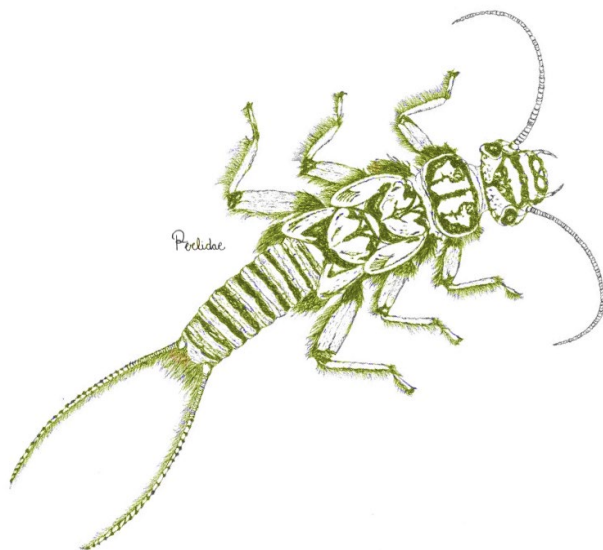


# Sediment pollution, chronic toxicity and metal bioaccumulation in freshwater macroinvertebrates from Pb/Zn mining districts

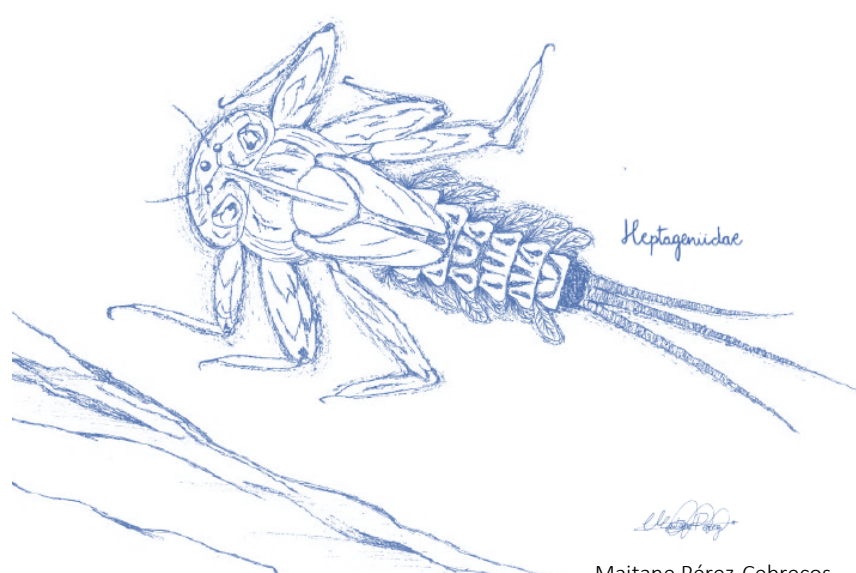
Iñigo Moreno-Ocio<sup>1</sup>, Pilar Rodriguez<sup>1</sup>,  
Maite Martínez-Madrid<sup>2</sup> & Leire Méndez-Fernández<sup>2</sup>

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Maitane Pérez-Cebrecos



Maitane Pérez-Cebrecos

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## 1. Introduction

## 2. Study area

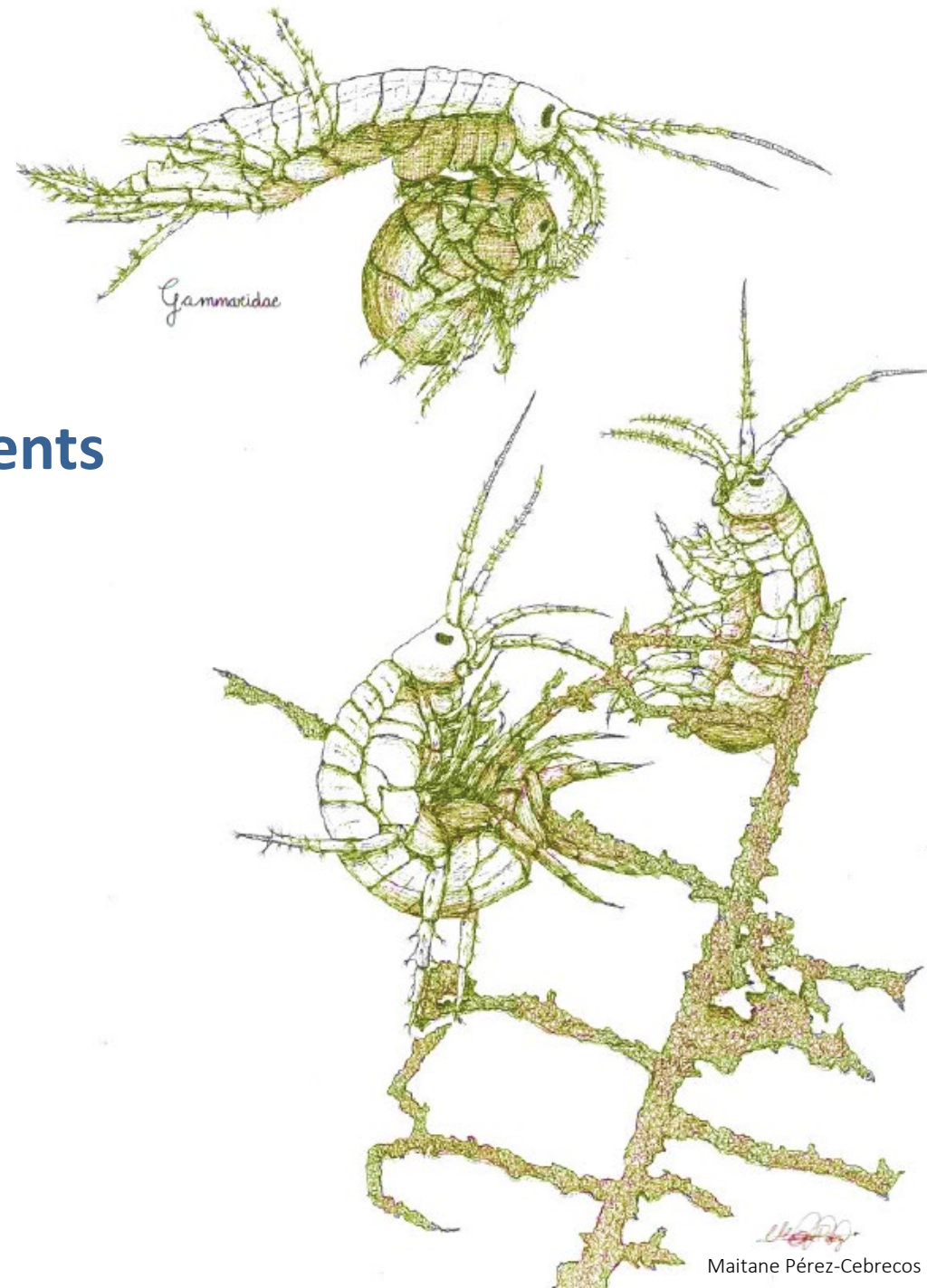
## 3. Sampling of macroinvertebrates and sediments

