Science of the Total Environment 634 (2018) 116–126

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Assessment of the effects of discontinuous sources of contamination through biomarker analyses on caged mussels

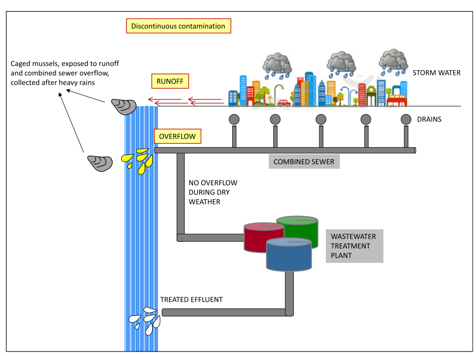
A. De los Ríos [a], B. Echavarri-Erasun [a], M.-H. Dévier [b,c], K. Le Menach [b,c], H. Budzinski [b,c], M. Ortiz-Zarragoitia [d,e], A. Orbea [d,e], J.A. Juanes [a], M.P. Cajaraville [d,e,*]

[a] Environmental Hydraulics Institute "IH Cantabria", University of Cantabria, c/ Isabel Torres no 15, 39011 Santander, Spain
[b] Univ. Bordeaux, EPOC, UMR 5805, LPTC unit, 351 crs de la Libération, F-33400 Talence, France
[c] CNRS, EPOC, UMR 5805, LPTC unit, F-33400 Talence, France
[d] Laboratory of Cell Biology and Histology, Department of Zoology and Animal Cell Biology, Faculty of Science and Technology, University of the Basque Country (UPV/EHU), Sarriena z/g, E-48940 Leioa, Basque Country, Spain
[e] Research Centre for Experimental Marine Biology and Biotechnology PIE, University of the Basque Country (UPV/EHU), Areatza z/g, E-48620 Plentzia, Basque Country, Spain

HIGHLIGHTS

- Heavy rains favor the discharge of contaminants from discontinuous sources.
- Chemical analyses succeeded in tracking contamination from discontinuous sources.
- Measured contaminants did not reach levels affecting mussels health.
- Changes in food availability provoked by rains may have affected mussels health.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 25 January 2018
Received in revised form 23 March 2018
Accepted 24 March 2018
Available online xxxx

Editor: D. Barcelo

Keywords:
Combined sewer overflow
Runoff
Caged mussels
Bioaccumulation
Biomarkers

ABSTRACT

The present study analysed potential adverse effects of discontinuous sources of contamination, namely the discharge of a combined sewer overflow (CSO) and of runoff in an urban area, the Bay of Santander (North Iberian Peninsula). Water samples and caged mussels were used to analyse concentrations of contaminants and biological responses. Mussels (*Mytilus galloprovincialis*) were transplanted to a marina receiving runoff from a petrol station and to a CSO discharge site. Samples were collected in synchrony with heavy rains along 62 days. Lysosomal membrane stability (LMS) and acyl-CoA oxidase (AOX) activity were measured as core biomarkers and were analysed at all sampling times. Histopathology of digestive gland and gonads, transcription levels of vitellogenin gene, volume density of black silver deposits and micronuclei formation were measured at initial and final stages of the transplant. Chemical analyses of metals, polycyclic aromatic hydrocarbons (PAHs) and endocrine disruptors were performed in water samples and mussel flesh. Mussels accumulated low concentrations of contaminants, which is in accordance with results obtained from exposure biomarkers. AOX activity decreased in all transplanted mussels after the first heavy rain, but this change seems to be related to the seasonal pattern of the enzyme activity. Mussels located close to the CSO discharge site showed a reduction in LMS after the first rain

* Corresponding author at: Laboratory of Cell Biology and Histology, Science and Technology Faculty, University of the Basque Country, Sarriena z/g, 48940 Leioa, Basque Country, Spain.
E-mail address: mirenp.cajaraville@ehu.eus. (M.P. Cajaraville).

https://doi.org/10.1016/j.scitotenv.2018.03.297

event, when compared to mussels before the transplant and to mussels from the reference location. However, this was attributable to natural environmental changes rather than to pollution. Values of the rest of analysed biomarkers were below threshold values reported for the study area.

1. Introduction

Diffuse contamination is the result of a variety of activities with no specific point of discharge that provokes an extensive release of contaminants in surface waters (EEA, 2013). In urban areas, the main diffuse contamination event is surface runoff, which is precipitation that neither evaporates nor penetrates the surface. Although runoff is mainly composed of rain, it is not free of contaminants. Rain adsorbs pollutants from the atmosphere as well as those existing in pavements and soils. Chemical properties of runoff depend on factors such as the nature of surfaces in the urban environment, the anthropogenic activities carried out in the area, and the natural processes operating within each catchment (Eriksson et al., 2007). In metropolitan areas runoff carries gasoline, motor oil, trash and other pollutants from roadways, as well as fertilisers and pesticides from residential lawns (Jartun et al., 2008; Wei et al., 2010; Jiang et al., 2012). Frequent contaminants are metals (mainly Pb) and polycyclic aromatic hydrocarbons (PAHs) (Rocher et al., 2004; Jartun et al., 2008). Runoff that enters the sewer network mixes with wastewater. During wet weather, sewer networks may cope with part of the contaminated rainwater (runoff) that is directed to the treatment plant. If rain flows entering the network system overpass the design threshold (in case of North Spain sanitation systems, 4–5 times wastewater flows), a diluted wastewater discharge takes place in certain points (combined sewer overflows-CSO) until reduction of rain flows.

