

# **A TOXICITY INDEX FOR THE VENICE LAGOON**

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## **Riassunto**

Nell'ambito del programma di ricerca promosso dal Co.ri.La sugli "Indicatori e indici di qualità ambientale per la laguna di Venezia", è stato sviluppato un indice di tossicità in grado di sintetizzare le risposte derivanti da più test di tossicità con diversi organismi acquatici applicati su diverse matrici estratte dal sedimento. L'indice di tossicità è uno strumento utile perché classifica la tossicità dei sedimenti lagunari in 5 classi di tossicità (assente, bassa, media, alta e molto alta), permette una facile visualizzazione delle informazioni e rappresenta un passo fondamentale nell'integrazione delle risposte ecotossicologiche con quelle chimiche ed ecologiche inerenti la comunità bentonica.

## **Abstract**

During the Research Program promoted by Co.ri.La on "Environmental quality indicators and indices for the Venice lagoon", a toxicity index was developed that can synthesise responses from different bioassays with a variety of aquatic organisms on various phases of exposure deriving from sediment. The toxicity index is a useful tool because it classifies sediment toxicity in five classes (absent, low, medium, high and very high), permits easy representation of the information and allows ecotoxicological responses to be integrated with chemical and ecological ones.

## **1 Introduction**

The research project (as part of line 3.11) aims to identify indicators and develop indices of environmental quality of the lagoon of Venice, based on the approach established by the Water Framework Directive (EU, 2000), which focus on the integration of chemical, physical and ecological parameters, in order to define ecological quality classes for the different water bodies. The main reasons for developing quality indices are chemoassessment and bioassessment; toxicity of environmental compartments is one element of bioassessment. We use toxicity bioassays related to sediment contamination at whole-lagoon scale, with the aim of identifying the most representative bio-indicators for the lagoon and developing a multimetric toxicity index.

Many toxicity bioassays for sediment assessment have been developed internationally in recent decades, involving various phases of exposure (pore water, fractionated and unfractionated organic extract and whole sediment),

conducted on a variety of aquatic organisms and considering a lot of endpoints, so that toxicity data are often difficult to interpret.

Toxicity indices are intended to combine bioassay results into a single response, synthesizing toxicity information and translating the results into simple terms for decision-makers. The representation of all results from the different sediment toxicity tests on a common, easily interpreted scale is the main and recognised application of environmental indices [Shin and Lam, 2001].

In this paper we briefly describe our research activities for developing the toxicity index; details on methods and results are reported in Losso et al. [submitted].

## 2 The conceptual model

We proposed a stepwise procedure to select, according to specific criteria, a minimum number of toxicity bioassays on suitable matrices in order to integrate their responses into a multimetric toxicity index (Fig. 1).

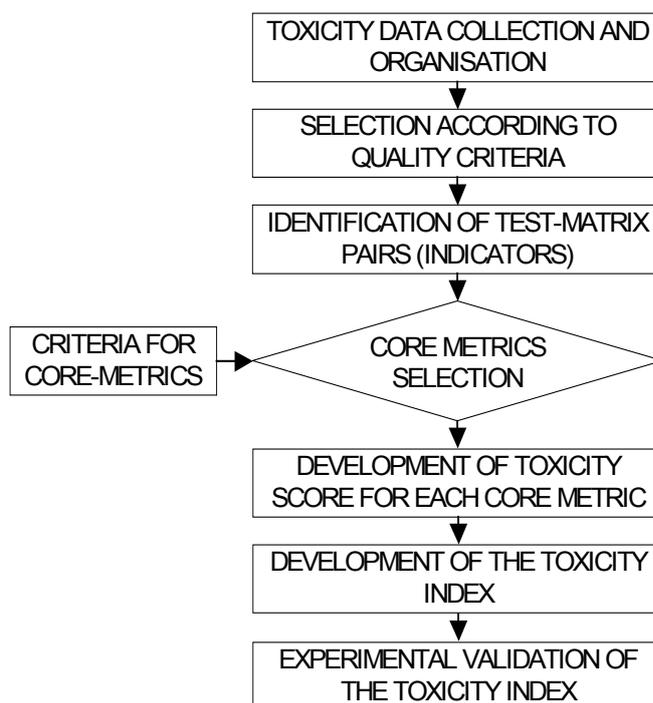


Fig 1 – Stepwise procedure to develop the toxicity index.

