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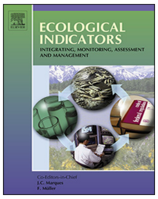

Original Articles

Development of a classification scheme for evaluating water quality in marine environment receiving treated municipal effluent by an integrated biomarker approach in *Meretrix meretrix*

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ABSTRACT

To evaluate the pollution level of marine environment received treated municipal effluent (TME), the water quality classification index (WQCI) capable of integrating biomarker results into a four-level pollution for effluent assessment was proposed and applied. The WQCI calculation was that of the integrated biomarker responses vesion 2 (IBRv2) index based on the expert system classification scale. In this research, mussels of *Meretrix meretrix* were treated with different effluent volume ratios (EVRs, 0–40%, v/v) for 12 days, six biomarkers of oxidative stress, metal exposure and neurotoxicity in both gills and digestive glands as well as the other two biomarkers of general health and genotoxicity in hemocytes were measured. Then based on the correlation analysis between the response of biomarkers and EVRs, the lysosomal membrane stability (LMS) and micronucleus frequency (MF) in hemocytes, glutathione (GSH) and acetylcholinesterase (AChE) in gills were chosen to calculate the IBRv2 and WQCI. The results showed that the control (EVR = 0%) was defined as Level I, EVR of 1–5% as Level II, EVR of 10–30% as Level III, and EVR of 40% as Level IV. In addition, two other sets of data of biomarkers in previous studies were used to test the applicability of the classification scheme established. The WQCI values and the evaluation results based on them were almost identical to those obtained by using the first set of data. This study is the first attempt to develop an index on water quality assessment of marine environment received discharged effluent from a biological perspective. The findings confirm that WQCI offers a potential measurement of water quality evaluation of marine environment and possible to be a water quality standard.

1. Introduction

Sewage treatment plant (STP) effluents discharged in marine waters are the major source of aquatic ecosystem pollution, a variety of pollutants which still have an effect on marine animals are incompletely eliminated during the treatment processes. Indeed, these effluents often contain well-known contaminants, such as heavy metals, fungicides, antibiotics, and some endocrine disrupting chemicals (EDCs) (Gagnon et al., 2006; Stamatisa et al., 2010; Jia et al., 2012; Katsiadaki et al., 2012; Liu et al., 2012). In China, the process of seawater chemical analysis and evaluation is to analyze the temporal and spatial distribution of water quality according to the national 'sea water quality standard' (GB 3097-1997) based on the main components and contents of seawater (Liu et al., 2002), including COD, Chl-a, pH, DO, phosphate and others(Zhang et al., 2011, Li et al., 2014; He et al., 2012), as well as 'discharge standard of pollutants for municipal wastewater treatment plant' (GB 18918-2002). Single factor pollution index and eutrophication index (Zhang et al., 2014) are also used to evaluate the pollution level and eutrophication level of seawater, respectively. All these parameters and evaluation methodologies are based on contents or concentration of chemical substances in the sea. However, some trace contaminants in discharge is under the detection limit and not be able to be detected, which is known to cause a variety of stress-related changes in aquatic organism health (Fatima and Ahmad, 2005; Blaise et al., 2008). In addition, chemical analysis alone may not reflect harmful effects of pollutants on aquatic organisms that pollutants discharged in waters have an effect on water organisms directly, biomarkers therefore should response to pollution like it is.

At first, a successful application of the biomarker approach by the environmental managers, decision makers, and other non-specialists is still hampered by the lack of a simple and reliable integration system able to overcome the obvious difficulties in relating changes in biomarker data to water quality, and classify the waters on a scale according to pollutant-induced changes in the health status of the

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organisms (Livingstone et al., 2000, Viarengo et al., 2000; Allen and Moore, 2004; Moore et al., 2004). To solve this problem, several integrative tools being able to integrate the response of a set of biomarkers in a single value and/or a graph have been developed (Beliaeff and Burgeot, 2002; Broeg et al., 2005; Chèvre et al., 2003; Hagger et al., 2008). Broeg et al. (2005) created the bioeffects assessment index (BAI) for the rapid evaluation of fish condition in the field based on a quantified health assessment index (HAI) devised by Adams et al. (1993). Similarly, the biomarkers at different levels of biological organisation were selected to calculate both the BAI and global biomarker index to make the life quality assessment of animals more reliable and biological effects monitoring more ecologically meaningful. However, in order to meet the specific needs of the Water Framework Directive, Hagger et al. (2008) developed the biomarker response index (BRI) following modification and refinement of the BAI and the HAI that had been developed previously for fish species (Adams et al., 1993; Broeg et al., 2005). The real merit of using biological indices, in our opinion, is not only to translate and simplify complex biological alterations into a pragmatic resource that can be used to inform risk assessment but also to provide an alternative to chemical analysis for evaluating environmental quality, because the deleterious effect of contaminants can only be reflected by aquatic organisms themselves rather than chemical concentrations. In order to obtain a quantification of the biological response able to reflect the stress syndrome induced by pollutant, potential algorithms must be capable of combining the biological meaning of various biomarker responses in selected sentinel organisms with the pollution level that represent the water quality of waters.

The aim of the present work was to develop a biological method for classifying pollution level of coastal seawater receiving STP effluent. An indicator, named water quality classification index (WQCI) was proposed by combining the integrated biomarker responses version 2 (IBRv2) index with the expert system classification scale. The IBRv2 value was calculated by integrating a suite of sensitive biomarkers in gills, digestive gland (DG) and hemocytes of *Meretrix meretrix*, which presented a significant linear response when exposed to the increasing concentrations of STP effluent.