Both runoff and CSO are climate dependent and, the latter, often infrequent (e.g. 30–50 days/year). Thus, they are considered discontinuous or intermittent contamination (Marsalek, 1990). Historically, environmental risk assessment has focused on the study of point source discharges, with known origin, frequency and composition (Preston, 2002). The problem of discontinuous contamination is the difficulty to anticipate flow volume and composition. Thus, in these cases, impact assessment must be based on the vulnerability of the receiving water systems, which is a function of its susceptibility and state of conservation (Gómez et al., 2014). The term susceptibility refers to the system's capacity to assimilate an external perturbation and is related to self-depuration. The highest rates of depuration occur in water systems with low flushing time (high dynamism) and low periods of residence for contaminants (Nilsson and Grelsson, 1995; Shen and Haas, 2004).

Nevertheless, not only characteristics of receiving waters are relevant in the assessment of the impact of discontinuous contamination. In the cases of runoff and CSO, both are consequence of rainfall but their composition and behavior can be far away from resembling each other. In CSO the initial moment of the overflow (called the first flush; Sartor and Boyd, 1972) is the most harmful in terms of contamination. During the first flush, at least 80% of the contaminants are released in the first 30% of the volume (Saget et al., 1995). Several studies have reported contamination derived from CSO, being oxygen depletion and increases in turbidity, metal concentrations and fecal indicator microorganisms the most frequent consequences (Iannuzzi et al., 1997; Weyrauch et al., 2010). Since pollutants of concern in urban areas are organic compounds and fecal microorganisms (Fletcher et al., 2008) models are focused on the behavior of these contaminants (Álvarez et al., 1999; Echavarri-Erasun et al., 2010; Juanes et al., 2005; Puente et al., 2002). In this context, Juanes et al. (2013) developed a specific method for the evaluation of contamination with no specific point of discharge, consisting on the calculation of the trajectory of discharged particles and the estimation of the potential affected area. However, these mathematic tools lack of ecological relevance if effects on aquatic organisms are not assessed. This issue has been commonly faced studying alterations in the structure of invertebrates inhabiting the bottoms of affected areas (Morrisey et al., 2003). Nevertheless, little importance has been paid on the effects that may occur at shorter exposures like, for instance, just after the rains that provoke the discharge. In these circumstances, it is necessary to evaluate biological effects that occur in the short-term.

In a previous work, we assessed the impact of treated and untreated urban effluents to estuarine and coastal waters applying a battery of biomarkers in mussels transplanted to discharge sites (De los Ríos et al., 2013). A similar approach has been applied in other works assessing urban pollution (Gagnon et al., 2006; Gagné et al., 2011; De los Ríos et al., 2012). With the hypothesis that intermittent contamination can produce alterations on marine organisms it would be possible to evaluate them using a similar procedure but taking into consideration the singularities of these type of discharges (frequency, duration, intensity). For that purpose, we transplanted caged mussels (*Mytilus galloprovincialis*) to sites affected by runoff and CSO discharges. As the degree of the effects derived from these sources depends on the rain intensity (Harremoës, 1988), analyses were performed on samples collected before and after heavy rain events. During mussel transplant, we assessed the presence of specific contaminants, their bioavailability and biological effects. We measured concentrations of metals, PAHs, phthalates, alkylphenols and bisphenol A. Metals and PAHs were measured in the two sites because they are present in CSO discharges and urban runoff (Morrisey et al., 2003; Gromaire et al., 2011). The rest of contaminants were measured only in the area of affection of the CSO discharge, since these contaminants are frequent in domestic sewage (Gasperi et al., 2008). To assess bioavailability of these contaminants, we measured their accumulation in transplanted mussels at initial and final stages of transplant.

To assess potential biological effects, we analysed a suite of biomarkers in transplanted mussels. Biomarkers are measurements at the molecular or cellular level that indicate exposure of the organism to pollutants (exposure biomarkers) and/or the magnitude of the response to such exposure (effect biomarkers) (McCarthy and Shugart, 1990). We analysed a battery of biomarkers, namely, lysosomal membrane stability (LMS), acyl-coA oxidase activity (AOX), digestive gland and gonad histopathology, autometallographic detection of metals, transcription of vitellogenin (*Vtg*) gene and micronuclei (MN) frequency. LMS, indicative of general health status (UNEP/RAMOGE, 1999), and AOX, exposure biomarker to organic contaminants (Cajaraville et al., 2003; UNEP/MAP, 2005; Cajaraville and Ortiz-Zarragoitia, 2006) were selected as core biomarkers (Sundt et al., 2006). Thus, they were analysed at all sampling times. Other biomarkers that respond to specific chemicals were analysed only at initial and final stages of the transplant. Through digestive gland and gonad histopathology, we measured metabolic and reproductive condition (Bignell et al., 2012). In addition, histological alterations can be associated to exposure to contaminants like PAHs and metals (Marigómez et al., 2006). Exposure to estrogenic endocrine disruptors was assessed through the measurement of transcription levels of *Vtg* gene in male gonads (De los Ríos et al., 2013). The bioavailability of metals was assessed through autometallography, which consists of the development of silver deposits on metal ions accumulated inside lysosomes (Soto and Marigómez, 1997a). Finally, as indicator of DNA damage caused by genotoxic compounds, we measured the frequency of MN in mussel haemocytes (Bolognesi et al., 2004).