### 2.1 First steps: the toxicological database for the Venice lagoon

We collected and organised all available toxicity data for the Venice lagoon. As first output, a toxicity database was created including the main information regarding the toxicity test (species, endpoint, test protocol), test matrix (matrix typology, protocol used for matrix preparation), study site (site name, site mark, coordinates, sampling year, depth and type), toxicity data (Percentage of Effect with standard deviation or, if calculable, EC50 and TU50 with confidence limits

at 95%), data quality (use of negative control, positive control, sediment control) and the reference for data availability.

Toxicity data were then selected according to quality criteria using the standard protocol and Quality Assurance/Quality Control criteria for sampling and preparing the test matrix and performing the toxicity bioassay.

All available test–matrix pairs for the lagoon were thus identified: they represent the TOXICOLOGICAL INDICATORS and are: sperm cell toxicity test with *Paracentrotus lividus* on pore water; sperm cell toxicity test with *P. lividus* on elutriate; embryotoxicity test with *P. lividus* on pore water; embryotoxicity test with *P. lividus* on elutriate; embryotoxicity test with *Mytilus galloprovincialis* on pore water; embryotoxicity test with *M. galloprovincialis* on elutriate; embryotoxicity test with *Crassostrea gigas* on pore water; embryotoxicity test with *C. gigas* on elutriate; test with *Vibrio fischeri* (Microtox® test) on solid-phase.

## 2.2 The selection of toxicological core-metrics

Toxicity core-metric (CM-tox) selection identifies the test-matrix pairs considered significant for the elaboration of the toxicity index. The selection was made according to specific criteria, suggested by the literature and expert judgment. We divided the criteria into four categories, based on the suggestion of Viaroli et al. [2004]:

- ECOLOGICAL SIGNIFICANCE (ES), regarding the species used and endpoint in relation to the study environment. This category includes the following criteria: species representativeness for the study environment, test representativeness for the investigated matrix and endpoint.
- UNCERTAINTY (U), concerning the uncertainty of bioassay response and including: test sensitivity, reproducibility, test species availability and influence of confounding factors.
- COST EFFECTIVENESS (CE) referring to the possibility of using the test for monitoring purposes: including execution rapidity, ease of execution and costs.
- DATABASE CHARACTERISTIC (DC), depending on the available information and regarding data abundance and result complementarity.

Comparative weights in the 0-1 range were assigned to each criteria. As a result, the selected CMs-tox for the Venice lagoon were:

- bioluminescence reduction with *V. fischeri* on solid phase;
- embryotoxicity test with *P. lividus* on elutriate;
- embryotoxicity test with *M. galloprovincialis* on elutriate;
- sperm cell test with *P. lividus* on pore water;
- embryotoxicity test with *C. gigas* on pore water.

## 2.3 The toxicity scores

We recently developed toxicity scores for elutriates [Losso et al., 2007]. These scores are based on five toxicity classes (absent, low, medium, high and very high). The first toxicity threshold, defining when a sample is toxic, is determined by a statistical approach, following the Minimum Significance Difference (MSD) suggested by Phillips et al. [2001]. The other toxicity thresholds, defining how toxic a sample is, are based on data distribution.

The same approach was used for the toxicity tests on pore waters as the tests on elutriate.

For the Microtox® test on solid-phase, such an approach is not applicable as the control response is from a solid matrix. For this reason the toxicity thresholds were defined only on data distribution; the first threshold defining when a sample is toxic was the 10<sup>th</sup> percentile (TU50=322, n=122).

#### **2.4 Developing the toxicity indices**

Two toxicity indices were developed for the Venice lagoon: the Toxicity Effect Index (TEI index) following an approach suggested in the literature and the Weighted Average Index (WATI Index).