## 4. Metal analyses

## 5. Lines of Evidence

## 6. Weight of Evidence

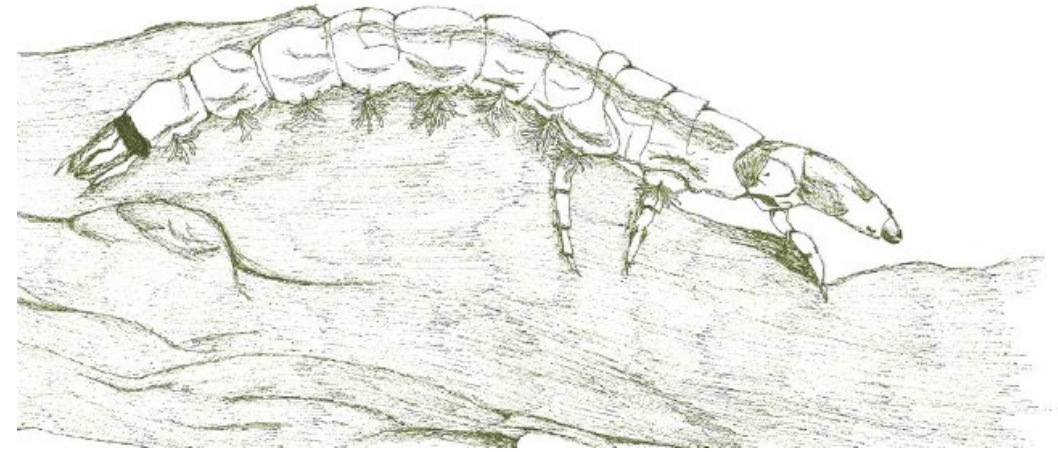
## 7. Conclusions



# 1.1. Introduction: Legislation



Type-specific reference  
conditions (WFD, 2000)



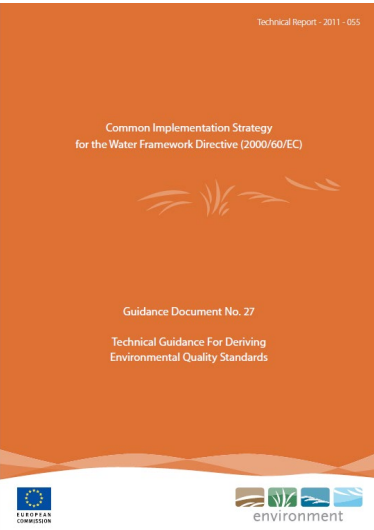
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Sediments and Biota as relevant matrices for long-term monitoring  
in Europe (EC, 2013)

- **Background** concentration is an additional line of evidence that should be considered in **EQS development** for naturally occurring substances such as **metals** (EC, 2013) → IMPORTANT in Areas affected by mining activities.

EQS developed and used during 2015-2021 (EC, 2013)

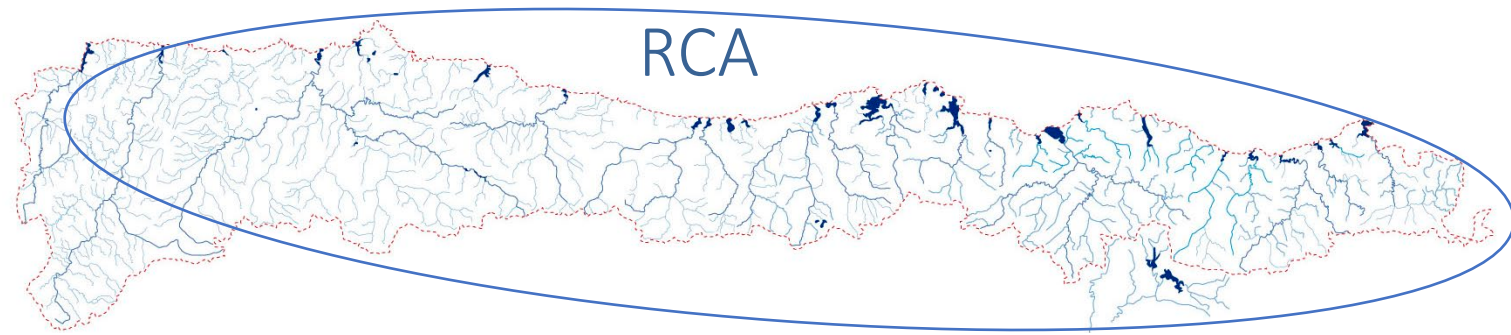
→ Extended till 2027!



## 1.2. Introduction: Definitions



REFERENCE CONDITION APPROACH (RCA): spatial network of minimally disturbed sites (WFD).



BACKGROUND/BASELINE levels: metal concentrations in the sediments or biota not due to local anthropogenic sources.

THRESHOLD levels: the upper limit of normal background/baseline metal fluctuations in sediments or biota related with no-effects at community level.

# 1.3. Introduction: Biomonitors

Promising tool to identify and assess sites having chemical tissue residues higher than reference levels.



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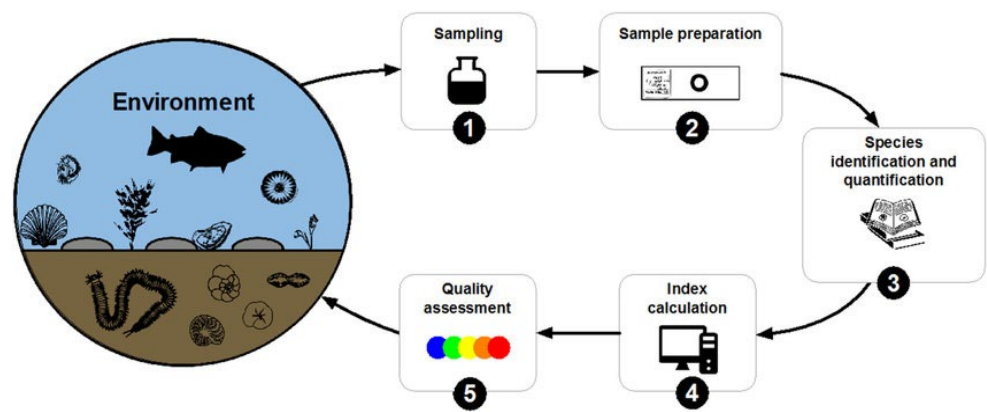


Fig. 1. Sampling strategies for bioaccumulation and ecological status assessment. From: Pawlowski *et al.* (2018). *Science of the Total Environment*, 637, 1295-1310.

Use of **several taxa** based on their departure from the baseline tissue residues.



Moreno-Ocio I., L. Méndez-Fernández, M. Martínez-Madrid, N. Costas, I. Pardo, P. Rodríguez. 2022. Developing As and Cu tissue residue thresholds to attain the good ecological status of rivers in mining areas. *Archives of Environmental Contamination and Toxicology*, 82, 379-390.

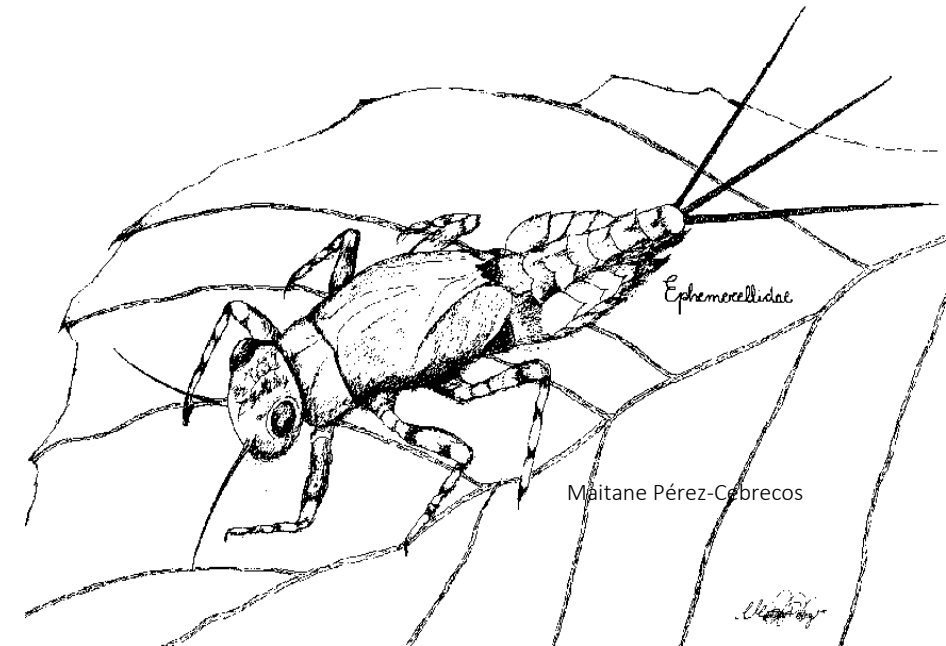
## 1.4. Objective:

To conduct an environmental risk assessment, based on the RCA, of sites influenced by Zn/Pb mining works, using three **Lines of Evidence**, LOE, in a decision-making process such as the **Weight of Evidence**, WOE.

