	74	22	4,1	20	3,67	5,08	91,24	18,47	28,76	0,7	23,42	167,1	31800	1,29	180	28,48
Pa2	76,2	22,1	4,2	21	3,67	5,08	91,24	18,47	28,76	0,7	23,42	167,1	31800	1,29	180	28,48
Pa2	71,8	21,9	4	19	3,67	5,08	91,24	18,47	28,76	0,7	23,42	167,1	31800	1,29	180	28,48
Pa3	83	20,01	3,8	51	1,82	38,53	59,65	19,81	23,78	0,04	18,61	162,5	22000	1,36	152,6	19,61
Pa3	86,1	20,11	4,03	53	1,82	38,53	59,65	19,81	23,78	0,04	18,61	162,5	22000	1,36	152,6	19,61
Pa3	79,9	19,91	3,57	49	1,82	38,53	59,65	19,81	23,78	0,04	18,61	162,5	22000	1,36	152,6	19,61
TM	99	22,1	4,2	160	0,2	7,8	92	1,1	1234	13,68	25,3	643,7	136000	2,01	230	42,5
TM	100,1	22,2	4,21	170	0,2	7,8	92	1,1	1234	13,68	25,3	643,7	136000	2,01	230	42,5
TM	97,9	22	4,19	150	0,2	7,8	92	1,1	1234	13,68	25,3	643,7	136000	2,01	230	42,5

Databases:

- Replica
- Metals
- Sediment characteristics
- Biomarkers
- Mortality
- .
- .
- .
- .

➔ not interpretable for advisors and managers.

➔ We need simplicity and interpretability

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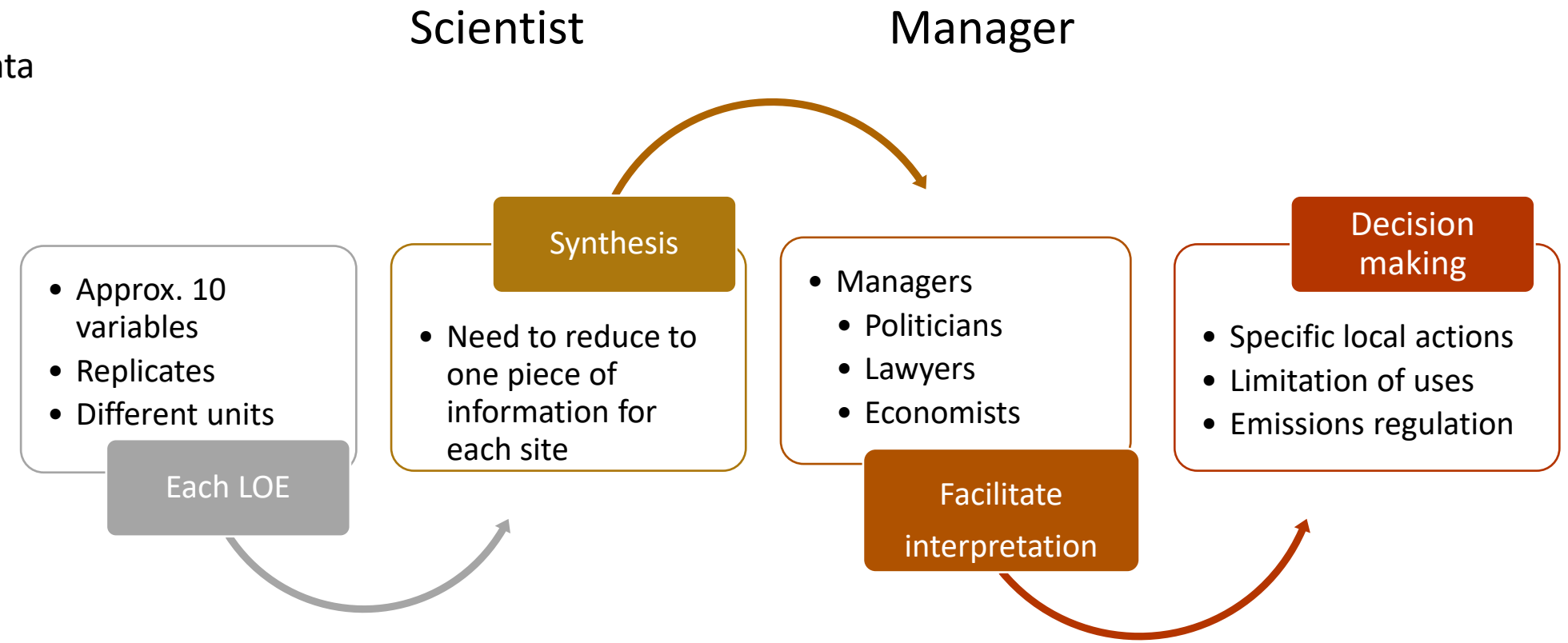
Integrated methods

Common issues:
There is always a lack of data due to:

- Infrastructure issues
- Lack of money
- Lack of interest in this type of data

Need to learn to use what is available and draw the best conclusions from what is there.



Adaptation of results
➔ integration

Interpretation of information
Representation of information

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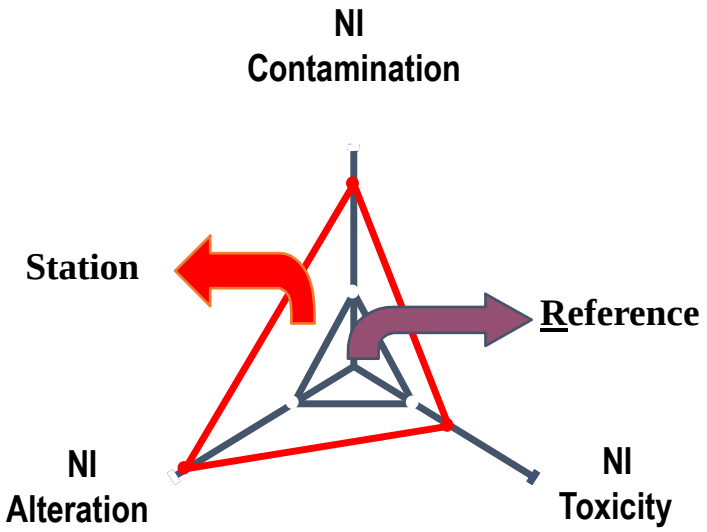
1. Triaxial Method

Oldest method, still used.

More advanced methodologies ➔ based on this triaxial method

Useful to understand how to interpret the results for monitoring

Mathematical, non-statistical approach: based on determining differences between groups



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POLLUTION INDEX calculated using pollution, in situ alteration and toxicity

Mathematical method, not statistical ➔ mean values

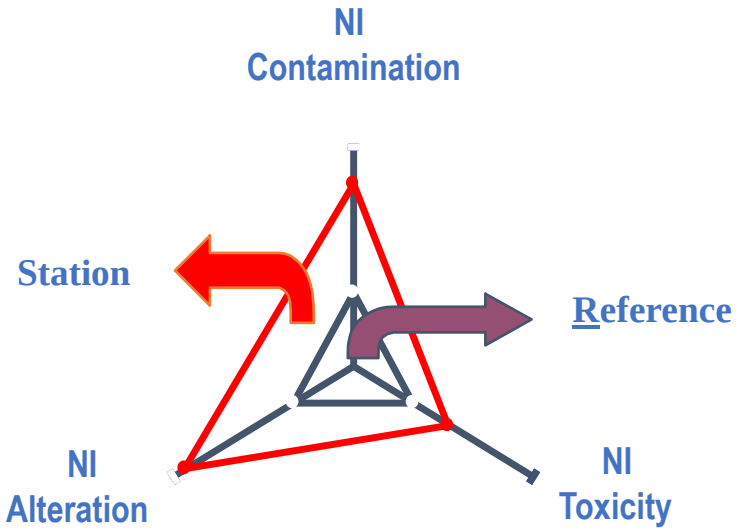
Only 3 LOEs can be included: contamination, in situ alteration and toxicity

Variables of an LOE are represented in index = axis of the triangle

Always includes 2 sites: reference and "problem"

Reference: always has the same area

Triangle area = pollution index: the greater the area, the greater the pollution ➔ station = more polluted than reference



$$RTR_i = \frac{\upsilon_i}{(\upsilon_i)_0} \qquad RTM_i = \frac{RTR_i}{(RTR\text{-}m_i)} \qquad NI = \frac{\sum_{i=n} RTM_i}{\left(\sum_{i=n} RTM_i\right)_0} \quad \forall i$$

DATABASES: 3 LINES OF EVIDENCE:
RTR matrix ("ratio-to-reference"): Normalize with reference to control
RTM ("ratio-to-maximum") matrix: Normalize with reference to maximum

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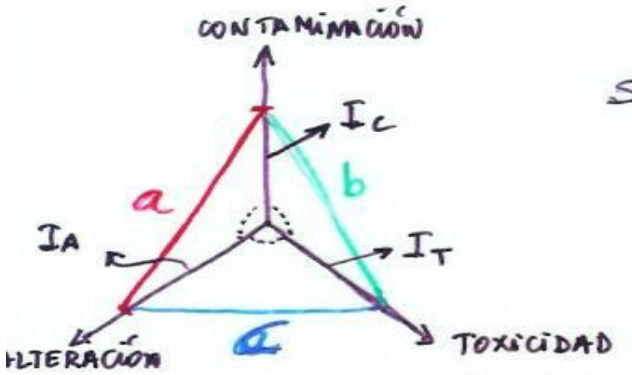

SISTEMA ISOMÉTRICO (120°)

- TEOREMA DEL COSENO.-
PARA EL MISMO ANGULO 120°

$$a = \sqrt{I_C^2 + I_A^2 - 2 I_C I_A (-0,5)}$$

$$b = \sqrt{I_C^2 + I_T^2 - 2 I_C I_T (-0,5)}$$

$$c = \sqrt{I_A^2 + I_T^2 - 2 I_A I_T (-0,5)}$$

CALCULO DEL ÁREA DE UN TRIÁNGULO CONOCIENDO LA LONGITUD DE SUS RESPECTIVOS LADOS.-

$$\text{SEMIPERÍMETRO} = S = \frac{a+b+c}{2}$$

$$A = \sqrt{S \cdot (S-a) \cdot (S-b) \cdot (S-c)} \quad \neq ?$$

$$P_{TRIAD} = A_{ESTACIÓN} - A_{REFERENCIA}$$

1,30 Área de triángulo con vértices (1,1,1).-

Calculation of the area of the triangle

Cosine theorem in isometric system (120º)

With regard to the length of each sides ➔
Toxicity, alteration and contamination index

Pollution index:

$$P_{triad} = A_{station} - A_{reference}$$

1,30
= triangle area
with vertex (1,1,1)

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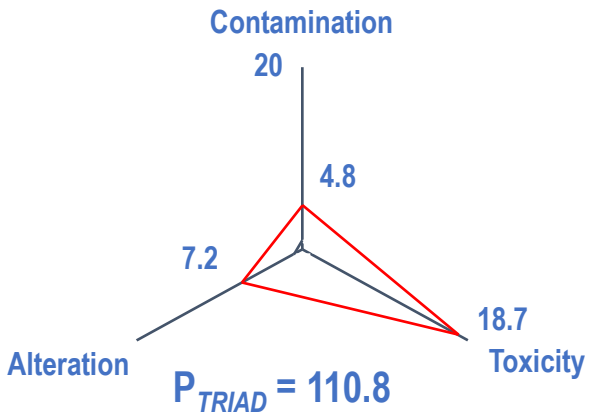
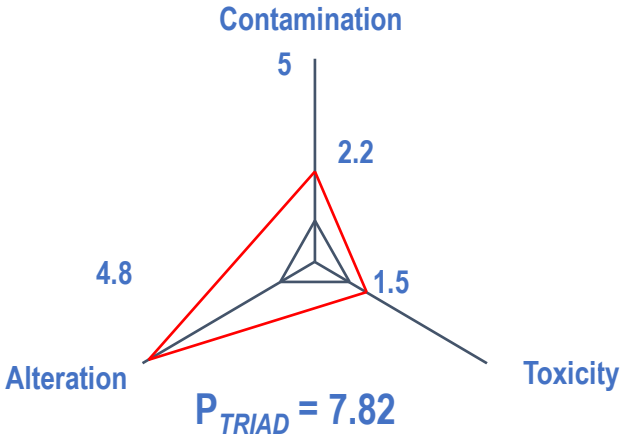
Final objective:

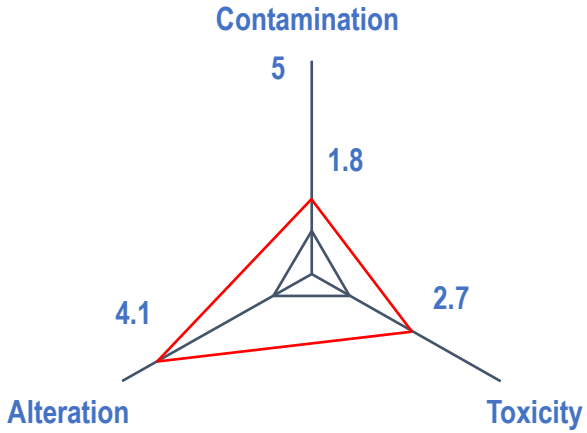
Calculation of the POLLUTION INDEX calculated using pollution, in situ alteration and toxicity



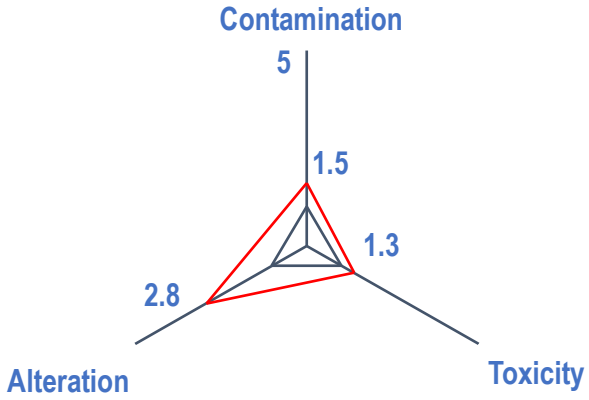
$P_{TRIAD} = 110.8$



$P_{TRIAD} = 7.82$



$P_{TRIAD} = 8.47$



$P_{TRIAD} = 3.25$

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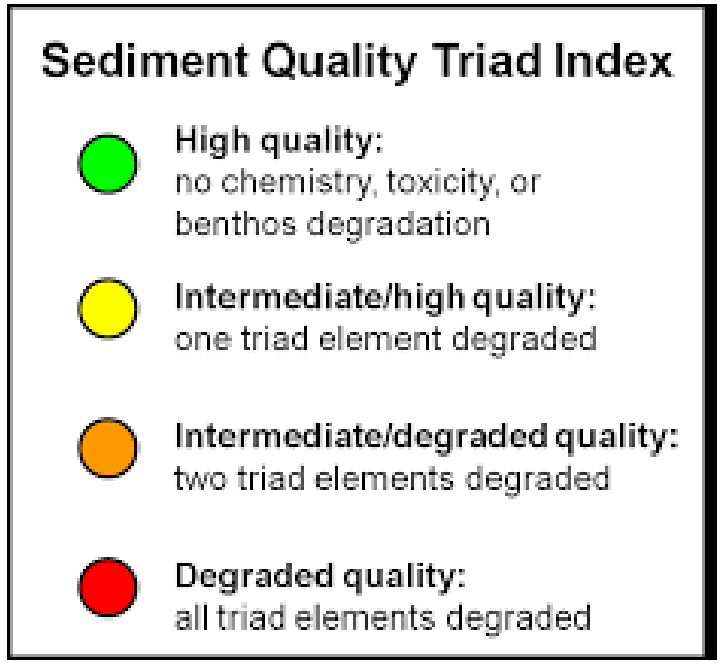
Information provided by differential Triad responses

Situation	Contamination	Toxicity	Alteration	Possible conclusions
1.	+	+	+	Strong evidence for pollution-induced degradation
2.	−	−	−	Strong evidence that there is no pollution-induced degradation
3.	+	−	−	Contaminants are not bioavailable
4.	−	+	−	Unmeasured chemicals or conditions exist with the potential to cause degradation
5.	−	−	+	Alteration is not due to toxic chemicals
6.	+	+	−	Toxic chemicals are stressing the system
7.	−	+	+	Unmeasured toxic chemicals are causing degradation
8.	+	−	+	Chemicals are not bioavailable or alteration is not due to toxic chemicals

Responses are shown as either positive (+) or negative (−), indicating whether or not measurable (e.g., statistically significant) differences from control/reference conditions/measures are determined.



