An integrative toolbox to evaluate the environmental quality of transitional waters: the case studies of Minho and Lima rivers

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ABSTRACT

The Directive 2000/60/CE (Water Framework Directive–WFD) intends establishing a framework for the protection of inland waters, transitional, coastal and groundwater. Accordingly, each member state has to protect, improve and recover the conditions of all aquatic ecosystems to achieve, by 2015, a good quality status for all water bodies. To attain these purposes, further specific measures for pollution control and for setting environmental quality standards will be required. Meeting these needs, the TEAM-Minho project aimed at establishing harmonized scientific criteria, which would allow typing, referencing and classifying the ecological status of transitional water bodies in southern Galicia and northern Portugal, including the transboundary Minho river estuary, and the transfer of results to the relevant public institutions, local social agents and society in general, in order to assist in the implementation of the WFD by providing a framework for the effective protection of transitional waters. This presentation will introduce the results obtained within the TEAM-Minho project regarding the application of sensitive, rapid and economic bioassays to establish reference conditions and for the evaluation and classification of the ecological status. Ecotoxicological assays, with species representative of different taxonomic and functional groups, from different trophic levels, were carried out for water and sediment samples collected at two case studies: the Minho and Lima river estuaries. The obtained results point to a different status of contamination of the water an sediment compartments at both transitional systems. The integration of the ecotoxicological indicators in the classification of the two water bodies within the WFD will be discussed.

KEY WORDS: *Transboundary waters, Water Framework Directive, Ecotoxicological toolbox, Transitional waters.*

INTRODUCTION

In 2000, the Directive 2000/60/EC (Water Frame Work Directive - WFD) of the European Parliament and of the Council was published and came into force in the European Union with the purpose of establishing a framework for community action in the field of water policy (European Community, 2000). The fundamental aim of this directive is to protect all water bodies (groundwater, inland surface waters, transitional waters, and coastal waters), by preventing their further deterioration, promoting a sustainable use of water, enhancing protection and improvement of the aquatic environment, ensuring a progressive reduction of groundwater pollution, and by contributing to mitigate the effects of floods and droughts (European Community, 2000; Chave, 2001). For this, all member states are committed to achieve a “good ecological and chemical status”, for all waters, by 2015.

One of the most important and innovative issues that the WFD introduced is an holistic approach involving a single system for water management through the concept of river basin districts, i.e. through natural geographical and hydrological units, instead of according to national administrative or political boundaries (European Community, 2000). As some river basin districts transverse national frontiers (e.g. Minho river basin) cooperation among member states is set as a challenge within the WFD for defining efficient river basin management plans. This strict cooperation is essential, since four elements must be concerted and applied homogeneously, among member states, within the river basin planning cycle: characterisation and assessment of impacts on river basin districts; environmental monitoring; setting of environmental objectives; and designing and implementing the program of measures needed to achieve them (European Community, 2000).

Additionally, under this water management holistic approach, the WFD entails not only physicochemical and ecological quality objectives to be set, but also that assessment methods must be developed to attain an effective monitoring of the aquatic ecosystems (Pollard and Huxman, 1998). On the basis of the characterisation and impact assessment carried out in accordance with the Article 5 and Annex II, Member States shall, for each period to which a river basin management plan applies, establish a surveillance monitoring programme (to assess long-term changes in water quality) and an operational monitoring programme (to provide information on the success of measures implemented to improve the water quality). Member States may also need, in some cases, to establish programmes of investigative monitoring (to identify the causes of a water body failing to achieve the environmental objectives) (Chaves, 2001).