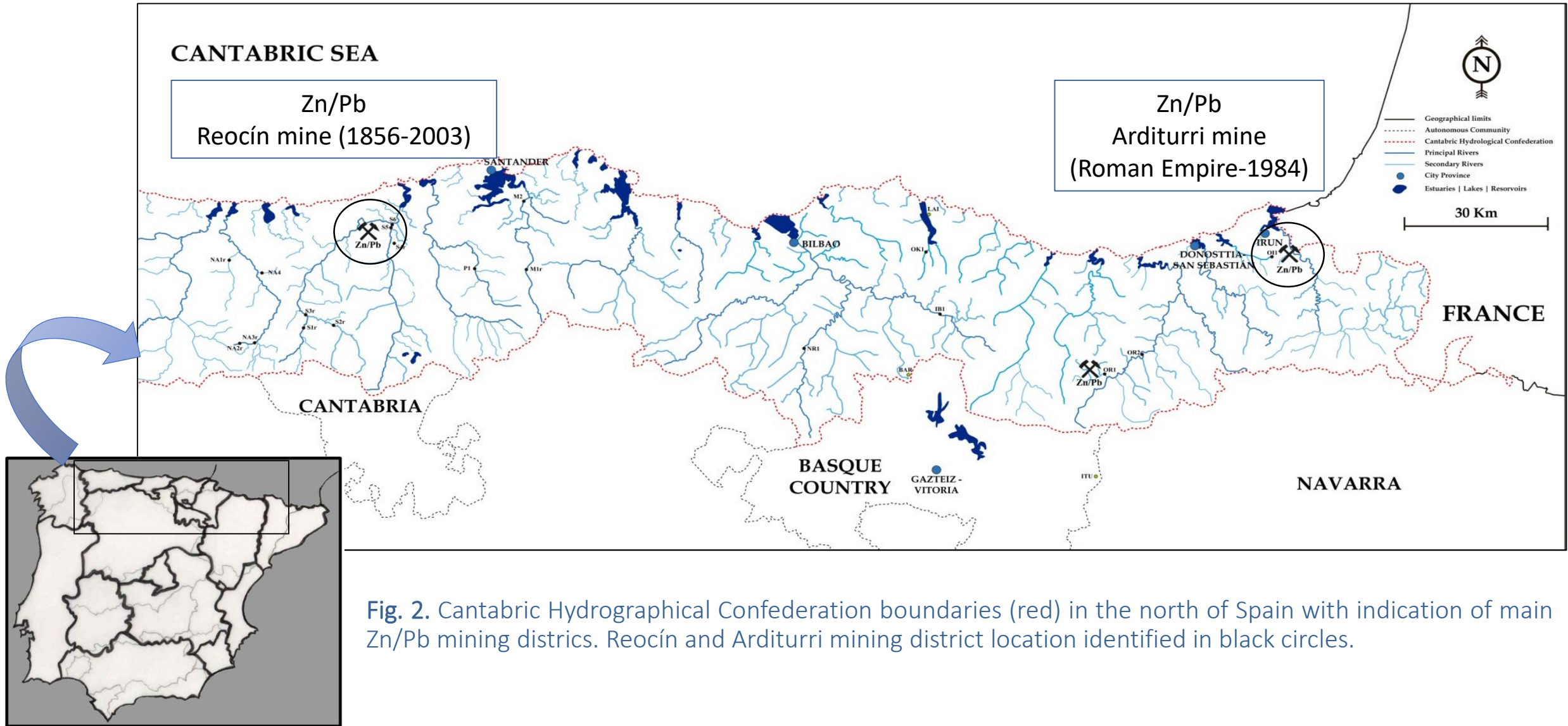
LOE 1: sediment chemistry

LOE2: field bioaccumulation

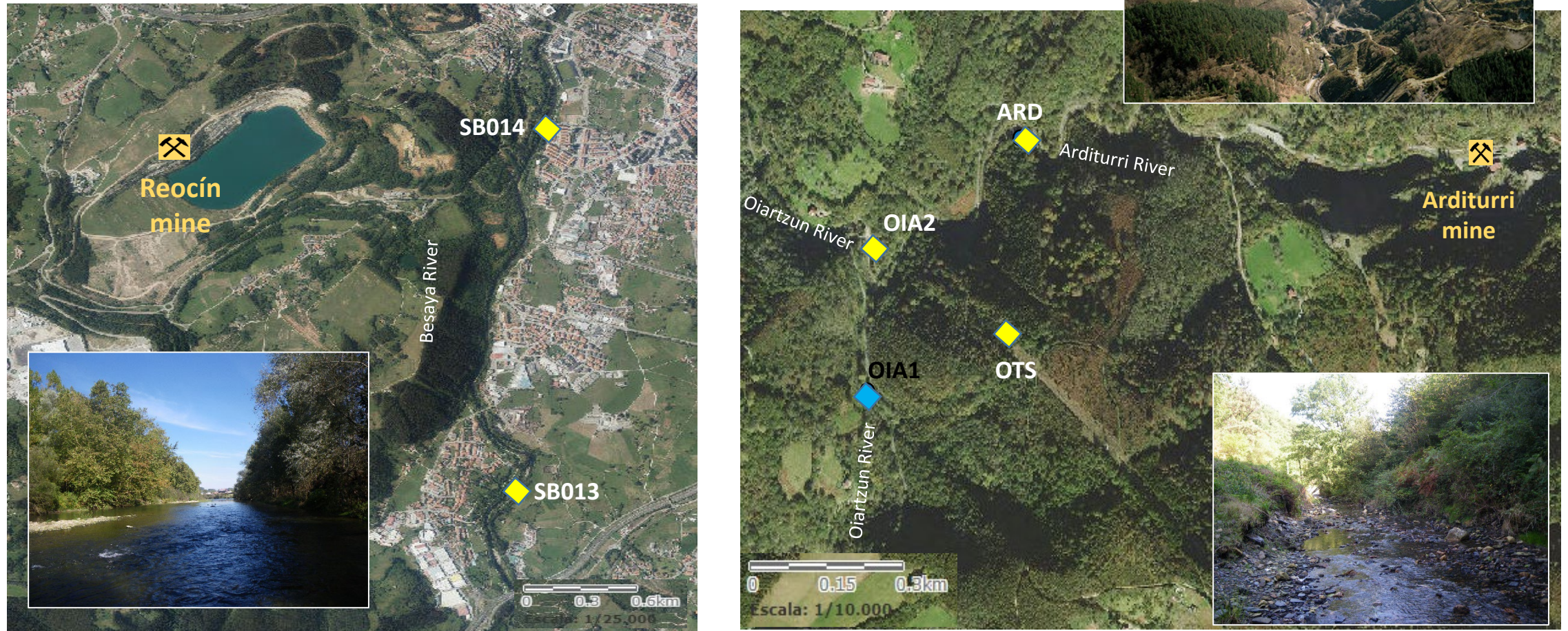
LOE 3: sediment bioassays



## 2. Study area: Reocín and Arditurri mines



## 2. Study area: Reocín and Arditurri mines



**Fig. 3.** Arditurri and Reocín mining districts with detailed representation of each Pb/Zn mine and sampling sites. In blue, OIA1 reference site and in yellow, OIA2, ARD, OTS, SB013 and SB014 test sites (Source: Google maps).