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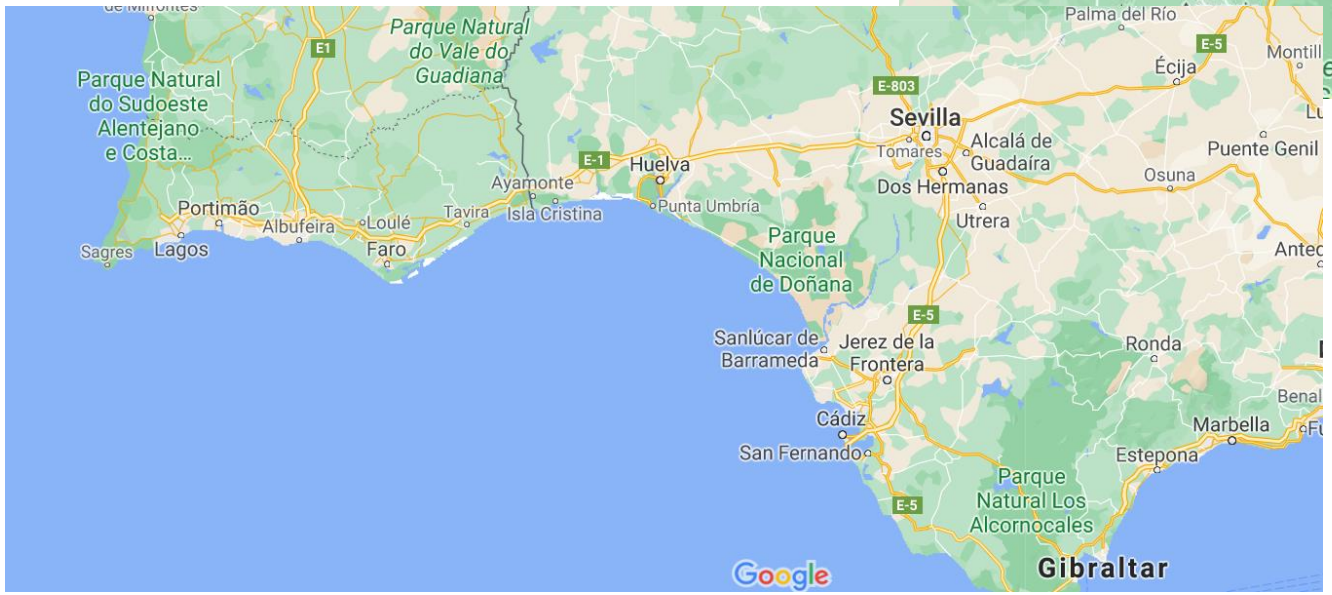
Case study:

4 different sites:
2 Spanish ports:
- Bilbao BI2 and BI3
- Passages PA2 and PA3



- Huelva HU2 and HU3
- Cadiz CA2

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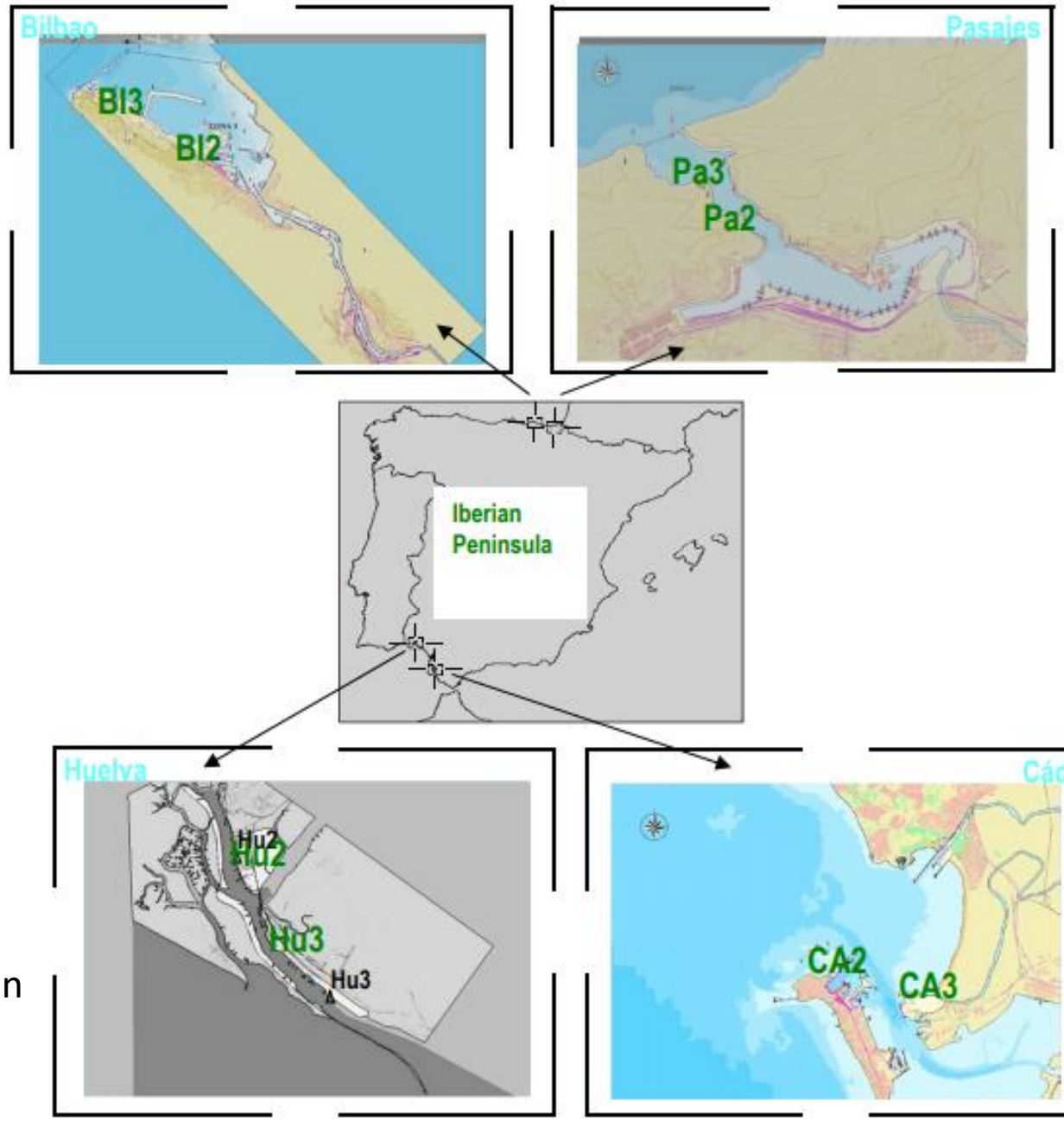
BI2 and BI3: Port of Bilbao (NNE, Spain)
Intense maritime traffic
Contaminants associated with organic compounds, especially hydrocarbons.



PA2 and PA3: Port of Pasajes (NNE, Spain),
Intense maritime traffic
Contaminants associated with organic compounds.

CA2 and CA3 (reference).
Port of Cadiz (CA2) and Inner Sac of the Bay (CA3) (SW, Spain)

Reference: CA3 (Bay of Cadiz)

- "clean"
- Well studied
- Well characterized
- Availability of sediment/in situ toxicity data

(DelValls and Chapman, 1998; Riba et al., 2004a,b)

HU2 and HU3: Port of Huelva (SW, Spain)
Heavy metal contamination
Mining activity

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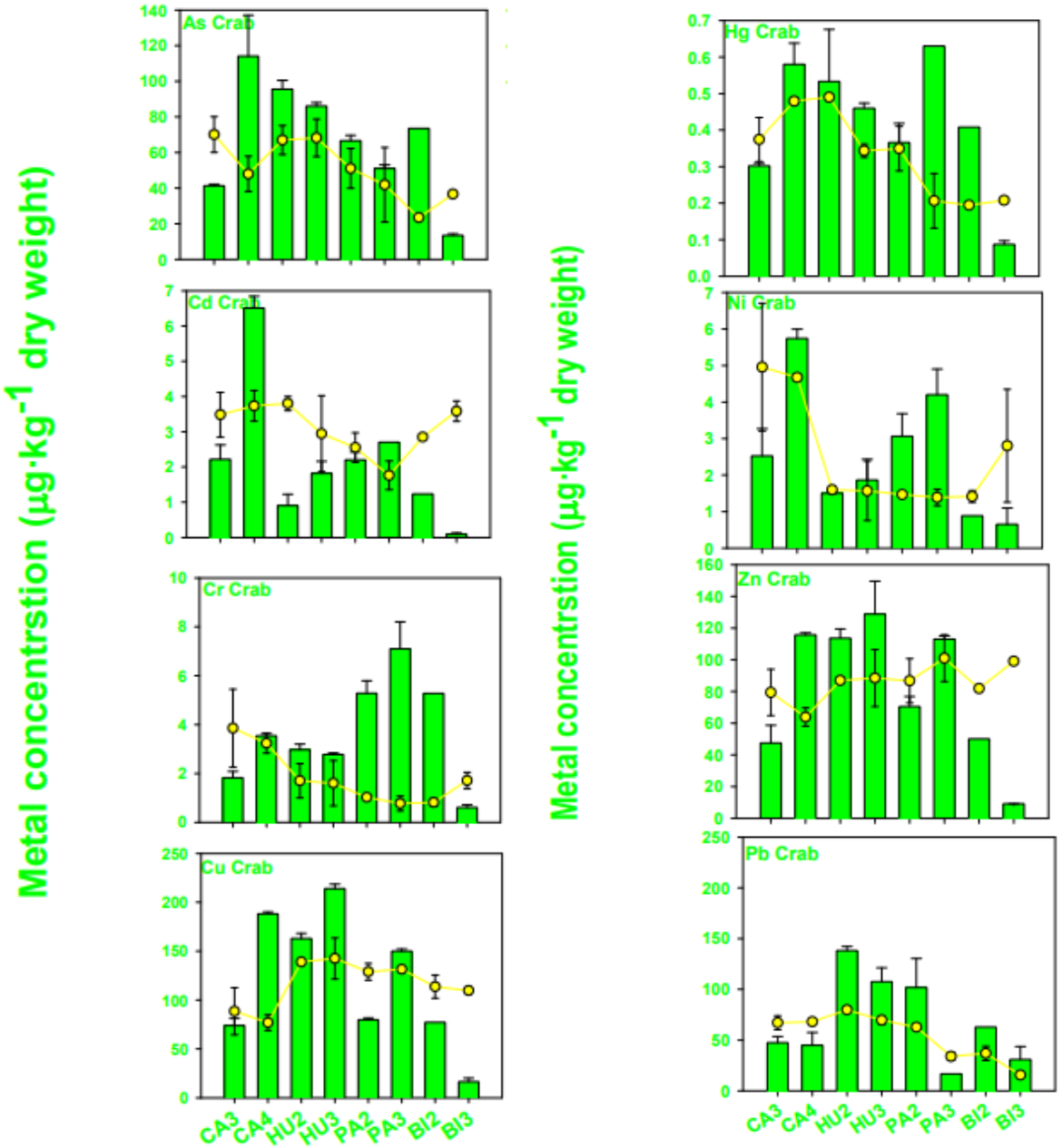

Carcinus maenas

- Individuos en jaulas/Lab
- 28 días
- Análisis químico en el sedimento

- MT (0, 7, 14,21,28)
- Actividad enzimática EROD, GST, GPX, GR (0, 7, 14, 21)
- Índice Gonadosomático
- Bioacumulación en branquia