Though the WFD demands the evaluation of a number of quality elements (hydromorphological, physicochemical, and biological) for the monitoring of water bodies, it does not totally specify in details the methods that should be used to do so. Therefore, new challenges are also posed to the scientific community in order to develop novel, cost effective and accurate methodologies capable of delivering appropriate data to be used by policy-makers and stakeholders so as to attain a successful implementation of the directive (de Jonge et al., 2006; Hering et al., 2010). These challenges constitute clear opportunities for the use of new biological indicators (e.g., biomarker analysis, bioassays, biological early warning systems), particularly within investigative monitoring, to be integrated with approaches that are already in use (e.g., chemical analysis and assessment of communities abundance and structure) (ICES, 2004; Allan et al., 2006, Quevauviller et al., 2008). Such new testing methodologies, namely bioassays, constitute important tools for establishing cause-effects relationships when classifying the ecological quality status of water bodies. Whereas physicochemical analysis intend to identify the cause of an effect, the ecological approach identifies the effects at the ecosystem level, the use of biomarkers or bioassays allows determining the causes of the ecological damage and ascertain if chemicals are the cause of failing to achieve a good quality status. Therefore, the integration of ecotoxicological methodologies, as quality elements, in the WFD may contribute to the operational monitoring and manage water bodies that fail to achieve a good ecological status through a more multidisciplinary and holistic perspective (ICES, 2004; Allan et al., 2006, Quevauviller et al., 2008).

According to the challenges identified above, the TEAM-MINHO project aimed at establishing harmonized scientific criteria, which would allow typing, referencing and classifying the ecological status of transitional water bodies in southern Galicia and northern Portugal, including the transboundary Minho river estuary. Furthermore, the transfer of results to the relevant public institutions (e.g. water administrative and management authorities), stakeholders and society in general, will also be performed in order to assist in the implementation of the WFD by providing a framework for the effective protection of transitional waters. To attain these main goals, several specific objectives were delineated, among which the provision of new sensitive, rapid and economic biological quality elements (bioassays) for establishing reference conditions and for the evaluation and classification of the ecological status of water bodies. The present work tackled this specific objective by carrying out ecotoxicological assays, with several fresh- and saltwater species, in two selected case studies: the Minho and Lima rivers.

METHODS

Case studies

Two case-studies of transitional water bodies were selected to carry out this study: the Minho river (a transboundary water body) and the Lima river. The two estuaries are characterized by an ambivalent character: though constituting especially productive areas and being ecologically sensitive harbouring protected areas by national and international laws (e.g., Natura 2000, SPA, RCM), they exhibit a high human population density and socioeconomic importance, thus being under several anthropogenic pressures (e.g., supply irrigation, drinking water, fishing, transit, hydroelectric, organic and inorganic pollution, non-native invasive species) (e.g., Sousa et al., 2006, 2008; Reis et al., 2009; Costa-Dias et al., 2010; Azevedo et al., 2011).

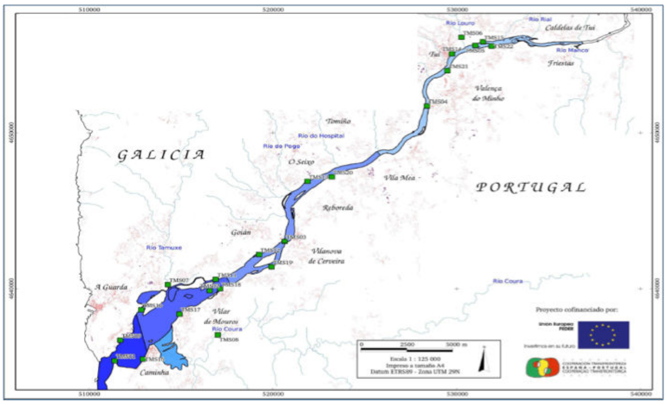

Figure 1. Scheme of the Minho River with indication of the water and sediment sampling sites.

The Minho river rises in Sierra de Meira (Spain) and, after a course of 300 km reaches the sea: its' mouth locates between Caminha (Portugal) and La Guarda (Spain). In the last 77 km marks the border between Portugal and Spain. Its basin has a total area of approximately 17,081 km^2, including 850 km^2 in Portugal. Its estuary is partially mixed or wedge saline. It extends along the river zone that is under tidal influence, over approximately 35-40 km (Figure 1). In this section, the rocky edge transit to abrupt edges with a predominance of fine materials (sand and silt), which, in view of the lower bathymetry of this portion of the estuary (maximum depth of 4 m), favours the appearance of flat islands by sedimentation, covered with reeds and meadows, or areas covered by scrub and tree or as well as the formation of sandbars and mudflats. The penetration of salt water is very limited, so that during the low tide water of the estuary is basically freshwater. The presence of dunes is limited and restricted to the mouth (INAG, 2001).