### 3. Sampling macroinvertebrates and sediments

- Macroinvertebrates: When possible, 3 replicates per taxon were collected.

10 selected macroinvertebrate taxa were sampled representing 5 feeding styles:

- ✓ 2 scrapers: Baetidae and Heptageniidae
- ✓ 3 filterers: Hydropsychidae, Ephemeridae and Simuliidae
- ✓ 2 predators: Rhyacophilidae and Perlidae
- ✓ 1 generalist: Ephemerellidae
- ✓ 2 deposit-feeders: Lumbricidae and Microdrili oligochaetes



Scrapers



Filterers



Predators



Generalist



Deposit-feeder

- Sediments:

- ✓ Composite sample of sediment from the upper 5–10 cm layer of fine sediment (stainless steel spade).
- ✓ Sediment air-dried and sieved through a 63- $\mu$ m mesh for analysis.

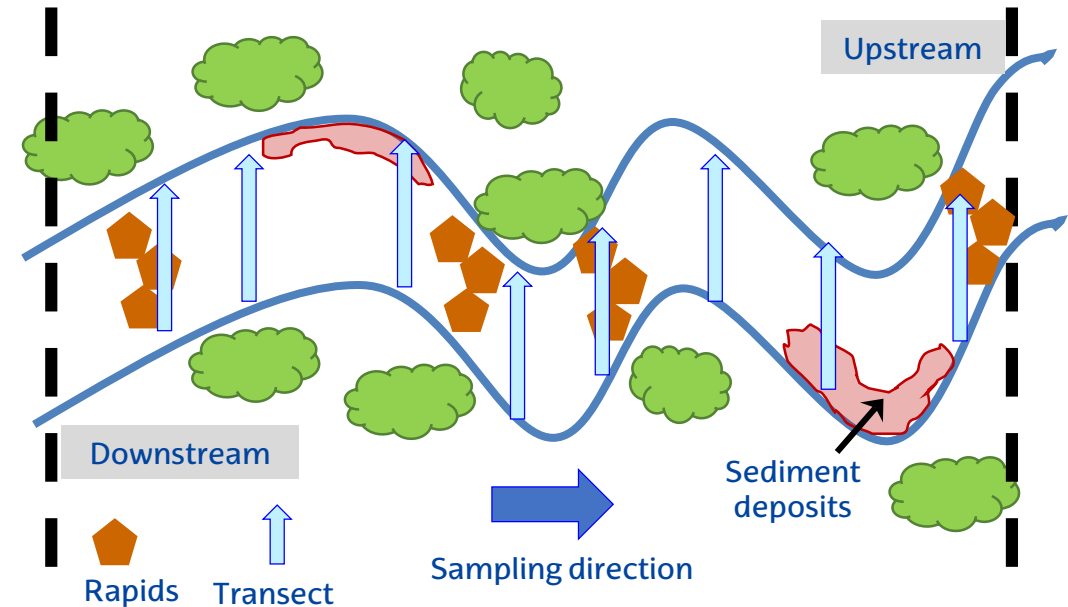
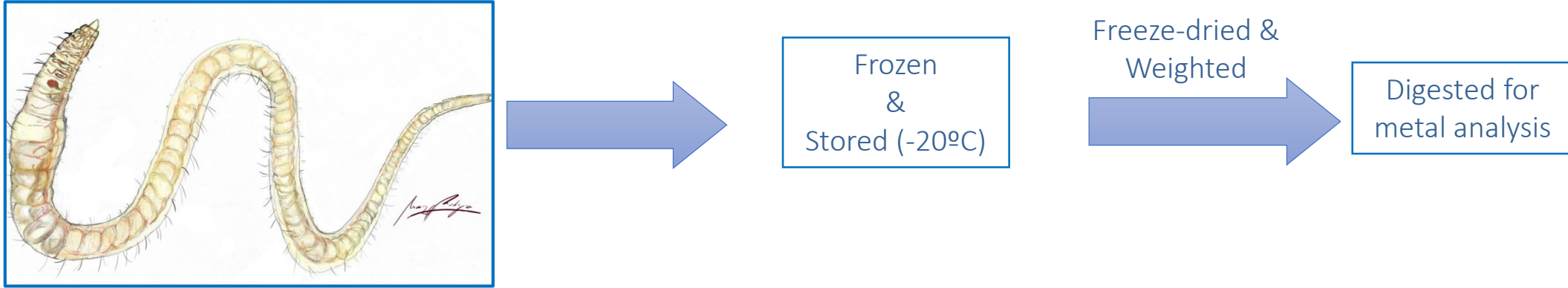


Fig. 4. Multi-habitat sampling scheme, in transects for the collection of macroinvertebrates. Sediments collected from deposits areas.

## 4. Metal Analyses

- Macroinvertebrate tissue residues (Méndez-Fernández *et al.*, 2013).



- Sediment → digested following EPA 3051.
- All samples measured by ICP-MS (7500ce, Agilent Technologies) at SGIker (UPV/EHU).

# 5. LOE 1: Sediment chemistry

Estimation of background threshold Cd, Pb and Zn concentrations using a RCA (31-55 sites) (database Méndez-Fernández, 2013).

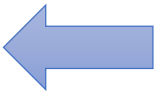
**Table 1.** Ecological background and thresholds estimated for sediment metal concentrations, in mg kg<sup>-1</sup> dw. The threshold effect concentration (TEC) and the probable effect concentration (PEC) for sediments from MacDonald *et al.* (2000) are also shown, for comparison.

| Ecological Backgrounds | Cd               | Pb               | Zn            |
|------------------------|------------------|------------------|---------------|
| Range                  | 0.08-2.20        | 3.1-126          | 12-411        |
| Mean (SD)              | 0.45 (0.50)      | 24.3 (18.5)      | 77.8 (59.4)   |
| n                      | 31               | 52               | 55            |
| Ecological Thresholds  | Cd               | Pb               | Zn            |
| Mean + 2SD             | 1.44             | 61.3             | 197           |
| TIF                    | 0.87             | 44.4             | 171           |
| P90 (95% CI)           | 1.33 (0.54-2.05) | 39.1 (30.9-62.3) | 136 (100-172) |
| TEC                    | 0.99             | 35.8             | 121           |
| PEC                    | 4.98             | 128              | 459           |