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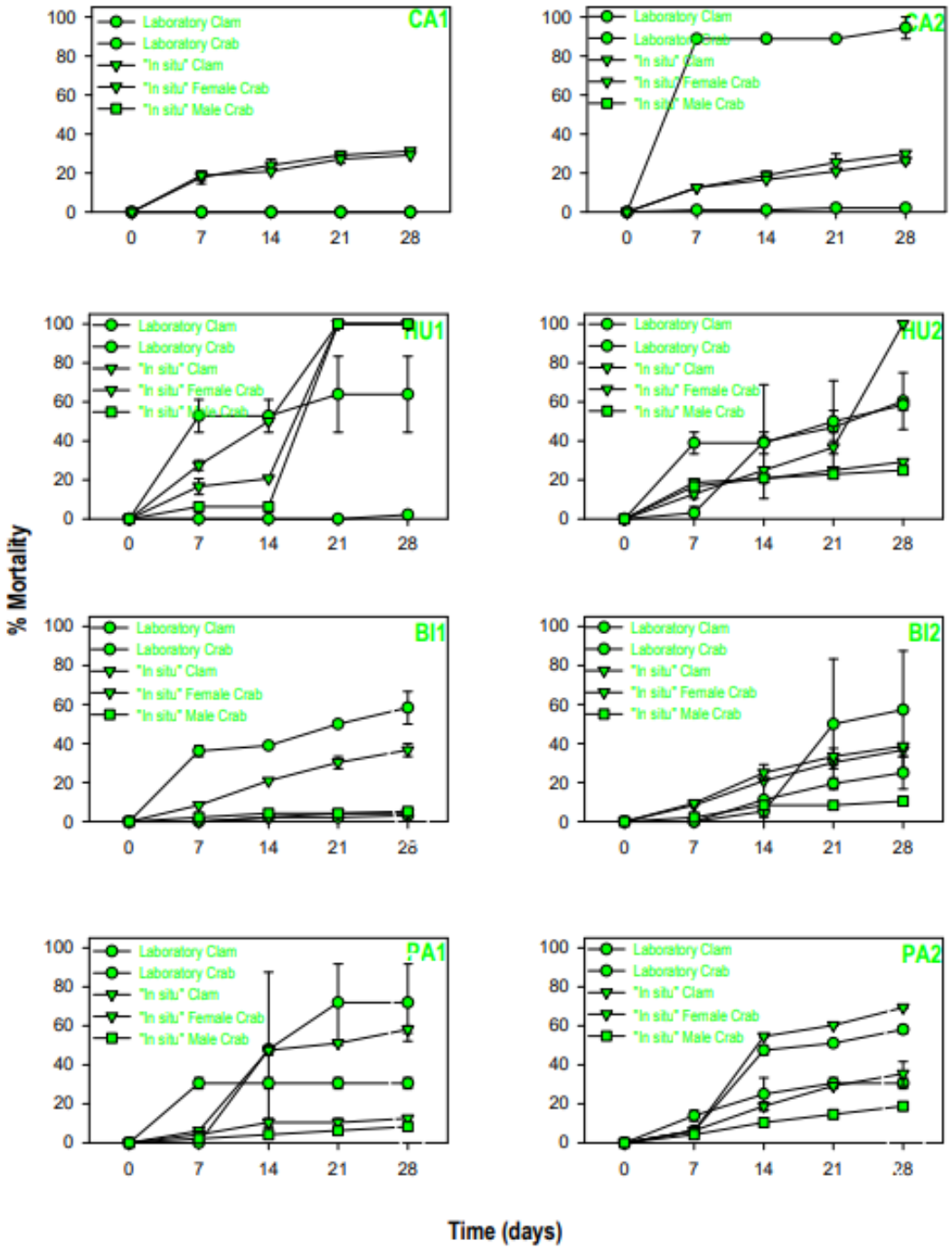
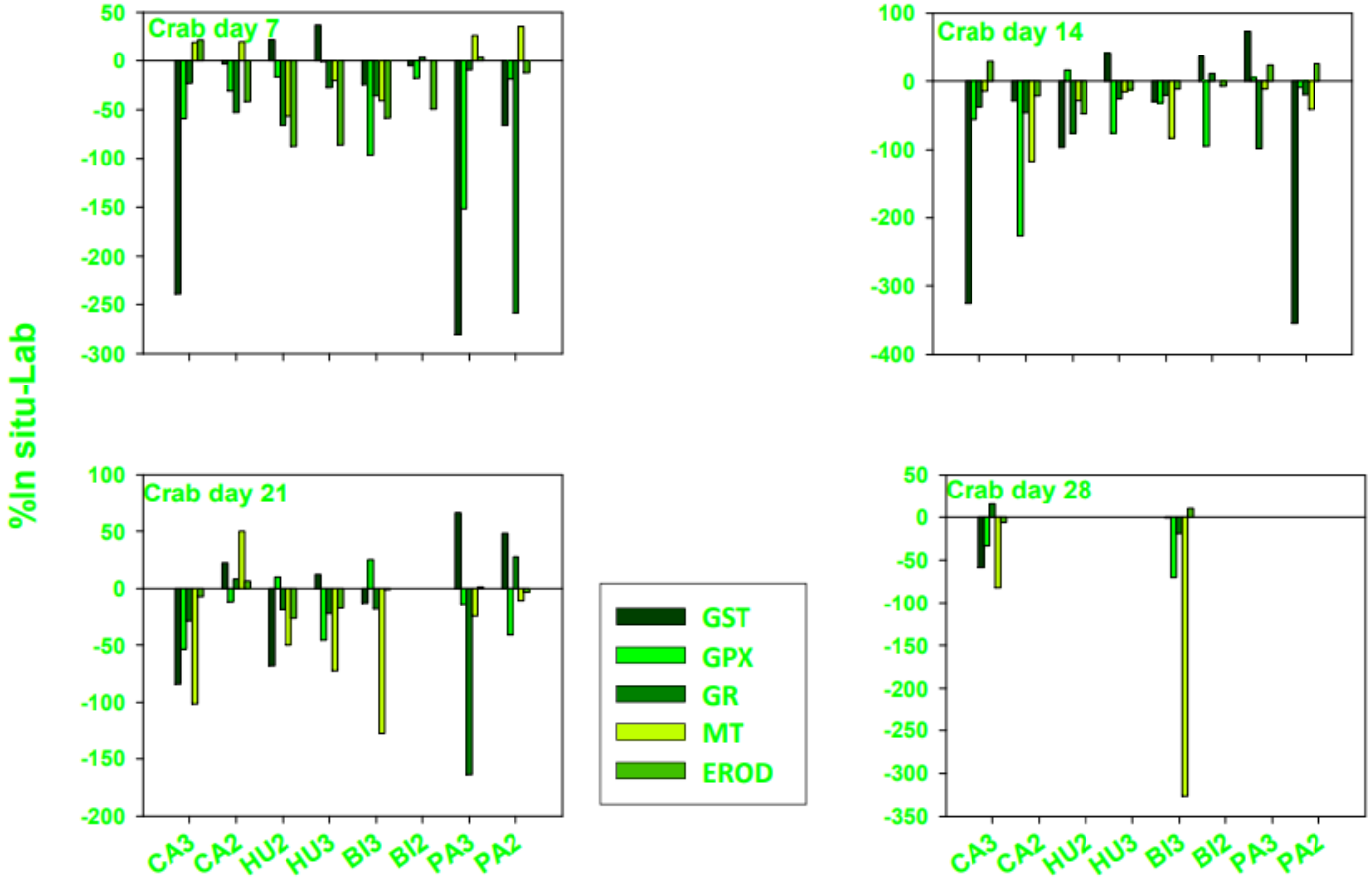
Biomarkers





3 LOEs:

- Laboratory toxicity: Carcinus means (females), sediments from all seasons, 28d: Mortality, biomarkers (GPx, GR, EROD, MT, VTG) HPTGills, HPThepato, HTPgonads
- In situ toxicity: Carcinus means (females), transplanted, 28d: Mortality, biomarkers (GPx, GR, EROD, MT) Gonadosomatic index, Heptasomatic index, HPTGills, HPThepato, HTPgonads
- [Contaminants]: metals, PCBs, PAHs

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CAN LAB	MOR28	GPX28	GR28	EROD28	MT28	VTG28	HPTGgill	HPTGhepato	HTPGgonads
CA3	1,00	426,00	30,57	23,36	32,40	170,30	1,00	0,60	0,50
CA2	94,40	710,00	29,00	18,00	23,04	410,00	2,00	0,50	0,50
HU2	63,87	669,90	32,32	15,65	197,75	368,51	2,00	1,00	0,33
HU3	58,33	710,00	52,00	23,00	69,50	210,00	1,50	2,00	0,33
BI3	58,33	776,58	38,10	18,84	35,50	324,28	2,00	2,00	2,50
B2	25,00	711,00	60,40	22,60	26,70	420,00	1,50	2,60	0,01
PA3	30,55	690,00	61,00	28,70	25,30	310,00	2,00	2,00	2,20
PA2	30,55	378,00	28,00	24,60	20,40	910,00	2,00	0,70	2,10

CAN IN SITU	MOR28	GPX28	GR28	EROD28	MT28	Gonadosomatic	Hepatosomatic I	HPTGgill	HPTGhepato	HTPGgonad
CA3	0,29	0,70	0,72	0,83	0,32	0,43	0,83	0,50	0,29	0,20
CA2	0,26	1,00	0,52	0,87	0,62	1,00	0,56	0,33	0,14	0,20
HU2	1,00	0,65	0,61	0,80	0,91	0,75	0,39	0,67	0,64	0,13
HU3	0,29	0,62	1,94	0,78	1,00	0,78	0,54	0,75	0,43	0,13
BI3	0,04	0,63	0,50	0,80	0,15	0,51	0,89	0,67	0,50	1,00
B2	0,39	0,34	1,00	0,93	0,48	0,30	0,76	0,83	0,64	0,00
PA3	0,12	0,54	0,57	1,00	0,43	0,17	0,94	1,00	0,71	0,88
PA2	0,35	0,52	0,96	0,89	0,45	0,29	1,00	0,67	1,00	0,84

	%GRAVES	%SAND	%FINES	%MO	AS	CD	CR	CU	FE	HG	MN	NI	PB	ZN	PCB	PAH
CA3	0,30	17,80	81,90	20,30	16,61	1,23	8,43	46,76	19625,00	0,28	294,40	16,90	17,61	135,50	0,00	0,01
CA2	0,05	40,42	59,53	13,75	30,77	1,32	14,94	202,80	26500,00	1,98	201,60	20,14	86,90	378,25	0,11	0,11
HU2	0,19	56,02	90,21	10,64	532,27	2,50	24,10	14,97	57125,00	1,99	303,60	7,10	384,70	1857,00	0,00	0,01
HU3	0,03	16,13	43,95	6,30	272,78	1,32	8,13	772,50	41250,00	1,20	354,45	128,55	217,60	1176,00	0,00	0,01
BI3	0,19	6,22	93,59	16,73	21,71	0,04	3,48	23,03	16980,00	0,18	191,35	15,72	285,90	122,35	0,00	13,9
B2	38,12	14,48	47,40	15,07	104,49	2,00	23,11	204,10	42000,00	1,43	396,60	32,00	147,50	777,50	0,23	66,77
PA3	1,82	38,53	59,65	19,81	23,78	0,04	18,61	162,50	22000,00	1,36	152,60	19,61	246,00	576,00	0,24	0,26
PA2	3,67	5,08	91,24	18,47	28,76	0,70	23,42	167,10	31800,00	1,29	180,00	28,48	293,70	763,00	0,74	1,06

Two matrices

RTR Matrix: Normalize with Control: Divide Each Variable by the Control Value (CA3)

RTM matrix: Normalize with maximum: divide each variable by the maximum value of this variable (in RTR)

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Matrix RTR: Normalize with control site

RTR

CAN LAB	MOR28	GPX28	GR28	EROD28	MT28	VTG28	HPTGgill	HPTGhepato	HTPGgonads
CA3	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00
CA2	94,40	1,67	0,95	0,77	0,71	2,41	2,00	0,83	1,00
HU2	63,87	1,57	1,06	0,67	6,10	2,16	2,00	1,67	0,67
HU3	58,33	1,67	1,70	0,98	2,15	1,23	1,50	3,33	0,67
BI3	58,33	1,82	1,25	0,81	1,10	1,90	2,00	3,33	5,00
B2	25,00	1,67	1,98	0,97	0,82	2,47	1,50	4,33	0,02
PA3	30,55	1,62	2,00	1,23	0,78	1,82	2,00	3,33	4,40
PA2	30,55	0,89	0,92	1,05	0,63	5,34	2,00	1,17	4,20

$$RTR_i = \frac{v_i}{(v_i)_c} \quad \text{and} \quad 1 = \frac{\sum_{i=1}^{n} RTR_i}{n} \; \forall \; i$$

CRAB IN SITU	MOR28	GPX28	GR28	EROD28	MT28	Gonadosoi	Hepatosom	HPTGgill	HPTGhepat	HTPGgonad
CA3	1,01	1,01	1,00	1,00	1,00	1,01	1,00	1,00	0,99	1,00
CA2	0,91	1,43	0,72	1,05	1,95	2,32	0,67	0,67	0,49	1,00
HU2	3,45	0,93	0,85	0,96	2,85	1,73	0,47	1,33	2,22	0,67
HU3	1,01	0,88	2,69	0,93	3,13	1,82	0,65	1,50	1,48	0,67
BI3	0,14	0,90	0,70	0,96	0,47	1,18	1,07	1,33	1,72	5,00
B2	1,33	0,48	1,39	1,12	1,49	0,69	0,92	1,67	2,22	0,00
PA3	0,43	0,77	0,79	1,20	1,34	0,40	1,14	2,00	2,46	4,40
PA2	1,22	0,74	1,33	1,08	1,41	0,67	1,20	1,33	3,45	4,20

	%GRAVES	%SAND	%FINES	%MO	AS	CD	CR	CU	FE	HG	MN	NI	PB	ZN	PCB	PAH
CA3					1,00	1,00	1,00	1,00	1,00	1,00	1,04	1,00	1,00	1,00	1,00	1,00
CA2					1,85	1,07	1,77	4,34	1,35	7,07	0,71	1,19	4,93	2,79	110,00	11,00
HU2					32,05	2,03	2,86	0,32	2,91	7,11	1,07	0,42	21,85	13,70	1,00	1,00
HU3					16,42	1,07	0,96	16,52	2,10	4,29	1,25	7,61	12,36	8,68	1,00	1,00
BI3					1,31	0,03	0,41	0,49	0,87	0,64	0,67	0,93	16,24	0,90	1,00	1390,00
B2					6,29	1,63	2,74	4,36	2,14	5,11	1,39	1,89	8,38	5,74	230,00	6677,00
PA3					1,43	0,03	2,21	3,48	1,12	4,86	0,54	1,16	13,97	4,25	240,00	26,00
PA2					1,73	0,57	2,78	3,57	1,62	4,61	0,63	1,69	16,68	5,63	740,00	106,00

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RTM MATRIX: NORMALIZE WITH THE MAXIMUM OF EACH VARIABLE

RTM: calculated with RTR results

=D37/max(D$37;D$43)

=SUMA(D69:L69)