2. Materials and methods

2.1. Sampling strategy and analysis

2.1.1. Sample collection

In this study, we conducted a simulation experiment of marine environment received discharged effluent in the laboratory. Effluent was collected from the Tuandao STP near the Jiaozhou Bay (Qingdao, China) (Fig. 1). Samples were collected at the effluent discharge outlet of the STP at 6 h time intervals. The four effluent samples collected over 24 h were mixed in equal volumes before the experiment and stored at 4 °C.

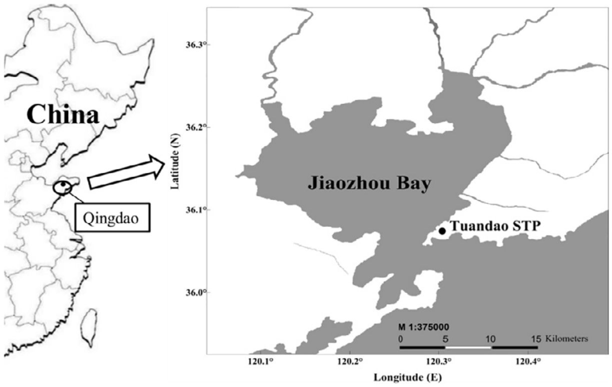

Fig. 1. Map of the study area and the sampling site (Tuandao sewage treatment plant, Tuandao STP).

Natural, clean seawater was collected from the Shilaoren sea area near Qingdao, China (with a pH of 7.90 ± 0.02 and salinity of 32‰).

Mussels as their characteristics that sessile, filter-feeding lifestyle, relatively low metabolic transformation rate, and propensity to bioaccumulate pollutants make them a useful bio-indicator species. In this work, *M. meretrix* with an average length of 40.1 mm, were collected from the culture zone of the Daguandao Sea in the Laoshan Bay area (Qingdao, China) and determined in clean natural sea water for 7 days. In the laboratory, six effluent volume ratios (EVRs, v/v) 1%, 5%, 10%, 20%, 30%, 40% and a control (0%) were set to simulate the marine environment from different distance of the STP outlet. Then, mussels were distributed into groups of 90 (two tiers) per glass cylinder and each with 10 L water containing different volumes of treated municipal effluent. Three glass cylinders of 90 clams were adopted per treatment. During all experimental periods, the clean natural seawater and effluents were changed daily in each glass cylinder. Mussels were fed with suspensions of the green alga *Chlorella pacifica* (1.3×10^7 cells per litre per day) and an air pump was used for continuous aeration to maintain a constant level of dissolved oxygen of 6 ± 0.5 mg L^{-1} during acclimation and exposure periods. Temperature (15 ± 1 °C), pH (7.90 ± 0.02) and a salinity (32‰) adjusted by sea crystal were kept consistent throughout the experiment.

2.1.2. Analysis methods

After different periods of time (0, 6 and 12 days), hemocytes of several bivalves were withdrawn for lysosomal membrane stability (LMS) and micronucleus frequency (MF) measurement, and mussels of each experimental aquarium were collected and gills and DG were dissected, then the homogenates (1:4, w (g)/v (ml)) of the samples were prepared in an ice-cold Tris-HCl buffer solution (0.02 M, pH 7.8). They were then centrifuged at 10,000*g* for 15 min at 4 °C to remove tissue debris. The supernatants were collected for measurement of protein content and the biomarkers except metallothioneins (MTs). For detection of MTs, the sample was homogenised (1:4, w/v) with cold Tris-HCl buffer solution containing 0.5 M sucrose, 0.5 mM phenylmethylsulphonyl fluoride, and 0.01% β-mercaptoethanol with a pH of 8.6. The supernatant of the homogenate was collected for biochemical analyses after centrifugation at 25,000*g* for 30 min at 4 °C. All assays were performed in triplicate.

To assess the water quality through general health of the animals, the following biomarkers were measured: LMS (Lowe et al., 1995); MF (Galloway et al., 2010); glutathione (GSH) (Zhang et al., 1993); acetylcholinesterase (AChE) (Ellman et al., 1961); MTs (Viarengo et al., 1997); glutathione reductase (GR), glutathione peroxidase (GP*x*), malondialdehyde (MDA) and the protein (by commercially-available assay kit, Nanjing Jiancheng Bioengineering Institute, China).

2.2. Water quality assessment

The WQCI was developed following modification of the expert system and the IBRv2 that had been developed and modified previously for marine mussels (Dagnino et al., 2007, Beliaeff and Burgeot, 2002, Sanchez et al., 2013).

The expert system represents a simple tool for risk assessment of the toxic impact of contaminants by ranking the level of stress syndrome induced by pollutants in marine mussels (Dagnino et al., 2007). This procedure, based on a number of previous studies, both in laboratory exposure experiments and in biomonitoring programmes, allows the classification of each biomarker result into one of four classes or alteration levels (AL), from NA (no alteration) to + + +/− − − (large alteration). From Table 1, the author thought that if alteration factor (AF, the ratio of the measured value to control) is within ± 20%, the organisms have no significant biological changes, if AF < 0.80 or AF > 1.20 and significant differences compared to the blank values, the organisms have the earliest physiological response, if AF < 0.50 or