Metal quotient: P90-Q

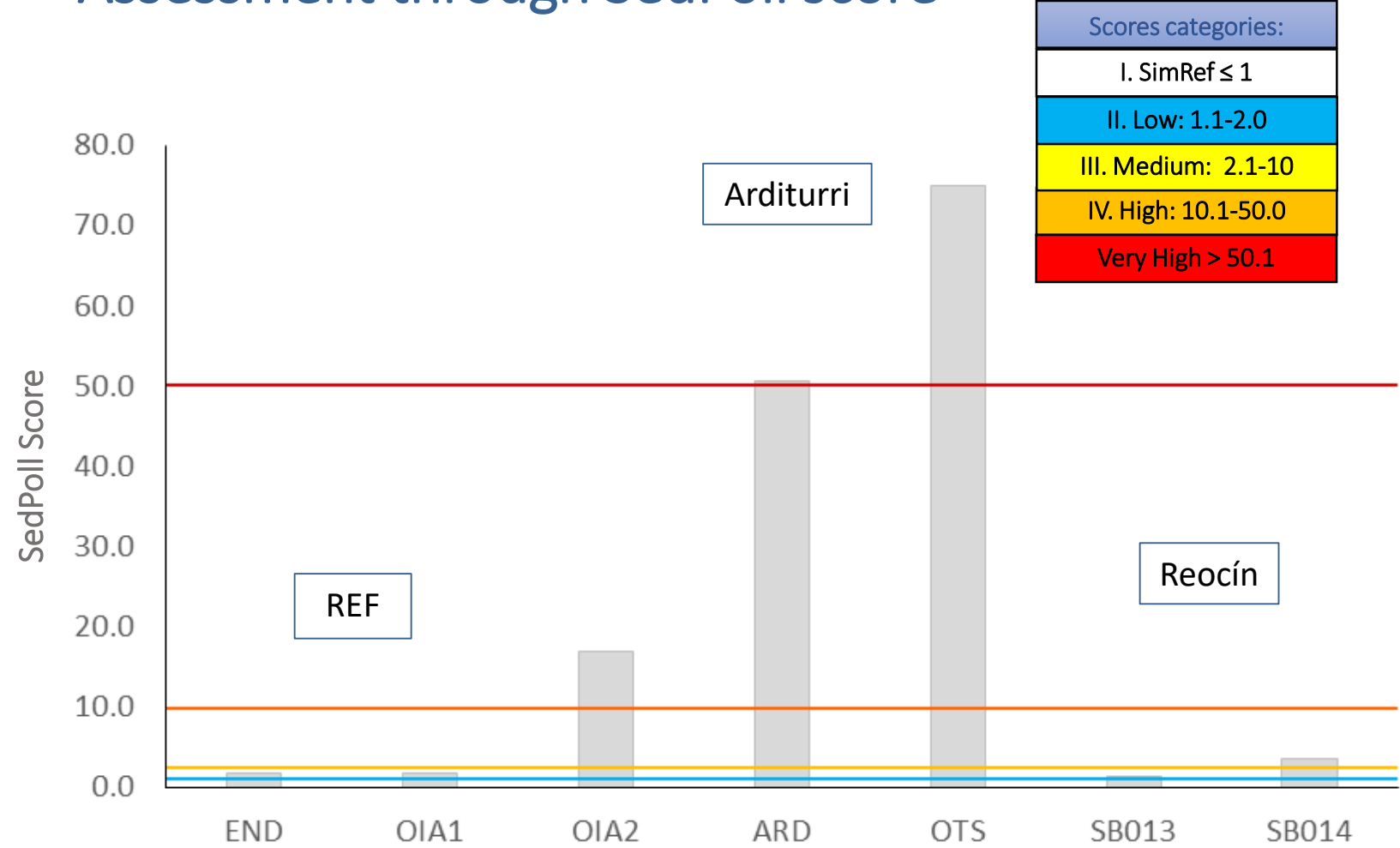


SedPoll Score  
for 3 metals



Méndez-Fernández, L., Casado-Martínez, C., Martínez-Madrid, M., Moreno-Ocio, I., Costas, N., Pardo, I., & Rodriguez, P. (2019). Derivation of sediment Hg quality standards based on ecological assessment in river basins. *Environmental Pollution*, 245, 1000-1013.

# Assessment through SedPoll score



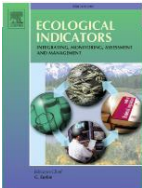
## Arditurri mining district

OIA2 → High metal concentration (SedPoll = 17.0);  
ARD & OTS → Very High metal concentration (SedPoll = 50.7 and 75.1, respectively)

## Reocín mining districts

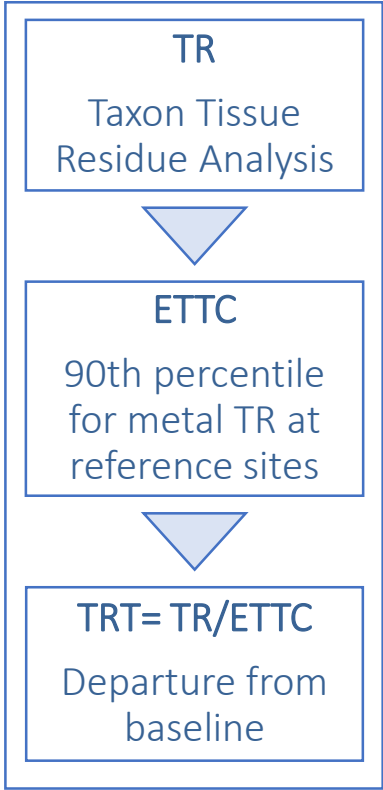
SB013 → Low metal concentration (SedPoll = 1.4);  
SB014 → Medium metal concentration (SedPoll = 3.6);

Fig. 5. SedPoll scores for sediment metal concentration of all study sites (Raw data in Table A1).



Costas, N., Pardo, I., Méndez-Fernández, L., Martínez-Madrid, M., & Rodríguez, P. (2018). Sensitivity of macroinvertebrate indicator taxa to metal gradients in mining areas in Northern Spain. *Ecological Indicators*, 93, 207-218.

# 5. LOE 2: Field bioaccumulation



Ecological Threshold  
Tissue Concentration  
(Rodriguez *et al.*,  
2018).



▶▶▶  $TRT = (\sum_i (TR_i / ETTC_i)) / n$  ▶▶

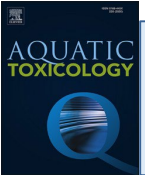
## INtegrated TISSue score

Site INTISS score  
Average TRT scores for a  
selection of metals and taxa  
(Rodriguez *et al.*, 2021)

| Scores categories: |
|--------------------|
| SimRef ≤ 1         |
| Low 1.1-2.0        |
| Medium 2.1-10      |
| High > 10          |



Rodriguez, P., Méndez-Fernández, L., Pardo, I., Costas, N., & Martinez-Madrid, M. (2018). Baseline tissue levels of trace metals and metalloids to approach ecological threshold concentrations in aquatic macroinvertebrates. *Ecological Indicators*, 91, 395-409.



Rodriguez, P., Moreno-Ocio, I., Martínez-Madrid, M., Costas, N., Pardo, I., & Méndez-Fernández, L. (2021). Proposal of integrative scores and biomonitor selection for metal bioaccumulation risk assessment in mine-impacted rivers. *Aquatic Toxicology*, 238, 105918.