Itox = M69/M$69

RTM

CAN LAB

	MOR28	GPX28	GR28	EROD28	MT28	VTG28	HPTGgill	HPTGhepato	HTPGgonads	SUM RTM TOX-CAN	NI TOX
CA3	0,01	0,55	0,50	0,81	0,16	0,19	0,50	0,23	0,20	3,16	0,99
CA2	1,00	0,92	0,47	0,63	0,12	0,45	1,00	0,19	0,20	4,98	1,56
HU2	0,68	0,86	0,53	0,54	1,00	0,41	1,00	0,38	0,13	5,54	1,74
HU3	0,62	0,92	0,85	0,80	0,35	0,23	0,75	0,77	0,13	5,42	1,70
BI3	0,62	1,00	0,62	0,66	0,18	0,36	1,00	0,77	1,00	6,20	1,95
B2	0,26	0,92	0,99	0,79	0,14	0,46	0,75	1,00	0,00	5,31	1,67
PA3	0,32	0,89	1,00	1,00	0,13	0,34	1,00	0,77	0,88	6,33	1,99
PA2	0,32	0,49	0,46	0,86	0,10	1,00	1,00	0,27	0,84	5,34	1,68

$$RTM_i = \frac{RTR_i}{(RTR\text{-}m_i)} \quad \text{and} \quad NI = \frac{\sum_{i=n} RTM_i}{\left(\sum_{i=n} RTM_i\right)_0} \; \forall \; i$$

CAN IN SITU

	MOR28	GPX28	GR28	EROD28	MT28	Gonadosomatic	Hepatosomatic I	HPTGgill	HPTGhepato	HTPGgonad	SUM RTM ALT-CAN	NI ALT
CA3	0,29	0,70	0,37	0,84	0,32	0,44	0,94	0,50	0,29	0,20	4,88	1,00
CA2	0,26	1,00	0,27	0,87	0,62	1,00	0,63	0,33	0,14	0,20	5,33	1,09
HU2	1,00	0,65	0,31	0,80	0,91	0,75	0,44	0,67	0,64	0,13	6,31	1,29
HU3	0,29	0,61	1,00	0,78	1,00	0,79	0,61	0,75	0,43	0,13	6,39	1,31
BI3	0,04	0,63	0,26	0,80	0,15	0,51	1,00	0,67	0,50	1,00	5,56	1,14
B2	0,39	0,34	0,52	0,93	0,48	0,30	0,86	0,83	0,64	0,00	5,28	1,08
PA3	0,12	0,54	0,29	1,00	0,43	0,17	1,06	1,00	0,71	0,88	6,22	1,27
PA2	0,35	0,52	0,49	0,90	0,45	0,29	1,13	0,67	1,00	0,84	6,63	1,36

contamination

	AS	CD	CR	CU	FE	HG	MN	NI	PB	ZN	PCB	PAH	SUM RTM	NI CONT
CA3	0,03	0,49	0,35	0,06	0,34	0,14	0,83	0,13	0,06	0,07	0,00	0,0001498	2,51	1,00
CA2	0,06	0,53	0,62	0,26	0,46	1,00	0,57	0,16	0,30	0,20	0,15	0,0016474	4,31	1,72
HU2	1,00	1,00	1,00	0,02	1,00	1,01	0,85	0,06	1,31	1,00	0,00	0,0001498	8,25	3,29
HU3	0,51	0,53	0,34	1,00	0,72	0,61	1,00	1,00	0,74	0,63	0,00	0,0001498	7,08	2,82
BI3	0,04	0,02	0,14	0,03	0,30	0,09	0,54	0,12	0,97	0,07	0,00	0,2081773	2,53	1,01
B2	0,20	0,80	0,96	0,26	0,74	0,72	1,12	0,25	0,50	0,42	0,31	1,0000000	7,27	2,90
PA3	0,04	0,02	0,77	0,21	0,39	0,69	0,43	0,15	0,84	0,31	0,32	0,0038940	4,17	1,66
PA2	0,05	0,28	0,97	0,22	0,56	0,65	0,51	0,22	1,00	0,41	1,00	0,0158754	5,89	2,34

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Copy Itox, Icont,Ialt values and paste into next table ➔ area and probability

SITES	N-Cont	N-tox	Nlalt	Ic2	It2	Ia2	a2	b2	c2	a	b	c	S	
CA3	1,00	1,00	1,00	1,00	1,00	1,00	3,00	3,01	3,00	1,73	1,73	1,73	2,60	1,69
CA2	1,72	1,57	1,09	2,94	2,48	1,19	6,01	8,12	5,39	2,45	2,85	2,32	3,81	7,43
HU2	3,29	1,75	1,29	10,79	3,05	1,67	16,71	19,59	6,98	4,09	4,43	2,64	5,58	28,11
HU3	2,82	1,71	1,31	7,95	2,92	1,72	13,37	15,70	6,88	3,66	3,96	2,62	5,12	21,71
BI3	1,01	1,99	1,14	1,01	3,98	1,30	3,46	7,00	7,54	1,86	2,65	2,75	3,63	5,52
B2	2,90	1,67	1,08	8,40	2,79	1,17	12,70	16,02	5,76	3,56	4,00	2,40	4,98	17,93
PA3	1,66	2,03	1,27	2,76	4,11	1,62	6,50	10,25	8,32	2,55	3,20	2,88	4,32	12,22
PA2	2,34	1,71	1,36	5,50	2,94	1,85	10,53	12,46	7,12	3,25	3,53	2,67	4,72	17,06

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AREA	P TRIAD
1,30	
2,73	1,43
5,30	4,00
4,66	3,36
2,35	1,05
4,23	2,93
3,50	2,20
4,13	2,83

P_{Triad} = Pollution index

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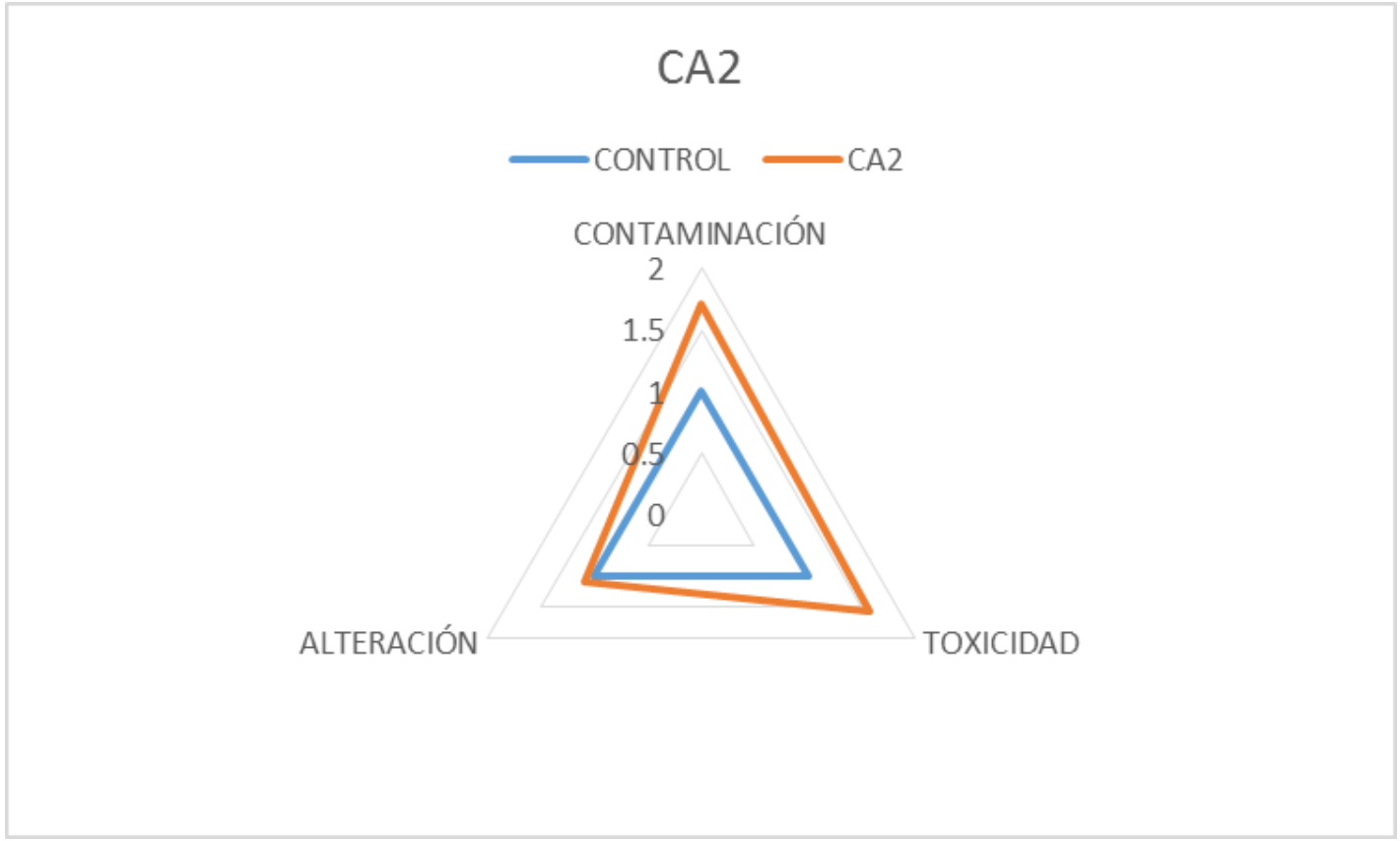
TRIAXIAL DIAGRAMS



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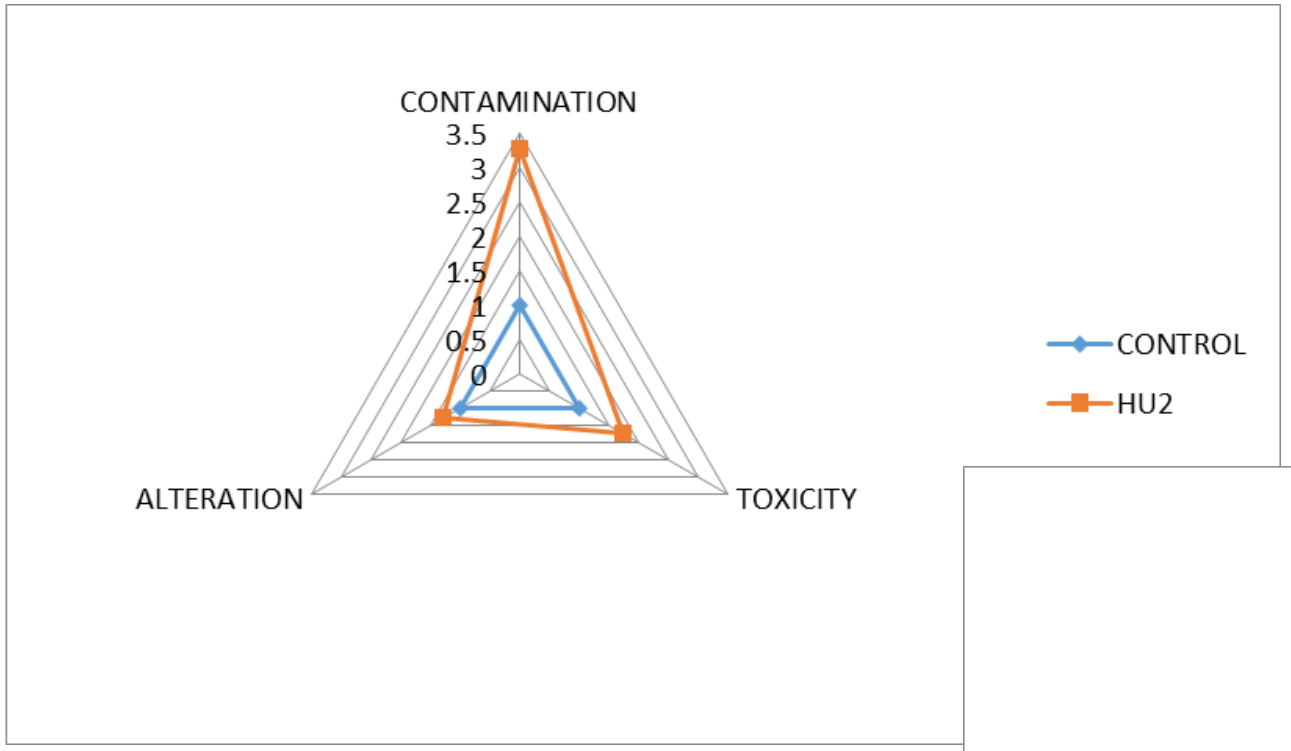