The Lima river rises in Spain in Sierra de San Mamed and its' mouth is located in Viana do Castelo (Portugal), after a course of 108 km. Its basin has an area of 2480 km^2 (1177 km^2 in Portugal: 47% and 1303 km^2 in Spain: 53%). It is bordered in the north by the watershed of the rivers Minho and Anchor, in the east by the Douro River basin and in the South by the Cávado river Basin. The Lima estuary develops into a relatively narrow valley, along approximately 20 km (Figure 2). The communication with the sea is through a narrow and deep channel (about 10 meters) and its mouth is artificialized, due to the construction of breakwaters protection for port facilities therein. It is dominated by an estuary tidal regime and river flows, partially mixed or saline wedge. The low estuary comprises a basin broad, flat and shallow. Along the canal, configure themselves small islands and wetlands with herbaceous vegetation typical of marshland coexisting with extensive sandy shores, which are uncovered during low tide. The high estuary consists of a narrow channel, whose depth decreases gradually upstream. On both banks of the estuary, the landscape is quite humanized, characterized by a dominance of agricultural areas with presence of small fragmented forest areas decreasing downstream (INAG, 2001; http://www.inag.pt/; http://www.icnb.pt/, last visited in February, 2013).

Figure 2. Map showing the location of water and sediment sampling sites at the Lima River (Picture adapted from http://maps.google.com).

Samples collection

To evaluate the ecological quality status of the water body masses, through the use of ecotoxicological methodologies, sub-superficial water and sediment samples were collected at the two study sites. Sampling was performed in spring (May 2012) and autumn (November 2012) for the Minho River and in summer (July 2012) and winter (February 2013) for the Lima River. Sub-superficial water samples were collected at seven and twelve sites, respectively at the Minho and Lima rivers, in the two sampling periods. Sediment samples were collected at twenty-two and twelve sites, respectively at the Minho and Lima rivers, only in the spring/summer campaigns (Figures 1 and 2).

The following physicochemical parameters were measured, *in situ*, at each sampling site: temperature, conductivity, salinity, pH, dissolved oxygen, turbidity and the potential redox.

Chemical survey

All sediment and sub-superficial water samples (dissolved and particulate phases) were characterized for major and minor elements, with special focus on metallic contamination (for Lima River). For the Minho River, only arsenic and mercury were determined. This characterization provided support information for the ecotoxicological assessments.

Imposex survey

The levels of imposex were assessed in the following gastropod species: *Nassarius reticulatus*, *Nucella lapillus*, both collected along the Minho and Lima estuaries, during low tides. In laboratory, the percentage of females affected by imposex (%I) and the vas deferens sequence index (VDSI) were determined for each sampling site.

Ecotoxicological assays

Bioassays were carried out for each sample of sub-superficial water and sediment that were collected in the two case studies. For freshwater and sediment samples collected at sites with very low salinities the following bioassays were carried out: 15-min bioluminescence inhibition with the bacteria *Vibrio fisheri* (decomposer), 72-h growth inhibition with the green microalgae *Pseudokirchneriella subcapitata* (primary producer), 48-h cumulative mortality and 21-d reproduction with the cladoceran *Daphnia magna* (primary consumer), 72-h reproduction with the epibenthonic crustacean *Heterosypris incongruens* (primary consumer), and 96-h embryonic development with the fish *Danio rerio* (secondary consumer). Regarding saltwater and sediments collected at higher salinities, the following assays were carried out: 15-min. bioluminescence inhibition with the bacteria *V. fisheri* (decomposer), 72-h growth inhibition with the algae *Nannochloropsis gaditana* and *Phaeodactylum tricornutum* (primary producers), 24-h mortality with the microcrustaceans *Artemia fransciscana* and *Brachionus plicatilis* (primary consumers, only for Lima river samples), embryonic development of *Paracentropus lividus* and *Mytilus galloprovincialis* (primary consumers, only for Minho river samples), 96-h mortality of *Siriella armata* and 10-d mortality assay with the amphipod *Corophium multisetosum* (secondary consumers, only for Minho river samples).