# Assessment through INTISS score

Table 2. Results of the Tissue-residue Ratio to Threshold (TRT) score for Cd, Pb and Zn and of the INtegrated TISSue (INTISS) score for all taxa. The INTISS score was also calculated for the four taxa present in all the study sites: Baetidae, Heptageniidae, Simuliidae and Rhyacophilidae.

| Scores categories: |
|--------------------|
| SimRef ≤ 1         |
| Low 1.1-2.0        |
| Medium 2.1-10      |
| High > 10          |

|           |       | Site             | Baetidae | Heptageniidae | Hydropsychidae | Ephemeraidae | Simuliidae | Rhyacophilidae | Perlidae | Ephemerellidae | Lumbricidae | Microdriles | INTISS   |        |
|-----------|-------|------------------|----------|---------------|----------------|--------------|------------|----------------|----------|----------------|-------------|-------------|----------|--------|
|           |       | [Cd, Pb, Zn] TRT |          |               |                |              |            |                |          |                |             |             | All taxa | 4 taxa |
| REF       | END   | 0.9              | 1.6      | 1.1           | 0.8            | 1.1          | 0.5        | 0.3            | 0.8      | 0.8            | 0.5         | 0.8         | 1.0      |        |
|           | OIA1  | 1.5              | 2.6      | 0.9           | 1.3            | 0.9          | 1.4        | 0.7            | 2.3      | 1.0            | 0.8         | 1.3         | 1.6      |        |
| Arditurri | OIA2  | 14.0             | 13.6     | 5.8           | 2.1            | 15.8         | 3.4        | 6.1            | 3.5      | 2.6            | -           | 7.4         | 11.7     |        |
|           | ARD   | 21.1             | 26.5     | -             | -              | 29.0         | 4.2        | -              | -        | -              | -           | 20.2        | 20.2     |        |
|           | OTS   | 18.7             | 21.0     | -             | -              | 26.0         | 3.5        | -              | -        | -              | 10.6        | 15.9        | 17.3     |        |
| Reocín    | SB013 | 0.8              | 1.3      | 0.7           | -              | 0.9          | 0.6        | -              | -        | 0.7            | -           | 0.8         | 0.9      |        |
|           | SB014 | 1.8              | 3.0      | 1.7           | -              | 1.8          | 1.0        | -              | 0.8      | 0.9            | 0.5         | 1.4         | 1.9      |        |

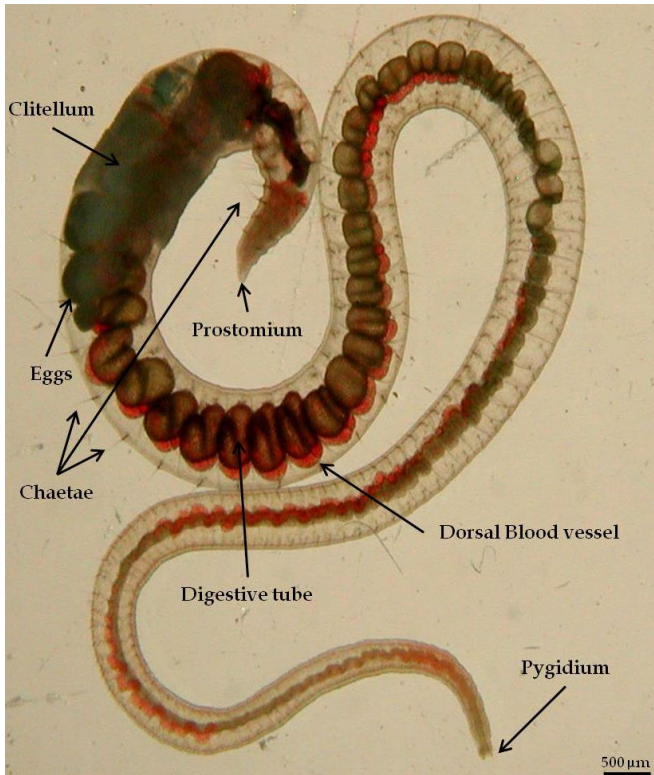
→ None or Low Risk of metal bioaccumulation in Reocín mining district

→ High Risk of metal bioaccumulation in Arditurri mining district

## 5. LOE 3: Sediment bioassay

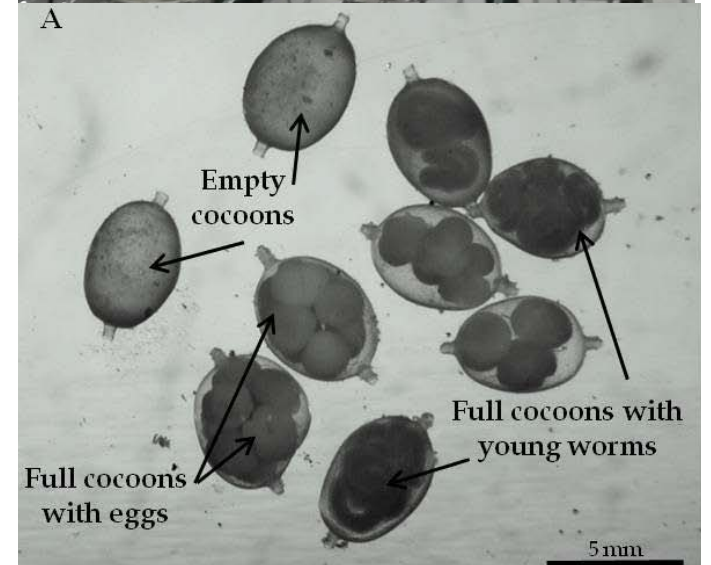
*Tubifex tubifex* sediment chronic toxicity test (28 days; ASTM, 2005)

- 5 replicates
- 4 worms/rep (mature)
- $22 \pm 1$  °C, in the dark, with aeration
- Food Supplement: 80 mg Tetramin



### Endpoints:

- Survival percentage (SUR)
- Reproduction:
  - Total Cocoons (TCC),  
Empty Cocoons (ECC)  
Total Young (TYG)
- Total Growth Rate (TGR= adult somatic biomass + cocoon biomass)



- Growth and reproduction impairment in OTS → High Pb concentrations.

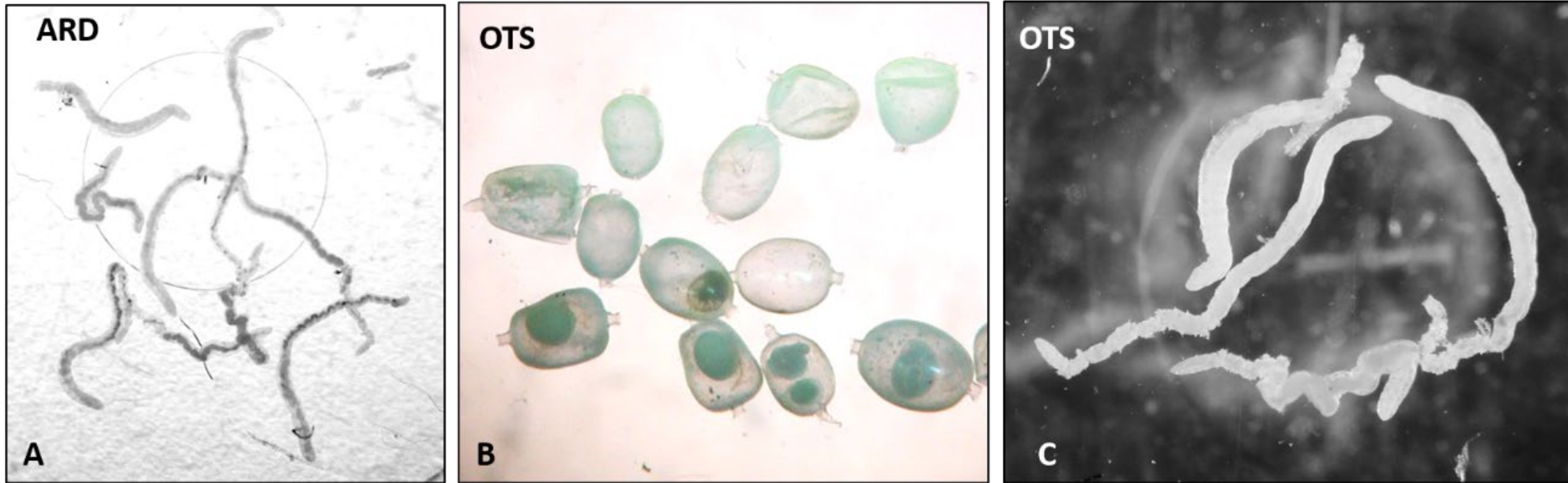


Fig. 6. *Tubifex tubifex* and its cocoons exposed to ARD and OTS. A and C, worms presenting autotomy and tail degradation; B, cocoons.