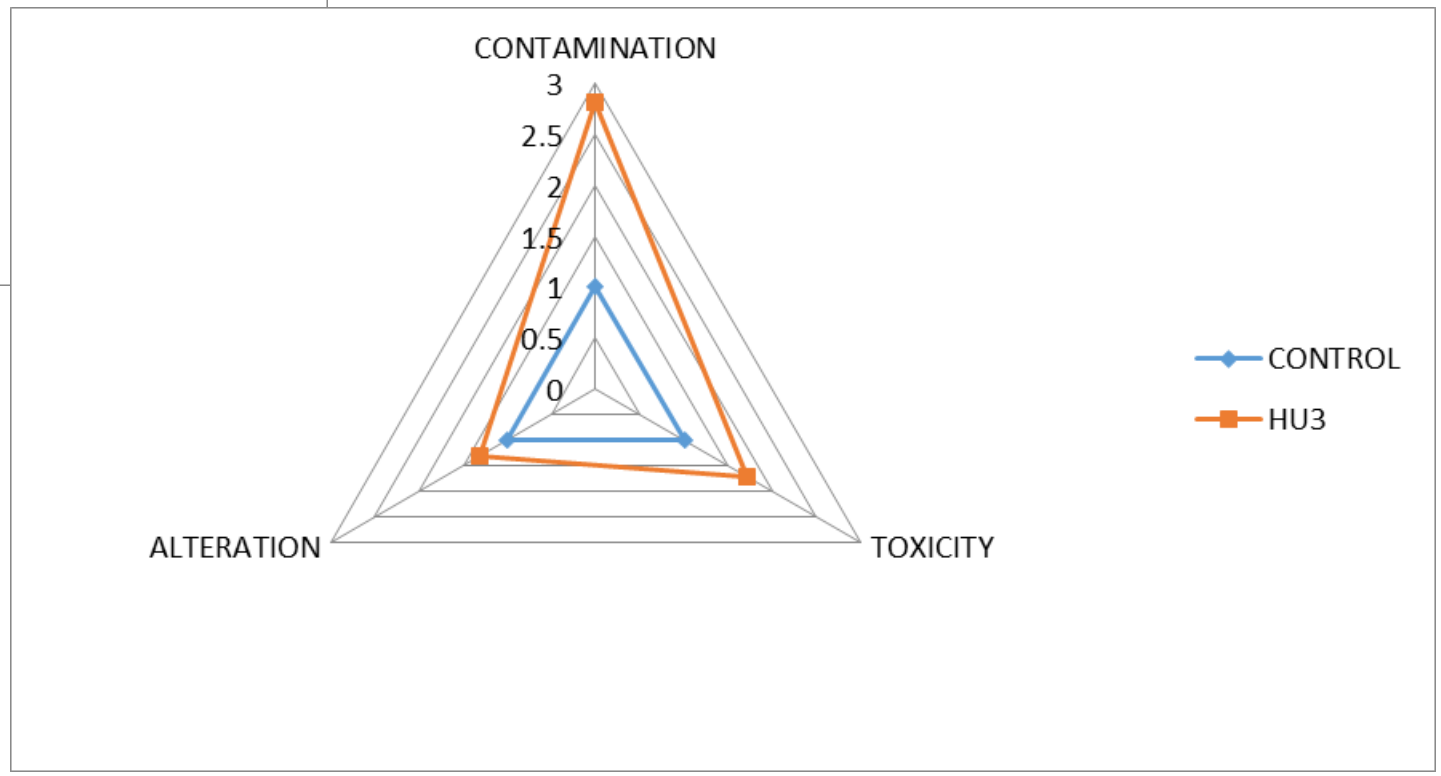

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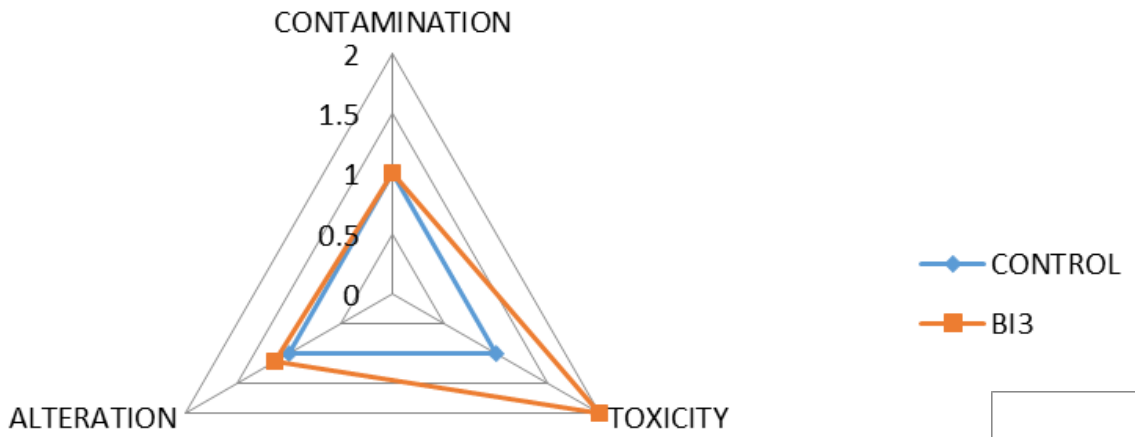

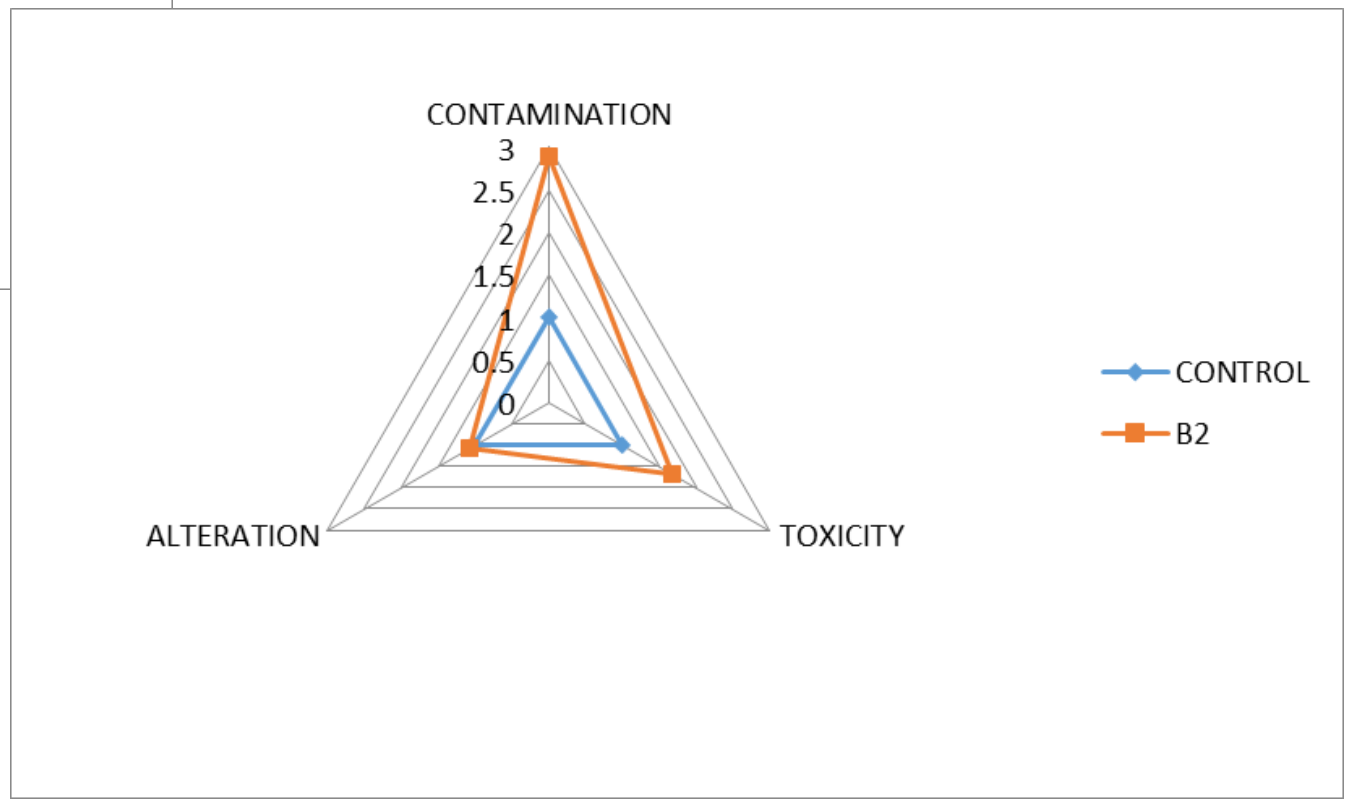

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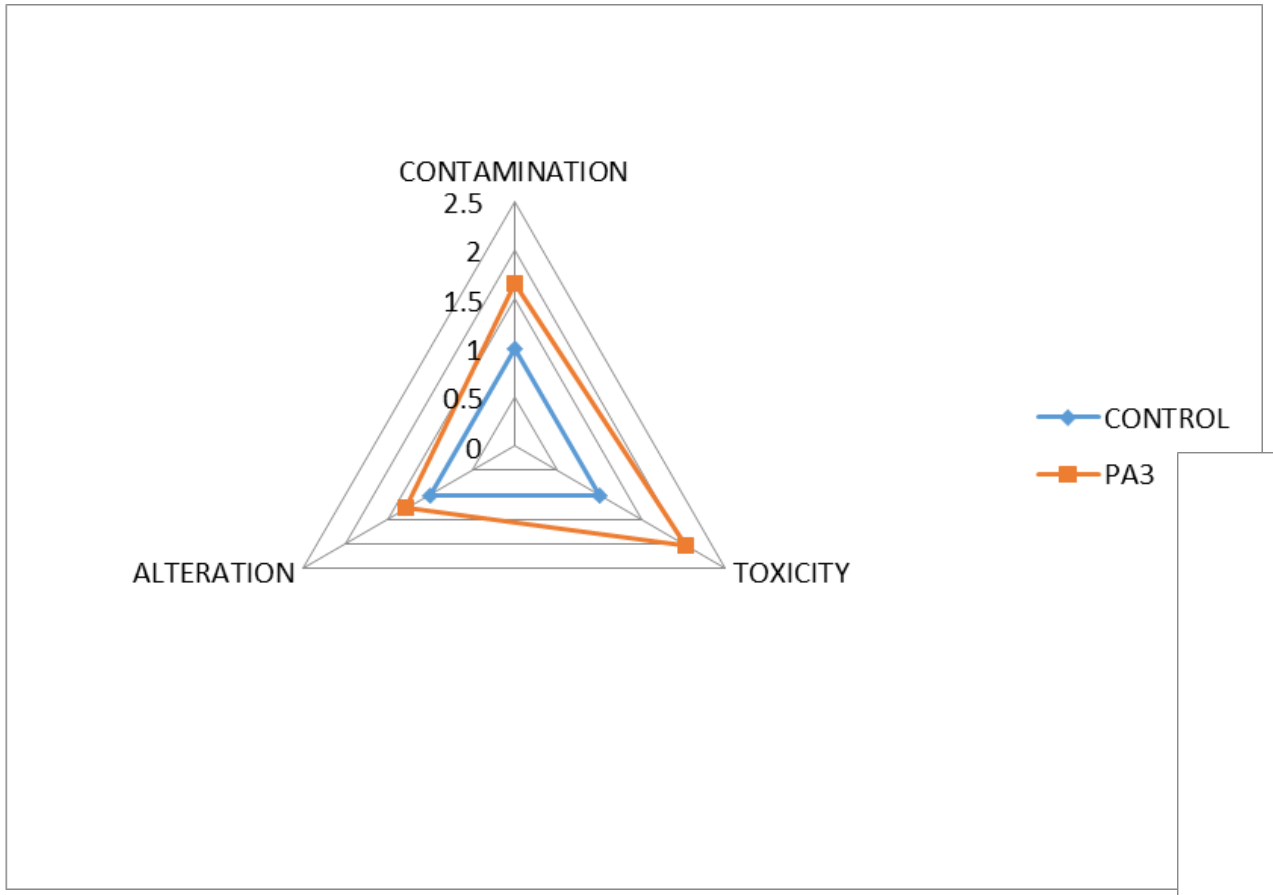

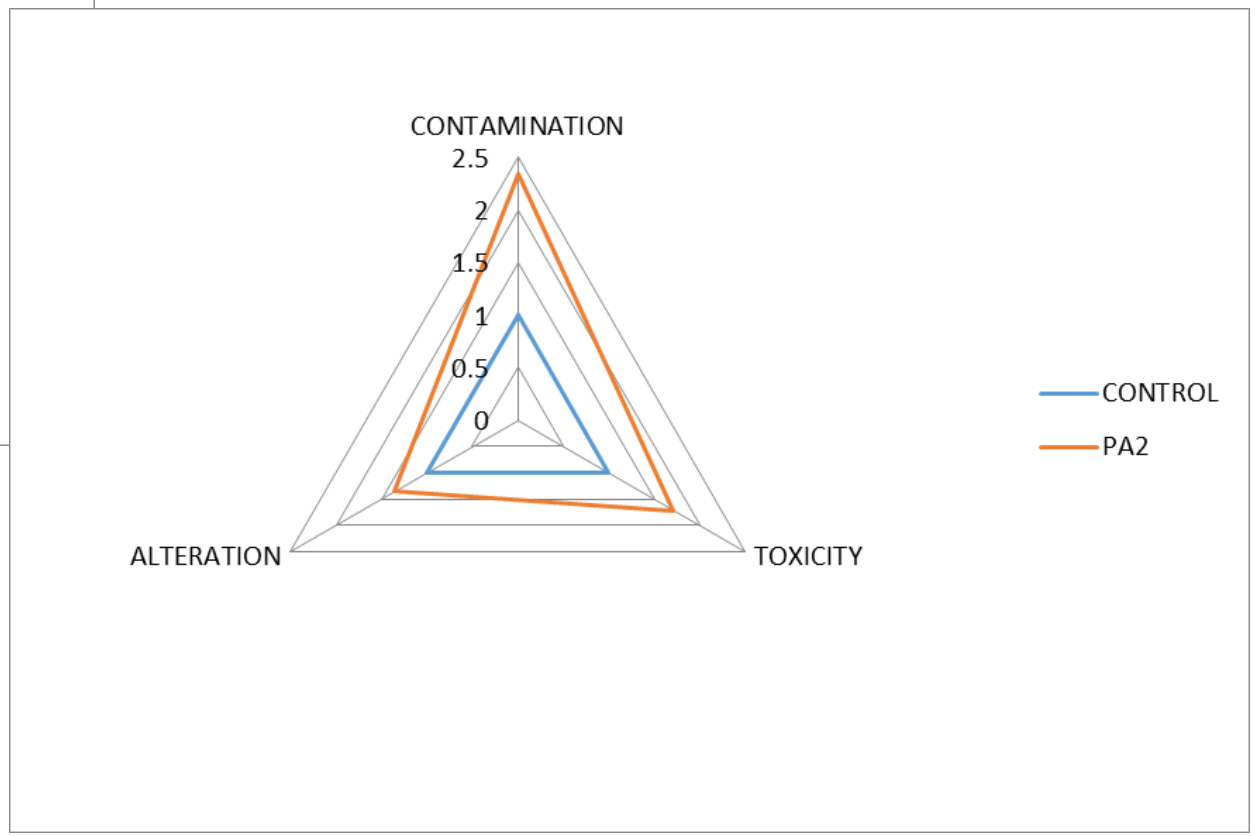

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- Easy to understand
- Easy to reduce information
- Easy to represent
- Informative and visible
- Represents situation of each station

➔ Good method for an initial screening

- I don't know if my effects are due to metal or organic contamination
- I don't know if my differences in toxicity and alteration are significant
- Does not separate responses related to reproduction and survival
- Toxicity indices are sometimes represented by 2 variables, others by 6, ...

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2. Factor Analysis

Need to relate which pollutant is responsible for my toxicity/effects data
For which pollutant it is necessary to develop SQVs
Information about the significance of the answers

- Huge amounts of data that come from the simultaneous observation of different variables
- Need for statistical analysis instruments that allow dealing with this great diversity.
- ➔Multivariate statistical methods: based on matrix calculus
- ➔They allow to combine the different aspects of the study in a single analysis.

SET OF MULTIVARIATE TECHNIQUES GLOBALLY KNOWN AS FACTORIAL METHODS

- Methodology to synthesize a large number of variables ➔ Most available information without significant loss of information.
- The new factors are a linear combination of the original variables.
- Statistical method ➔ significance information
- Determination of sediment quality values
- Understand the relationships between variables and their relevance to the problem being studied.