The TEI index was calculated starting from the SED-TOX index [Bombardier and Bermingham, 1999], which is not applicable to the Venice lagoon as it is, because other toxicity bioassays are considered and toxicity ranges are different. We therefore introduced substantial modifications to the SED-TOX. Briefly, the TEI index starts from the toxicity values expressed as TU, which are normalised to dry weight. Data are thus normalised to the TU<sub>lim</sub>, i.e. the minimum toxicity revealed by the test. Normalised data are summarised, taking into account the sensitivity weight of each CM-tox. Finally, the logarithm of the weighted sum is considered. TEI values were classified in five toxicity classes:  $0 \leq \text{TEI} \leq 0.3$  for absent toxicity;  $0.3 < \text{TEI} \leq 0.7$  for low toxicity;  $0.7 < \text{TEI} \leq 1.23$  for medium toxicity;  $1.23 < \text{TEI} \leq 1.8$  for high toxicity;  $\text{TEI} > 1.8$  very high toxicity.

The WATI index started from the toxicity classes determined by the toxicity scores. A score was assigned from 0 to 4 to each toxicity class, where 0 defines the absence of toxicity and 4 very high toxicity. The scores of each CM-tox were summarised and weighted according to the comparative weights (at 0-1 range) assigned by the criteria for CM-tox selection. The WATI values were classified in five equivalent sub-ranges, determining five toxicity classes:  $0 \leq \text{WATI} \leq 0.2$  absent;  $0.2 < \text{WATI} \leq 0.4$  low;  $0.4 < \text{WATI} \leq 0.6$  medium;  $0.6 < \text{WATI} \leq 0.8$  high;  $0.8 < \text{WATI} \leq 1$  very high.

### **3 Application of the toxicity indices to the Venice lagoon**

The indices were applied to the available toxicity data, concerning 110 sediment samples collected between 1994 and 2005 from 21 sites (Figure 2) in the lagoon of Venice, most of which were sampled twice at different seasons. Despite the experimental activity (in the field and laboratory) to fill the gaps in the lagoon toxicity dataset, there are only 52 sediment samples with more than one CM-tox. The TEI index showed values from 0 to 2.42: 10 samples were classified as toxicity absent, 14 as low, 13 as medium, 10 as high and 5 as very

high. For the WATI index, 11 samples were classified as toxicity absent, 16 as low, 6 as medium, 7 as high and 12 as very high. Both indices evidenced that samples with very high toxicity are from the industrial area of Porto Marghera, but high or medium toxicity was also attributed to sediment samples from sites far removed from direct contamination. Toxicity indices also highlighted the high temporal variability of sediment toxicity: for example, the Tresse site, in front of the industrial area, showed low toxicity in summer 2000, absence of toxicity in winter 2001, medium toxicity in summer 2001 and high toxicity in winter 2003.

Figure 2 reports an example of data visualization: the central circle in the pie chart shows the integrated toxicity according to the WATI index while the slices represent each CM-tox toxicity.