- Stimulatory response in sites with higher Zn concentrations.

(Raw data in Table A1)



# Assessment through Probability ellipses

Database of 60 reference sites from the North of Spain.

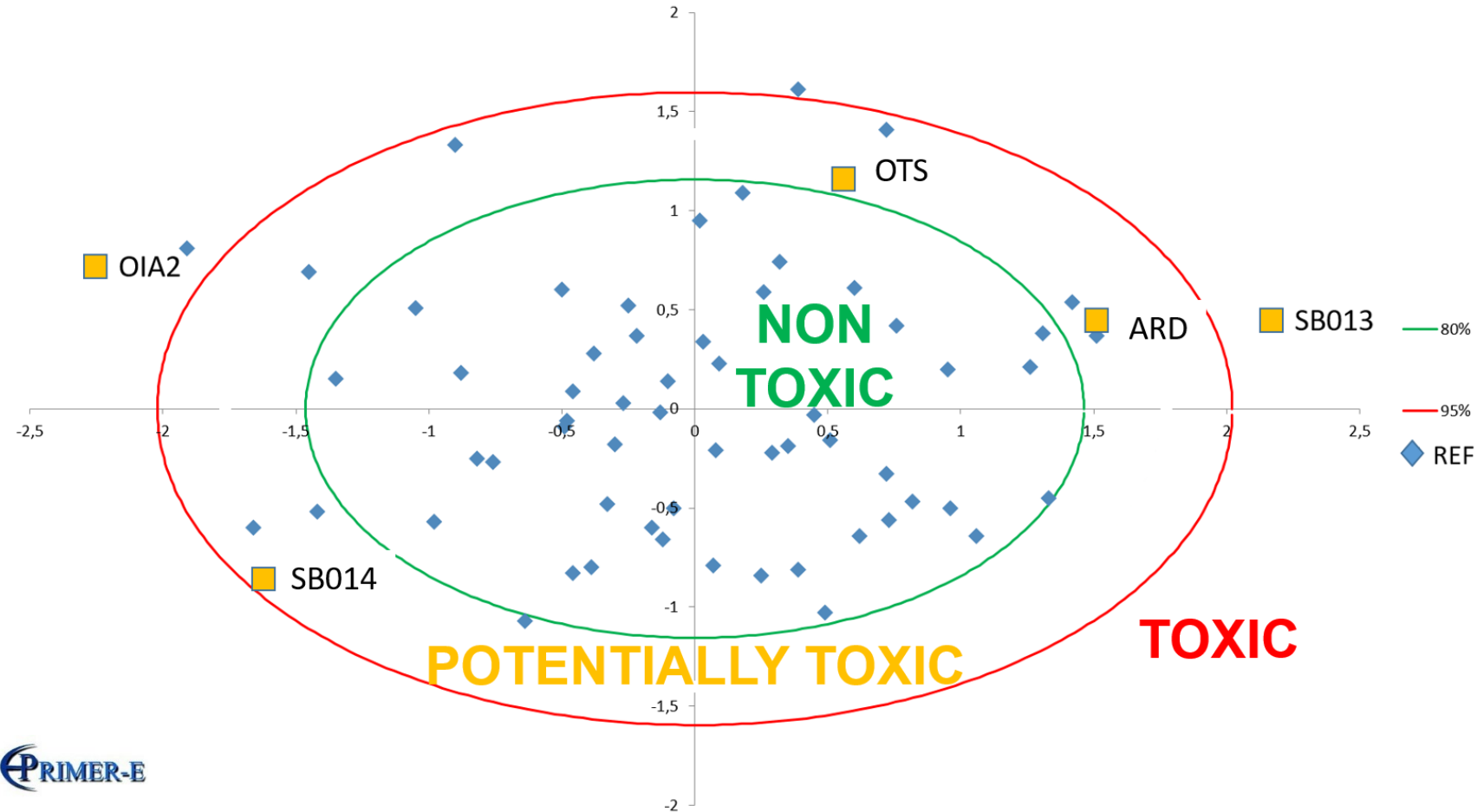


Fig. 7. Sediment toxicity assessment through probability ellipses in the nMDS ordination space for reference sites (in blue). Test sites from Ariturri and Reocín mining districts are represented in yellow. More Information on Fig. A1.

# 6. Weight of evidence (WOE) framework

## LOE 1: Sediment chemistry

- SedPoll I
- ⦿ SedPoll II and III
- SedPoll IV and V

## LOE 2: Bioaccumulation

- INTISS ≤1
- ⦿ INTISS 1.1-10.0
- INTISS >10.0

## LOE 3: Sediment chronic toxicity

- Non-Toxic
- ⦿ Potentially Toxic
- Toxic

Table 3. Decision matrix for Weight of Evidence (WOE) categorizations of the study area based on three Lines of Evidence (LOE): sediment chemistry, sediment chronic toxicity, and metal bioaccumulation on field biomonitors.

- Adverse effects are unlikely.
- ⦿ Adverse effects may occur.
- Adverse effects are expected.

| Site  | Sediment Chemistry | Metal Bioaccumulation | Sediment Toxicity |
|-------|--------------------|-----------------------|-------------------|
| OIA2  | ●                  | ●                     | ●                 |
| ARD   | ●                  | ●                     | ⦿                 |
| OTS   | ●                  | ●                     | ⦿                 |
| SB013 | ⦿                  | ○                     | ●                 |
| SB014 | ⦿                  | ⦿                     | ⦿                 |

HIGH RISK of adverse effects

## 7. Conclusions

The capacity of aquatic ecosystems to maintain a healthy macroinvertebrate assemblage, was affected due to metal pollution in the sediments, evidenced by metal bioaccumulation in aquatic organisms and by the toxic effects observed in laboratory organisms.



*THANK YOU SO MUCH  
FOR YOUR ATTENTION!!!*

*Questions?*





*Acknowledgements* - This investigation has been supported by the research project of the Spanish Government CGL2013-44655-R, (MINECO). Dr. Moreno-Ocio was supported by a predoctoral fellowship (2017-2021) from the University of the Basque Country (UPV/EHU). The contribution to this meeting was possible thank to the economical support from the UPV/EHU. I am specially grateful to Dr. Pérez-Cebrecos for letting me the use of her magnificent drawings, as well as to S. Aveleira, a very motivated biology student that conducted her graduate project with *T. tubifex* and in her kindness offered her drawings for their use.