FACTOR ANALYSIS:
FACTORIAL ROTATION: Matrix transformation by rotation:

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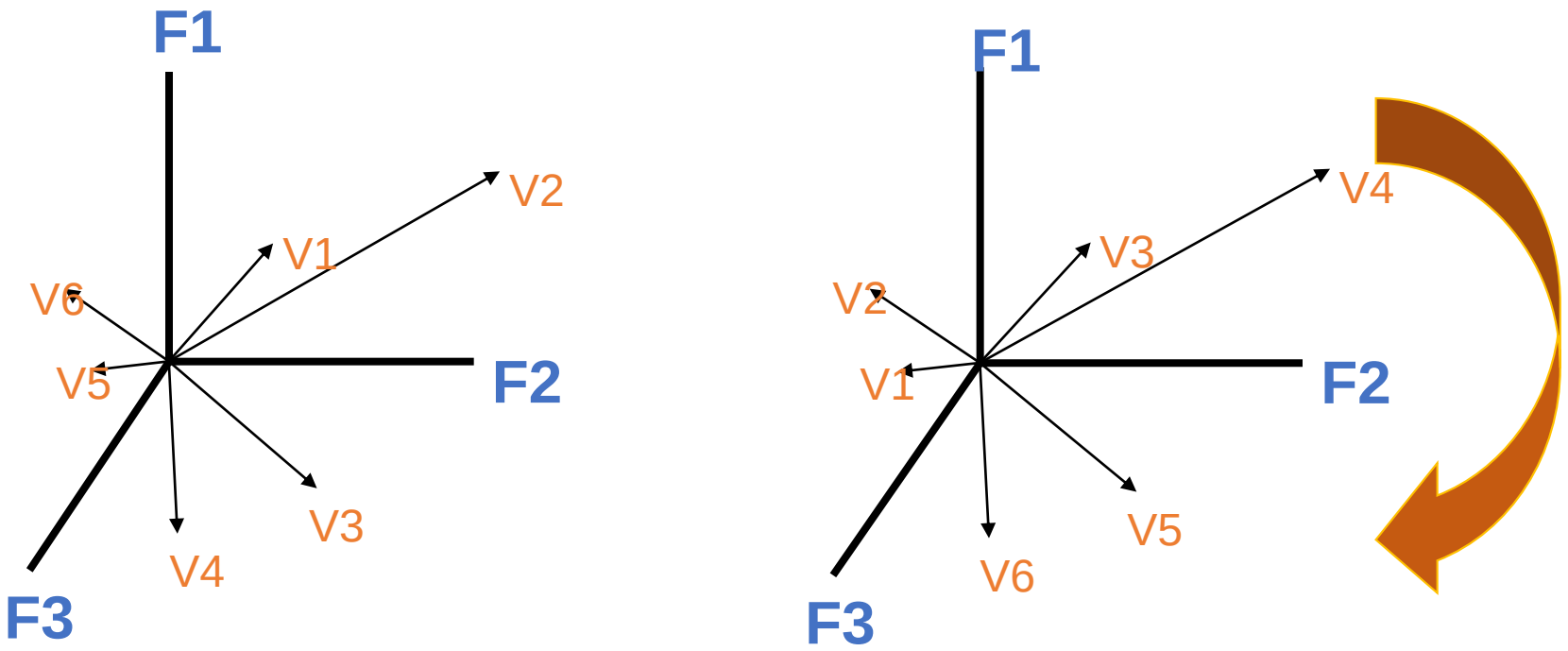
Change of the factorial matrix pursued by the Principle of Simple Structure.
Orthogonal rotation method that minimizes the number of variables with high weight on each factor ➔ Simplifies the interpretation of factors.
Maximizes the variance explained by each factor ➔ significant differences, not noise
VARIMAX Rotation (Kaiser, 1958)



Reduction of the number of variables
➔ Get new variables called factors
Factors are linear combinations of the original variables
Possibility of representation of these factors ➔ simple and interpretable

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We obtain 3 tables

1. Percentage that explains the variance.

FACTOR	PERCENTAG DE LA VARIANZA	PORCENTAGE ACUMULADO DE LA VARIANZA
1	32,4	32,4
2	20,2	52,7
3	16,7	69,3
4	10,8	80,1
5	8,9	89,0
6	7,0	96,0
7	3,9	100

Variance ➔ information on the difference of the variables.
Increasing the number of variables ➔ increases noise

- Sometimes not all correlations can be explained
- What is the percentage of variance I want to explain?

We want to explain ≥ 75% of the variance.
We cannot loose > 25% of the data.

2. Matrix of rotated elements (Varimax).

	FACTOR 1	FACTOR 2	FACTOR 3	FACTOR 4
As	0,90	-0,21	0,18	0,09
Cd	0,85	-0,10	-0,05	-0,39
Cr	0,64	0,62	-0,27	0,09
Cu	0,12	-0,16	0,74	-0,15
Fe	0,97	0,08	0,18	-0,04
Hg	0,74	0,01	-0,08	0,16
Ni	0,10	-0,17	0,76	-0,17
Pb	0,46	0,18	0,09	0,80
Zn	0,94	0,07	0,20	0,16
PCBS	-0,04	0,73	-0,33	0,22
PAHS	0,05	0,43	0,30	-0,29
MORLAB	0,30	-0,70	0,01	0,46
GPXLAB	0,08	-0,42	0,58	0,35
GRLAB	-0,08	0,39	0,82	0,01
ERODLAB	-0,48	0,65	0,29	-0,08
METLAB	0,83	-0,24	-0,02	0,17
VTGLAB	0,14	0,53	-0,47	0,34
HPTGLAB	0,17	-0,02	-0,19	0,92
HPTHLAB	-0,10	0,30	0,83	0,14
HPTGOLAB	-0,58	0,24	-0,15	0,73
MORS	0,94	0,01	-0,24	-0,06
GPXS	-0,08	-0,83	-0,42	-0,01
GRS	0,22	0,03	0,69	-0,28
ERODS	-0,25	0,68	-0,06	0,08
MTS	0,80	-0,27	0,34	-0,05
GSIS	0,37	-0,88	-0,04	-0,05
HPTS	-0,75	0,61	-0,14	0,14
HPTGS	0,02	0,65	0,62	0,31
HPTHS	0,20	0,82	-0,02	0,45
HPTGOS	-0,58	0,24	-0,15	0,73

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Weight that each variable has in each factor:

F1 = a[As] + b[Cd] + c[Cr] .....

Coefficients a, b, c, ... = weight of each variable in each factor

The higher the coefficient ➔ the greater the weight of the variable within F1
The higher the coefficient ➔ the greater the probability that the relationship exists

To define each factor: only values ≥ 0.4

Positive F1 related with : As; Cd; Cr;.....
Negative F1 related with: ERODlab; HPTGOlab; HPTs; HPTGOs ➔ Not associated with any negative contaminants

3. Factor Weight for each study area

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SITE	FACTOR 1	FACTOR 2	FACTOR 3	FACTOR 4
CA2	-0,82	-0,17	-0,78	-1,79
CA3	-0,08	-1,39	-0,87	-0,09
HU2	2,02	-0,35	-0,54	0,52
HU3	0,38	-0,73	1,84	-0,31
BI3	-1,20	-0,72	0,15	1,17
BI2	0,38	1,19	0,72	-0,96
PA3	-0,70	0,87	0,54	0,91
PA2	0,02	1,30	-1,06	0,55

Weight factor F = Obtained by substituting the numeric values of [ ], %, ... for each site

F1 = a[As] + b[Cd] + c[Cr] …….

F1 = positive ➔ explains that my [As], [Zn],..... are related to the + observed effect
F1 = negative ➔ negative values related to negative coefficients

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Database
In situ and laboratory biomarker results
Characteristics and sediment chemistry
Community alteration

STATGRAPHICS Plus - Untitled StatFolio - [BASE DE DATOS MIECA.sf3]

File Edit Plot Describe Compare Relate Special SnapStats!! View Window Help

	ESTACIÓN	GST	GPX	EROD	HPTBR	MET	%GRAVAS	%ARENAS	%FINOS
1	1	19,02	588,1	1,33	1	17,37	0,19	99,77	0,04
2	2	52,1	710	1,8	2	25,24	0,05	40,42	59,53
3	3	38,1	510	1,1	1	32,5	0,3	17,8	81,9
4	4	30,3	456	1,1	2	43,7	0,03	0,38	99,59
5	5	59,26	385,83	1,4	2	189	0,07	9,71	90,22
6	6	41,74	456	1,2	2	208,05	0,19	56,02	90,21
7	7	37	567	1,56	1,5	198	0,03	16,13	43,95
8	8	34	889	4,5	2	30,3	2,39	20,28	77,33
9	9	24	711	5,6	1,5	27	38,12	14,48	47,4
10	10	20,09	680	1,98	2	37,6	0,19	6,22	93,59
11	11	31	690	3,7	2,25	34,6	0,84	28,87	70,29
12	12	28	769	4,2	2	21,5	3,67	5,08	91,24
13	13	19	640	3,1	2	28,4	1,82	38,53	59,65
14	14	6,45	594,4	1,1	2	145	0,2	7,8	92
15									
16									

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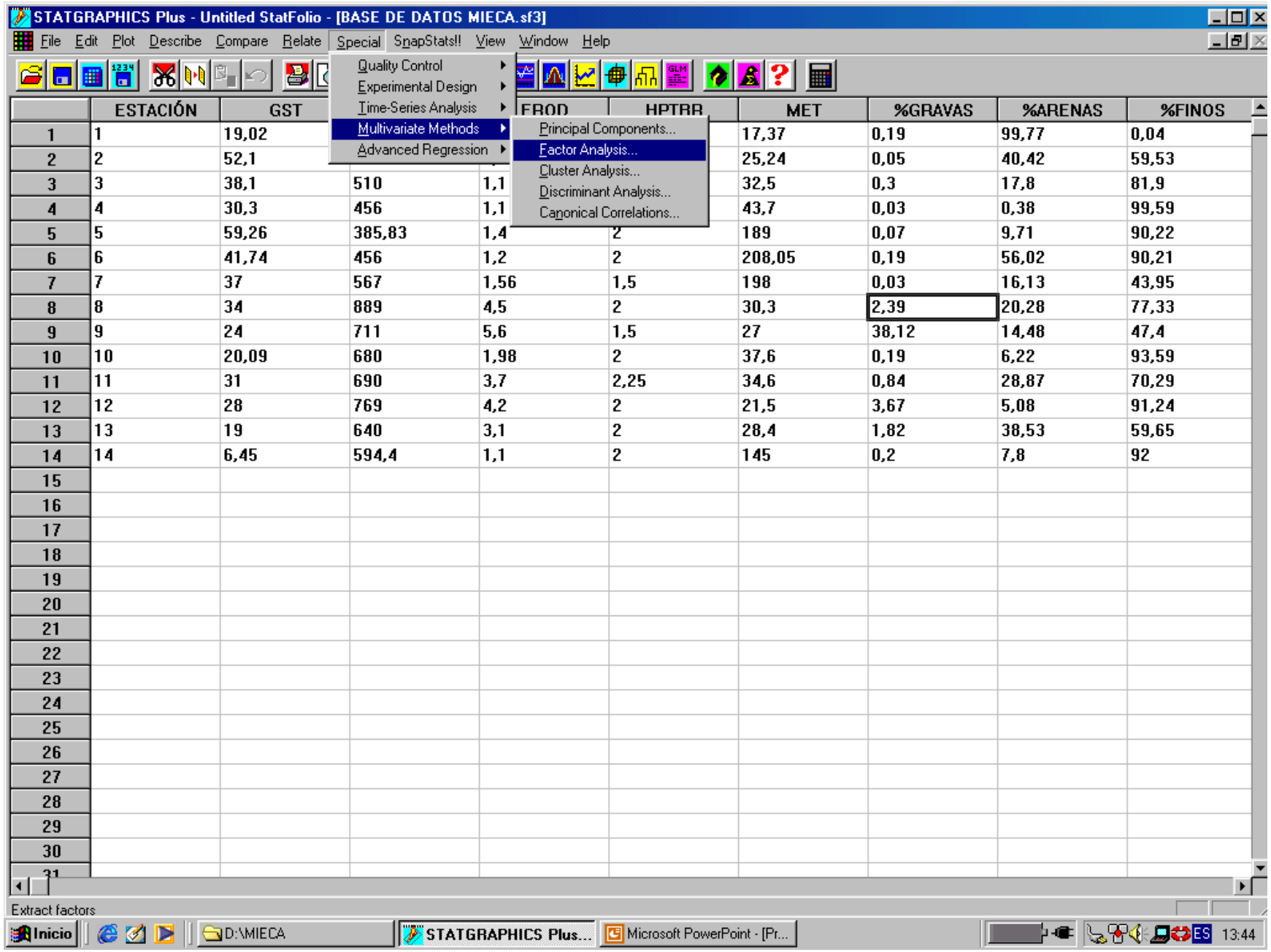

	ESTACIÓN	GST				MET	%GRAVAS	%ARENAS	%FINOS
1	1	19,02				17,37	0,19	99,77	0,04
2	2	52,1				25,24	0,05	40,42	59,53
3	3	38,1	510	1,1		32,5	0,3	17,8	81,9
4	4	30,3	456	1,1		43,7	0,03	0,38	99,59
5	5	59,26	385,83	1,4	2	189	0,07	9,71	90,22
6	6	41,74	456	1,2	2	208,05	0,19	56,02	90,21
7	7	37	567	1,56	1,5	198	0,03	16,13	43,95
8	8	34	889	4,5	2	30,3	2,39	20,28	77,33
9	9	24	711	5,6	1,5	27	38,12	14,48	47,4
10	10	20,09	680	1,98	2	37,6	0,19	6,22	93,59
11	11	31	690	3,7	2,25	34,6	0,84	28,87	70,29
12	12	28	769	4,2	2	21,5	3,67	5,08	91,24
13	13	19	640	3,1	2	28,4	1,82	38,53	59,65
14	14	6,45	594,4	1,1	2	145	0,2	7,8	92
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Describe