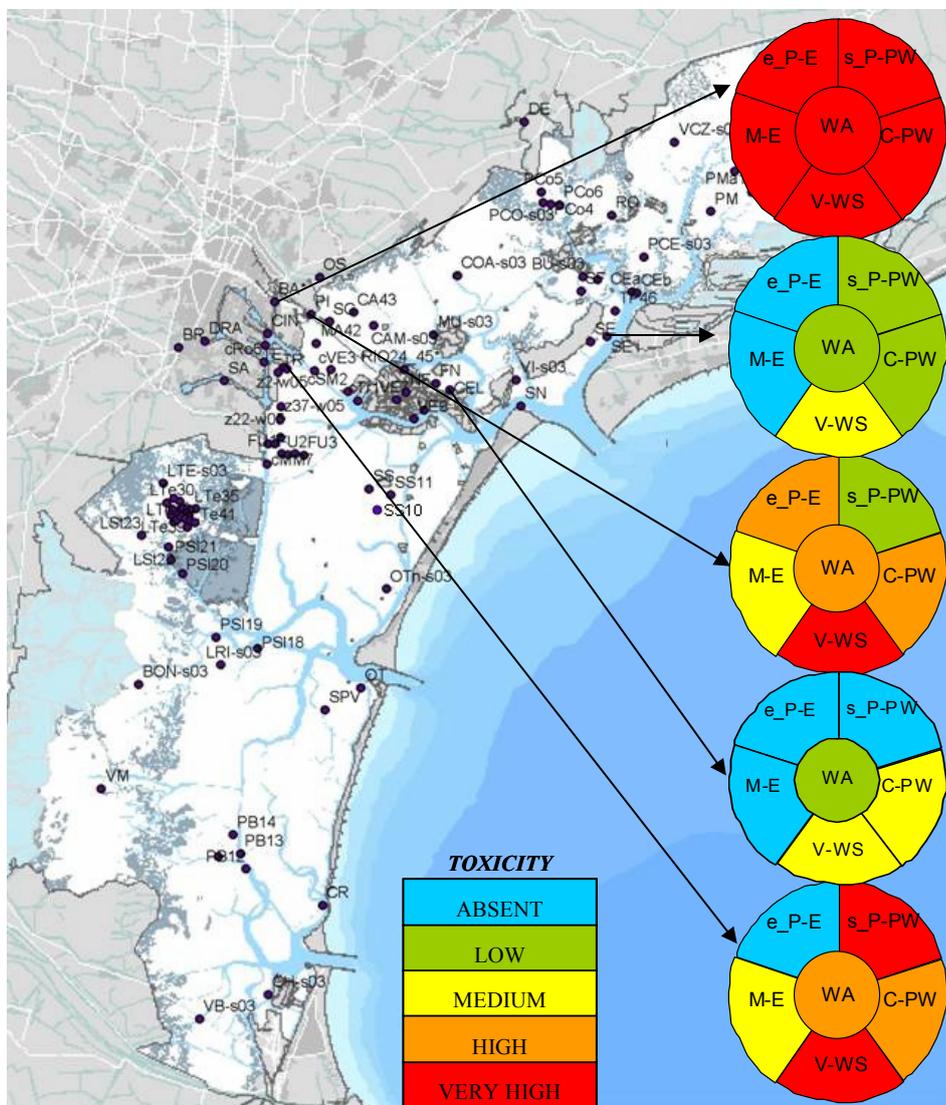


Fig 2 – Location of investigated sites and example of toxicity data visualization (Microtox test on solid-phase= *b\_Vf\_sp*; embryotoxicity test with *P. lividus* on elutriate=*e\_Pl\_e*; embryotoxicity test with *M. galloprovincialis* on elutriate=*e\_Mg\_e*; sperm cell test with *P. lividus* on pore water=*s\_Pl\_pw*; embryotoxicity test with *C. gigas* on pore water=*e\_Cg\_pw*).

Lastly, some considerations on the two indices. The TEI index is more complex to calculate than the WATI index; to be applied, it needs a characterisation of the sediment sample, i.e. the water content in fresh and centrifuged sediment. For the TEI index we calculated the error due to the sensitivity variability of biological response that is valuable using the sensitivity of each CM-tox through

the reference toxicant; we demonstrated that this variability is quite low for the CMs-tox used, validating the choice of a 5 toxicity classes ranking. In the WATI index, simpler than the TEI index in conception and calculation, the toxicity data is ranked twice (first for the toxicity scores and then for the index). This guarantees easy treatment of each data processing step, but has the drawback of introducing a higher error in data treatment as the toxicity information is approximated twice, increasing the truncation errors.

The responses of both indices obviously depend on the available toxicity data; in particular, we showed how the absence of one CM-tox influences the indices results and demonstrated that the lack of Microtox test on solid phase causes the greatest error.

### Conclusions

We developed a toxicity index for integrating responses from a battery of toxicity bioassays, using a stepwise procedure. Among all available indicators for the Venice lagoon, the 5 most representative core-metrics were selected according to specific criteria. These 5 CMs-tox were integrated using two mathematical approaches, and two toxicity indices (TEI and WATI) were developed. Both indices, applied to the toxicity dataset for the lagoon, were able to discriminate among sites with different quantitative and qualitative chemical contamination, so they can be considered as evidence in sediment quality assessments. A final validation of the two indices requires proper field measurements.

The use of one of the two indices can be a useful tool for decision-making processes as this integrates toxicity responses deriving from toxicity bioassays with different sensitivity applied on a different test matrix. Moreover the classification in five toxicity classes (absent, low, medium, high, very high) can be easily visualised or depicted on a coloured map.

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