# Annex

**Table A1.** Sediment (Sed) metal concentration (mg kg<sup>-1</sup> dw), ecotoxicity endpoints and metal tissue residues (TR) (µg g<sup>-1</sup> dw) in *T. tubifex* sediment bioassay (28 days). Probability of H<sub>0</sub> rejection in ANOVA and Dunnett’s t-test comparisons of each case with the respective ITU control are shown as \*p ≤ 0.05; \*\*p ≤ 0.01; \*\*\*p ≤ 0.001.

|        | Sed Metals |       |        | Endpoints   |               |               |                 |                  | TR Metals     |                |                |
|--------|------------|-------|--------|-------------|---------------|---------------|-----------------|------------------|---------------|----------------|----------------|
| Site   | Cd         | Pb    | Zn     | SUR         | TCC           | ECC           | TYG             | TGR              | Cd            | Pb             | Zn             |
| ITU 1  | 0.5        | 16.3  | 53.3   | 90.0 ± 13.7 | 33.8 ± 8.1    | 19.4 ± 6.5    | 82.4 ± 28.2     | 0.029 ± 0.007    | 0.4 ± 0.2     | 9.4 ± 2.5      | 447 ± 24       |
| END    | 1.11       | 96.6  | 282    | 95.0 ± 11.2 | 45.8 ± 0.8**  | 27.8 ± 2.8*   | 116.0 ± 34.1    | 0.041 ± 0.004**  | 7.0 ± 5.5***  | 13.4 ± 4.4     | 723 ± 64**     |
| OIA 1  | 0.97       | 85.8  | 336    | 95.0 ± 11.2 | 43.8 ± 4.4**  | 25.8 ± 4.1    | 130.8 ± 24.4    | 0.044 ± 0.003*** | 0.5 ± 0.3     | 17.3 ± 4.2**   | 626 ± 13       |
| OIA 2  | 16.9       | 457   | 3,629  | 100.0 ± 0.0 | 50.4 ± 2.7*** | 29.8 ± 2.9**  | 182.0 ± 11.0**  | 0.058 ± 0.006*** | 0.8 ± 0.2     | 254 ± 68.2***  | 1,399 ± 148*** |
| ARD    | 27.8       | 1,91  | 11,221 | 100.0 ± 0.0 | 44.0 ± 3.4**  | 26.8 ± 2.8*   | 146.0 ± 6.3**   | 0.047 ± 0.002*** | 1.4 ± 0.3**   | 320 ± 22.0***  | 2,423 ± 206*** |
| OTS    | 16.2       | 4,600 | 12,974 | 100.0 ± 0.0 | 28.4 ± 4.8*   | 22.2 ± 4.8*   | 29.0 ± 5.6**    | 0.018 ± 0.003**  | 13.6 ± 2.4*** | 4,219 ± 576*** | 2,514 ± 71***  |
| ITU 2  | 0.60       | 16.2  | 41.0   | 100.0 ± 0.0 | 31.6 ± 2.9    | 16.8 ± 2.2    | 103.8 ± 14.2    | 0.018 ± 0.006    | 0.17 ± 0.16   | 8.1 ± 2.0      | 371.1 ± 46.3   |
| SB 013 | 0.63       | 72.6  | 250    | 95.0 ± 11.2 | 50.0 ± 4.2*** | 28.4 ± 2.6*** | 228.6 ± 26.1*** | 0.050 ± 0.003*** | 0.05 ± 0.01** | 12.9 ± 1.4**   | 340.8 ± 22.5   |
| SB 014 | 1.24       | 119   | 921    | 100.0 ± 0.0 | 45.2 ± 6.1*** | 24.6 ± 5.6*   | 165.0 ± 62.1    | 0.060 ± 0.010*** | 3.23 ± 3.65   | 35.7 ± 10.2*** | 377.8 ± 32.4   |

Probability ellipses classification of toxicity variables using a RCA of 60 reference sites:  
nMDS analysis and Euclidean distances

Simpacted sediments approach:

- Error Type I: is the probability of classifying sites as Toxic when they are not
- Error Type II is the probability of classifying sites as Non Toxic when they really are

For instance, taking 5% as the probability of error, setting Type II < 0.05 requires the use of the 95% probability ellipse which implies a disturbance level of 35% in impacted sediments→ this would set errors Type I and II below 0.05 → this sets the outside band: Toxic (T) or very different from reference condition.

The inner band that identified sites as Potentially Toxic (PT) or possibly different from reference from the Non Toxic (NT) (or equivalent to reference) was set using the 80% probability ellipse, that was able to detect a 30% red in studied endpoints.

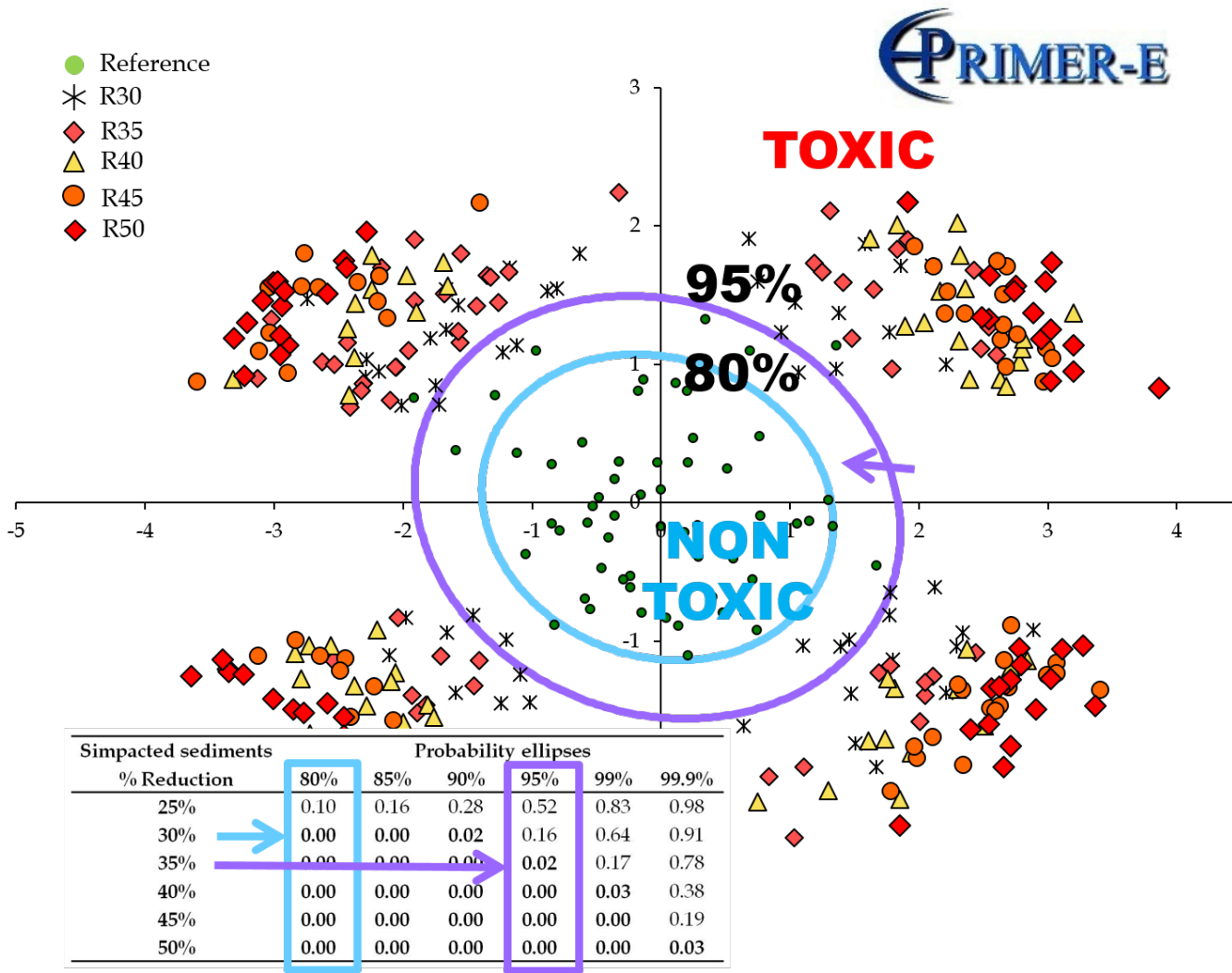


Fig. A1. Construction of probability ellipses in the nMDS ordination space for reference sites (in green). Impacted sediments included by different symbols.