➔ multivariate methods

➔ factor analysis

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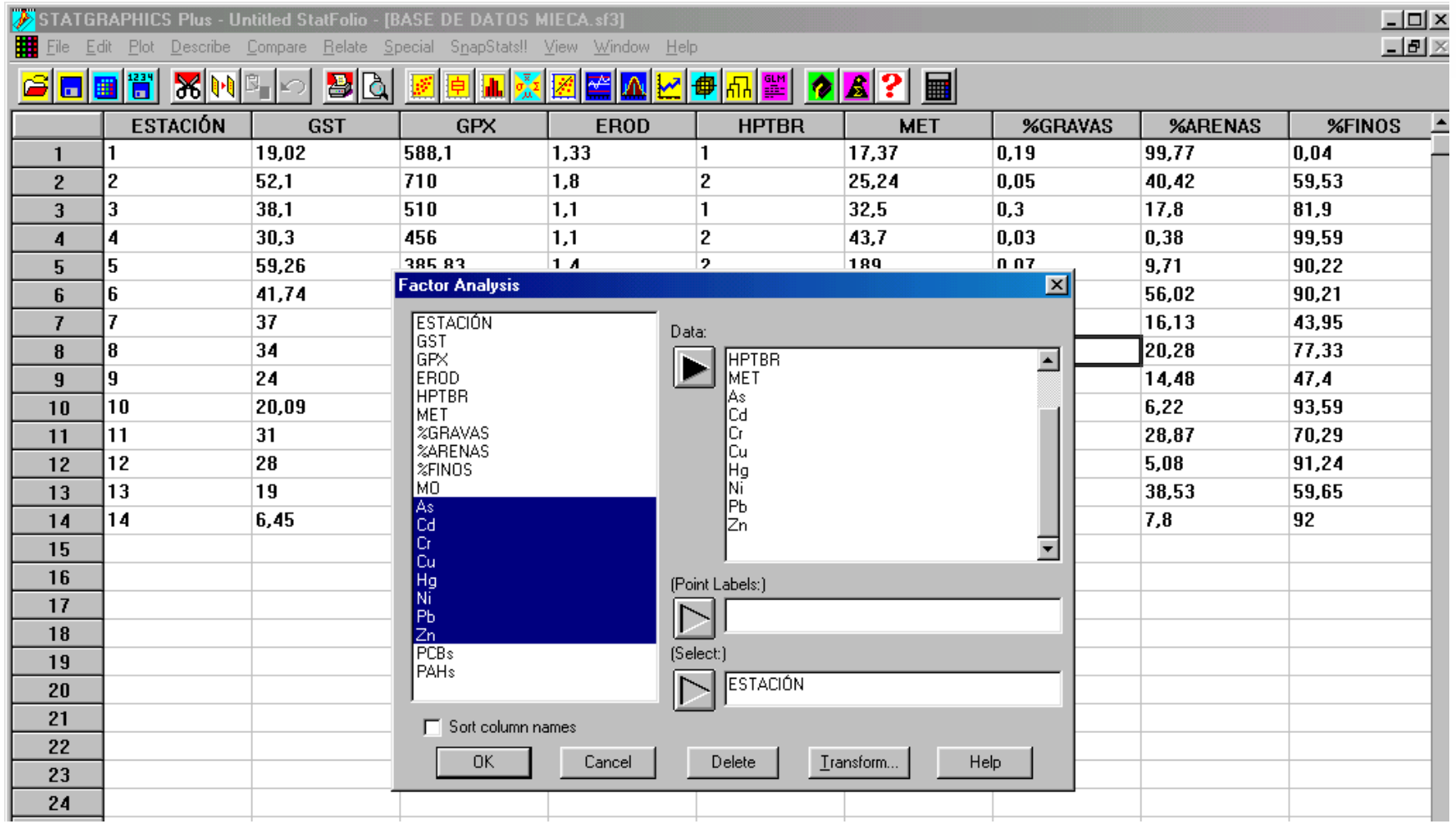

	ESTACIÓN	GST	GPX	EROD	HPTBR	MET	%GRAVAS	%ARENAS	%FINOS
1	1	19,02	588,1	1,33	1	17,37	0,19	99,77	0,04
2	2	52,1	710	1,8	2	25,24	0,05	40,42	59,53
3	3	38,1	510	1,1	1	32,5	0,3	17,8	81,9
4	4	30,3	456	1,1	2	43,7	0,03	0,38	99,59
5	5	59,26	385,83	1,4	2	189	0,07	9,71	90,22
6	6	41,74						56,02	90,21
7	7	37						16,13	43,95
8	8	34						20,28	77,33
9	9	24						14,48	47,4
10	10	20,09						6,22	93,59
11	11	31						28,87	70,29
12	12	28						5,08	91,24
13	13	19						38,53	59,65
14	14	6,45						7,8	92
15									
16									
17									
18									
19									
20									
21									
22									
23									
24									

Select variables to be included in the analysis

➔ Select all except “station”

➔ Data

➔ Select “STATION”

➔ (Select)

➔ OK

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STATGRAPHICS Plus - Untitled StatFolio - [Factor Analysis (ESTACIÓN)]

File Edit Plot Describe Compare Relate Special SnapStats!! View Window Help

Lbl: Row:

Analysis Summary

Data variables:
GST
GPX
EROD
HPTBR
MET
As
Cd
Cr
Cu
Hg
Ni
Pb
Zn

Selection variable: ESTACIÓN

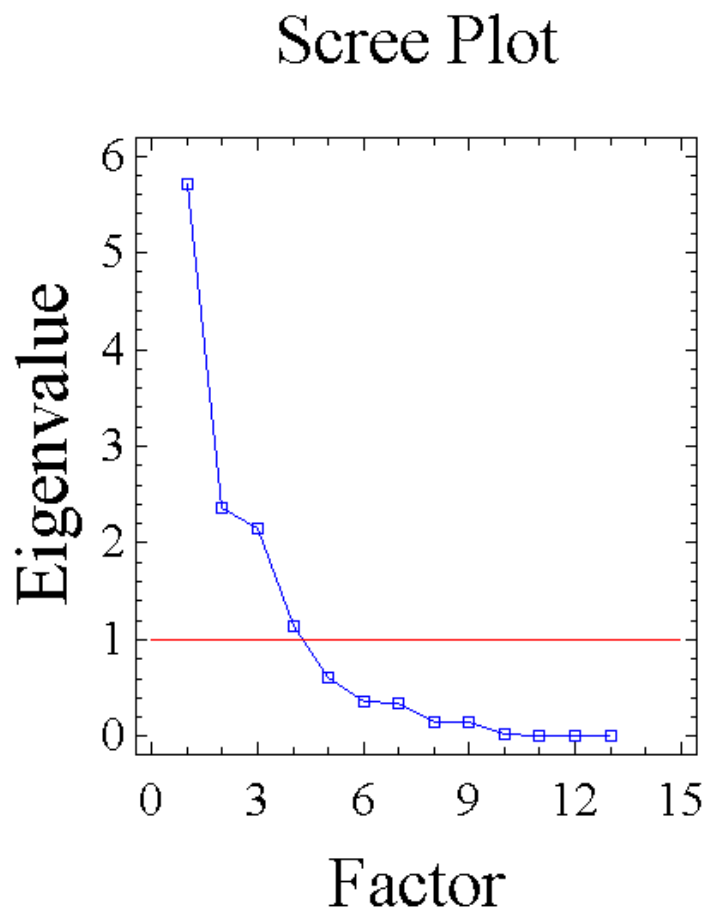
Data input: observations
Number of complete cases: 14
Missing value treatment: listwise
Standardized: yes

Type of factoring: principal components
Number of factors extracted: 4

Factor Analysis

Factor Number	Eigenvalue	Percent of Variance	Cumulative Percentage
1	5,71183	43,937	43,937
2	2,35753	18,135	62,072
3	2,14662	16,512	78,584
4	1,1338	8,722	87,306
5	0,61468	4,728	92,034
6	0,369547	2,843	94,877
7	0,336389	2,588	97,465
8	0,152227	1,171	98,636
9	0,143331	1,103	99,738
10	0,0215821	0,166	99,904
11	0,00925071	0,071	99,975
12	0,00319891	0,025	100,000
13	0,0000149635	0,000	100,000

Variable	Initial Communality
GST	1,0
GPX	1,0
EROD	1,0
HPTBR	1,0
MET	1,0
As	1,0



Use the right mouse button to select options

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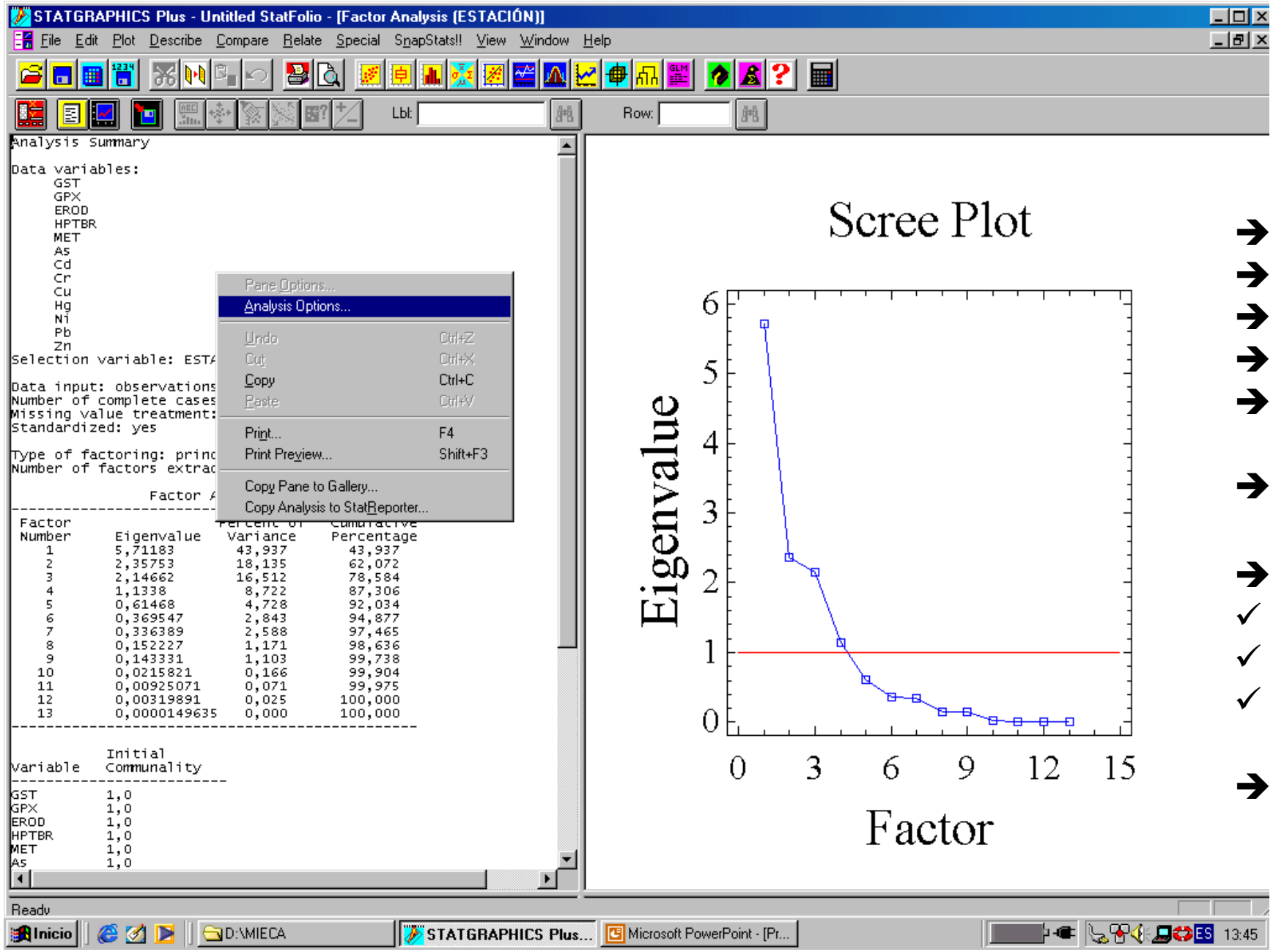

➔ New window:
➔ Listwise
➔ Principal components
➔ Varimax
➔ Nº factors: 13

➔ OK

➔ Tables & Graphs
✓ Analysis summary
✓ (after)rotation
✓ Factor scores

➔ OK

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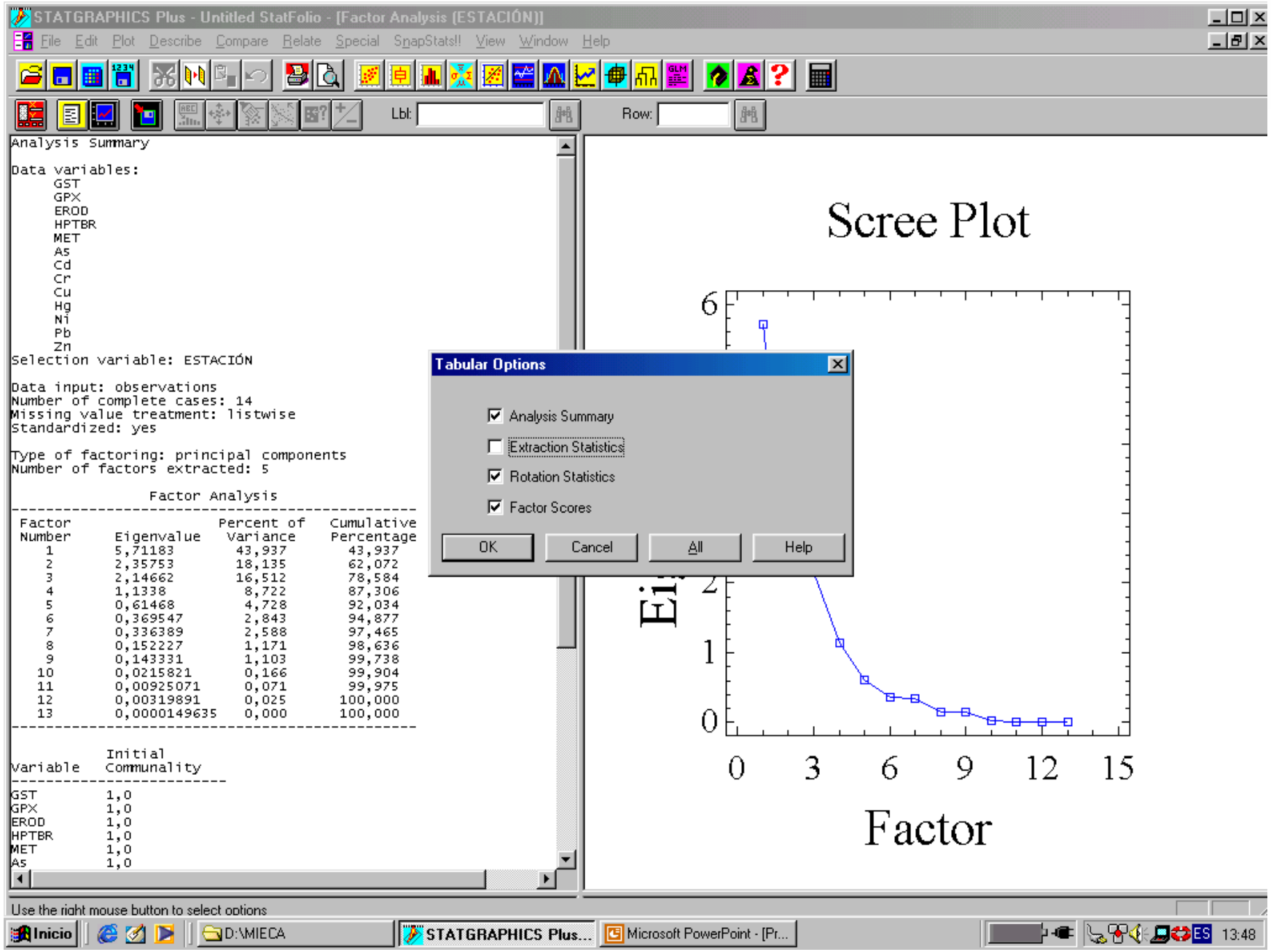