Additionally, *N. reticulatus* females with very low levels of imposex were exposed, during 28 days, to sediments collected from both rivers in order to assess the increment of VDSI and female penis length.

RESULTS

Chemical survey

Generally, the analysed metallic contaminant levels were low in both systems, both in the sub-superficial water and sedimentary compartments and well within the quality guidelines proposed in the WFD for good chemical status.

Imposex survey

Both indicator species were present near the mouth of the estuaries (either inside and in the adjacent coast) and females with imposex occurred at all surveyed sites. In the Minho estuary the percentage of females affected by imposex (%I) across sampling sites ranged between 55-94 in the case of *N. lapillus* and 47-72 for *N. reticulatus*; the VDSI of *N. lapillus* varied from 0.6 to 1.6 whilst for *N. reticulatus* varied between 0.6 and 0.7. In the Lima estuary the imposex levels were higher: the %I varied between 82-100 for *N. lapillus* and 69-100 for *N. reticulatus* and the VDSI ranged between 1.0-3.2 and 1.3-3.8, for each species respectively.

Ecotoxicological assays

In general, sub-surface water samples collected at the Minho and Lima Rivers exerted no significant adverse effects for all tested species (both fresh- and saltwater). As well, results obtained for sediments collected at higher salinities, in the Minho River, reveal lack of toxicity for the algae *P. tricornutum* and the mussel *M. galloprovincialis.* However, these sediments were moderately toxic for the sea urchin *P. lividus*, except for the sample collected closer to the mouth of the estuary. Regarding sediments collected at sites with low salinity (in Minho River), a significant inhibition of bioluminescence production by the bacteria *V. fisheri* and of growth of the microcrustacean *H. incongruents* was provoked by exposure to most of the sampled sediments. Also, elutriates of some of the collected sediments induced a significant decrease of bioluminescence of *V. fisheri*, and a decrease in the heart beat and body length of the fish species *D. rerio*,

Only sediments collected at two sites (one located at the harbour-near the river mouth, and the other was the sampling site located most upstream) in the Lima River induced significant toxic effects in the bacteria *V. fisheri* and in the growth of the microalgae *N. gaditana*. However, elutriates of these sediments only induced significant adverse effects in the growth of the microalgae *P. tricornutum* and *N. gaditana*. No significant effects were observed for the bacteria, artemia and rotifer.

Finally, the ecotoxicological assays revealed higher induction of imposex when using sediments collected from the Lima river comparing to Minho, pointing again to an higher tributyltin contamination at this southern system.

CONCLUSION

Imposex surveys were performed to assess the impact of the priority hazardous substance (TBT) on the ecosystems and the quality status of benthic invertebrates. Based on the low VDSI values observed for both indicator species, and according to OSPAR (2005), it is expected that adverse effects in the more sensitive taxa of the ecosystem caused by exposure to TBT are unlikely to occur in Minho. In contrast, the impact is comparatively higher in Lima estuary as VDSI levels are superior, denoting a risk of adverse effects in the more sensitive taxa.

The ecotoxicological assays suggest that special attention should be given to the ecological status quality of some sites located at both the Minho and Lima Rivers, given the sublethal adverse effects that were observed in the model species exposed to the sediments. These results also suggest that this matrix constitutes a sink and source of contaminants at those places.

In addition, the ecotoxicological assays revealed to be sensitive tools, being capable of detecting contamination of the sediments, despite the apparent good chemical status of water. Similar conclusions can be drawn for the use of imposex to evaluate ecological status of water body masses. However, it must be highlighted that comparisons with levels of other pollutants, namely organic, must still be carried out.

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