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STATGRAPHICS Plus - Untitled StatFolio - [Factor Analysis (ESTACIÓN)]

File Edit Plot Describe Compare Relate Special SnapStats!! View Window Help

Lbl: Row:

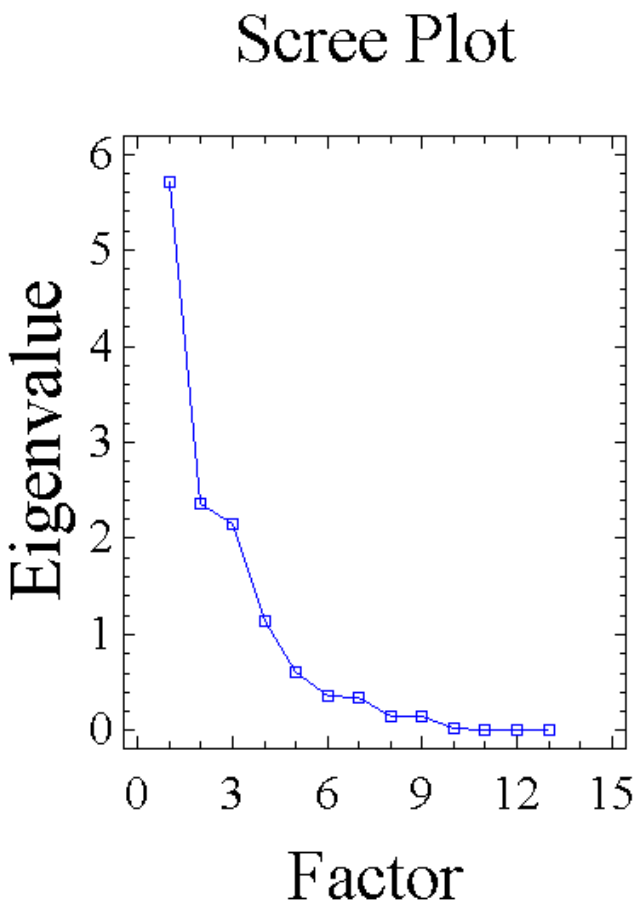
```
Analysis Summary

Data variables:
     GST
     GPX
     EROD
     HPTBR
     MET
     As
     Cd
     Cr
     Cu
     Hg
     Ni
     Pb
     Zn
Selection variable: ESTACIÓN
```

Factor Loading Matrix After Varimax Rotation

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
GST	-0,52354	0,695426	-0,327056	0,0680169	0,065821
GPX	-0,0800673	-0,252093	0,878967	0,0245089	0,14617
EROD	-0,17167	0,140557	0,94365	-0,0261155	0,067606
HPTBR	0,123219	0,212694	0,133615	-0,0245511	0,945883
MET	0,343941	0,446216	-0,556223	0,477523	0,123572
As	0,823976	0,422239	-0,322645	0,127929	0,079410
Cd	0,955375	0,163276	-0,118351	0,0309006	0,007837
Cr	0,331108	0,754609	0,208067	-0,120928	0,413501
Cu	0,247382	0,738622	-0,265413	0,346345	-0,07577
Hg	0,388771	0,774224	0,0144256	0,104157	0,277787
Ni	0,0800564	0,10232	0,0149562	0,977204	-0,04132
Pb	0,980196	-0,0101013	-0,0684388	0,0665904	0,116207

Table of Factor Scores

Row	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
1	-3,27792	-4,89529	-0,305616	-1,68136	-3,40311
2	-2,19443	0,67781	0,467994	-0,627921	0,632463
3	-3,18818	-2,68494	-1,36778	-0,990861	-2,95236
4	-2,47695	-2,16089	-1,13059	-1,01151	-0,472913
5	4,38419	8,23921	-4,70098	2,33206	1,61369
6	2,12062	2,91245	-2,97018	0,11713	1,31185
7	0,218388	1,18188	-2,38184	4,42082	-1,17835
8	-2,13341	-1,26293	3,72446	-0,746531	0,593495
9	-1,01583	-0,31673	3,36683	-0,516539	-0,323124
10	-2,54054	-3,8296	0,902803	-1,08035	-0,611556



Use the right mouse button to select options

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Three tables are obtained

1. Table in which the weight of each variable in each factor is represented.
2. Table showing the variance explained in each factor.
3. Table in which the weight of each factor in each case is observed.

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SITE	FACTOR 1	FACTOR 2	FACTOR 3	FACTOR 4
CA2	-0,82	-0,17	-0,78	-1,79
CA3	-0,08	-1,39	-0,87	-0,09
HU2	2,02	-0,35	-0,54	0,52
HU3	0,38	-0,73	1,84	-0,31
BI3	-1,20	-0,72	0,15	1,17
BI2	0,38	1,19	0,72	-0,96
PA3	-0,70	0,87	0,54	0,91
PA2	0,02	1,30	-1,06	0,55

	FACTOR 1	FACTOR 2	FACTOR 3	FACTOR 4
As	0,90	-0,21	0,18	0,09
Cd	0,85	-0,10	-0,05	-0,39
Cr	0,64	0,62	-0,27	0,09
Cu	0,12	-0,16	0,74	-0,15
Fe	0,97	0,08	0,18	-0,04
Hg	0,74	0,01	-0,08	0,16
Ni	0,10	-0,17	0,76	-0,17
Pb	0,46	0,18	0,09	0,80
Zn	0,94	0,07	0,20	0,16
PCBS	-0,04	0,73	-0,33	0,22
PAHS	0,05	0,43	0,30	-0,29
MORLAB	0,30	-0,70	0,01	0,46
GPXLAB	0,08	-0,42	0,58	0,35
GRLAB	-0,08	0,39	0,82	0,01
ERODLAB	-0,48	0,65	0,29	-0,08
METLAB	0,83	-0,24	-0,02	0,17
VTGLAB	0,14	0,53	-0,47	0,34
HPTGLAB	0,17	-0,02	-0,19	0,92
HPTHLAB	-0,10	0,30	0,83	0,14
HPTGOLAB	-0,58	0,24	-0,15	0,73
MORS	0,94	0,01	-0,24	-0,06
GPXS	-0,08	-0,83	-0,42	-0,01
GRS	0,22	0,03	0,69	-0,28
ERODS	-0,25	0,68	-0,06	0,08
MTS	0,80	-0,27	0,34	-0,05
GSIS	0,37	-0,88	-0,04	-0,05
HPTS	-0,75	0,61	-0,14	0,14
HPTGS	0,02	0,65	0,62	0,31
HPTHS	0,20	0,82	-0,02	0,45
HPTGOS	-0,58	0,24	-0,15	0,73

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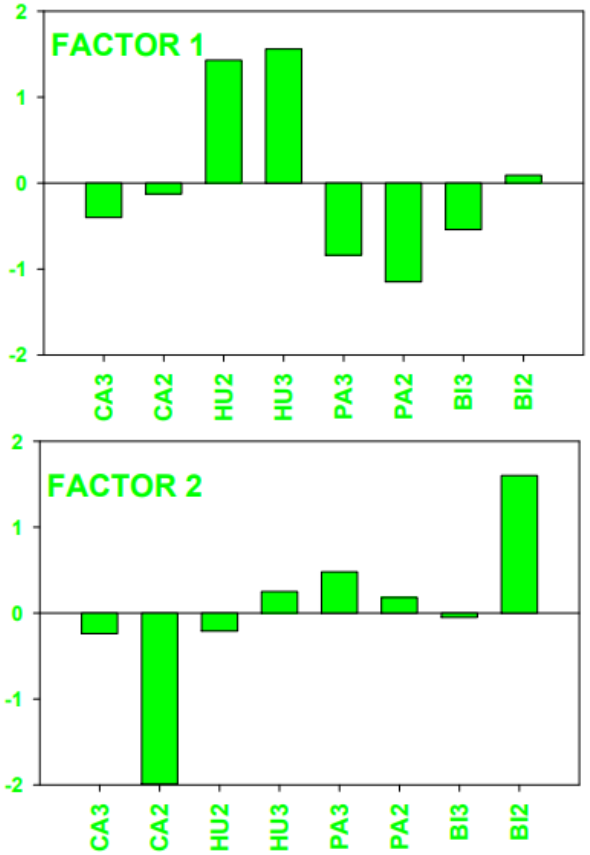
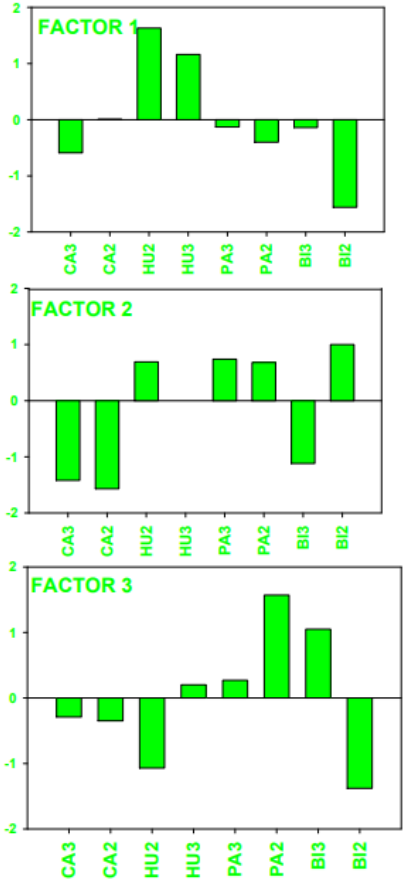


Análisis de Factores: *Carcinus maenas*

Factor 1: (Positivo) Inducción de MTs y exposición a metales en el laboratorio e in situ

(Negativo) Inducción de GPX y EROD en el laboratorio y campo y PCBs

Factor 2: (Positivo) Inducción de GR y EROD inducción en el laboratorio y campo debido a PAHs, Mn, Cr



Análisis de Factores: *Carcinus maenas*

Factor 1: As, Cu y Pb en el sedimento y en los tejidos biológicos sin asociarse a daños histopatológicos

Factor 2: Bioacumulación de Cu en individuos expuestos in situ y daños histopatológicos en hepatopáncreas y gónada.

Factor 3: Ni, Cr y Hg en el sedimento y en individuos expuestos en el laboratorio junto con daño en el hepatopáncreas

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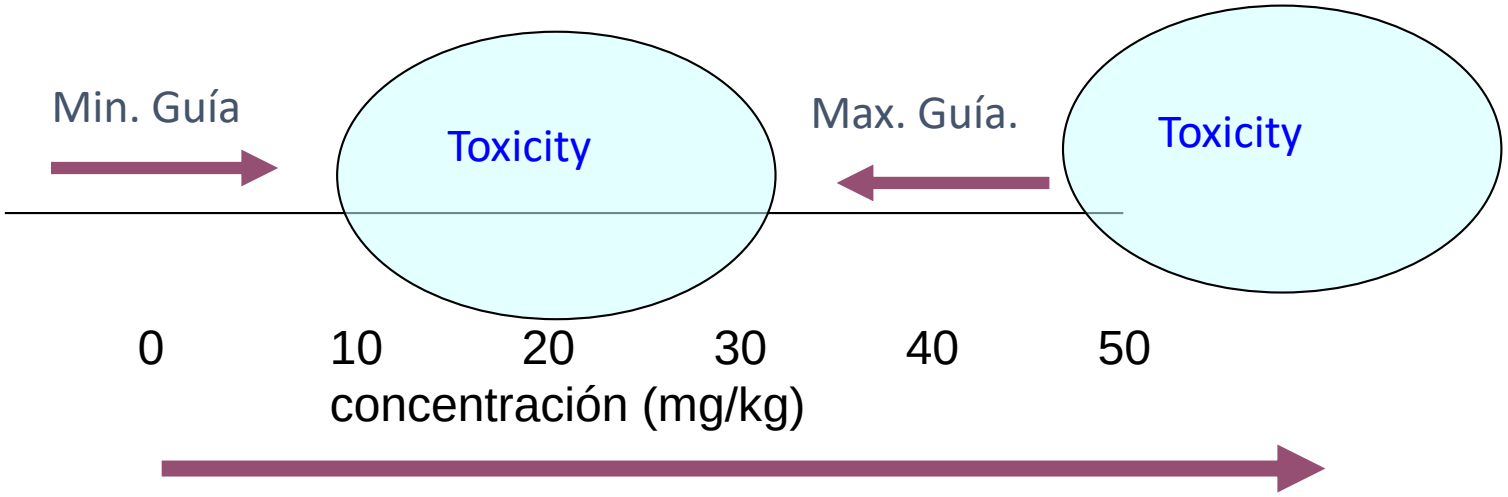
QUALITY INDICES: SEDIMENT QUALITY GUIDES

➔ Relate the concentration of a pollutant to an adverse effect:

Maximum Guide: Minimum concentration above which there is an associated adverse effect.

Minimum Guide: Maximum concentration below which there is no associated adverse effect.



¡GRACIAS! Thank you Faleminderit Hvala.

Miriam Hampel
E-mail: miriam.hampel@uca.es

UCA Universidad de Cádiz

MEP&M
Development of Regional Joint Master Program in Maritime Environmental Protection and Management