Marine Pollution Bulletin xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Marine Pollution Bulletin

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

The usefulness of transplantation studies in monitoring of metals in the marine environment: South African experience

R. Greenfield [a], K. Brink [a], N. Degger [a,b], V. Wepener [c,*]

[a] Centre for Aquatic Research, Department of Zoology, University of Johannesburg, P.O. Box 524, Auckland Park 2006, South Africa
[b] School of Biological Sciences, The University of Hong Kong, Pokfulam Road, Hong Kong
[c] Water Research Group (Ecotoxicology), School of Biological Sciences, Unit for Environmental Science and Management, North-West University, Private Bag X6001, Potchefstroom 2520, South Africa

ARTICLE INFO

Keywords:
Metal bioaccumulation
Spatial and temporal
Perna perna
Harbours

ABSTRACT

Due to their close proximity to the point sources of pollution, estuaries and harbours are exposed to metals. Mussels are used worldwide to monitor marine pollution due to their ability to take up contaminants and the ease of transplantation. Mussels were collected from two reference sites and transplanted in Richards Bay Harbour (2006 and 2010) and in three harbours. Transplanted and resident mussels were removed after 6 weeks exposure and metal concentrations were analysed using ICP-MS techniques. Transplanted mussels had higher metal concentrations than the resident mussels. This was attributed to regulation of metals by the resident mussels. Metal regulation was greater in mussels that are continuously submerged as opposed to those that undergo tidal influences. For monitoring it is essential that the reference population for transplantation is considered carefully as upwelling events in the pristine reference location results in significant increases in metal bioaccumulation.

1. Introduction

Mussels have been used in monitoring programs since the early 1970s with the onset of the global mussel watch program (Goldberg, 1975). The program proposed to use sentinel organisms as an approach to monitor various selected pollutants. Various bivalves (especially mussels and oysters) were found useful organisms due to their worldwide distribution, ubiquitous abundance, sedentary lifestyle and ability to bio concentrate pollutants (Goldberg, 1975). The sedentary nature of bivalves also provides the ability to monitor temporal trends within the bioavailable fraction within the environment (Wu et al., 2007). Mussels have also been used in South African marine biomonitoring since 1974, but have mainly concentrated on resident populations (Wepener and Degger, 2012).

The use of mussels in active monitoring/transplantation studies has been found to be a valuable tool as most land derived effluent reaches the intertidal zone where natural mussel populations occur (Smolders et al., 2003). Transplantation studies have been applied successfully as indicators of environmental change on a spatial and temporal scale in Sydney Harbour (Honkoop et al., 2003), Boston Harbour (Hunt and Slone, 2010) and the Scheldt Estuary (Wepener et al., 2008).

Harbours do not only play an essential economic role, but also function as an important ecosystem, that provides sheltered refuge, feeding grounds and nursery area for a wide range of organisms (Wepener et al., 2008). Consequently monitoring of harbours for contaminants is vital for the protection of marine coastal environments as a whole. South Africa's premier bulk cargo port is Richards Bay Harbour. It is South Africa's largest and most modern cargo handling port (Jury and Guyon, 2009). In 1998 Richards Bay Harbour was the world's largest single coal terminal (Walmsley et al., 1998), which also exports a large range of chemicals including metals (Greenfield et al., 2011). Cape Town Harbour is situated on one of the world's busiest trade routes. The harbour handles container ships, fishing vessels, Cruise ships and is an important port for ship repair. The port handled 2775 vessels for a gross tonnage of 51,000, 519-gt for the 2011/2012 financial year (Ports and ships, nd). Port Elizabeth Harbour is one of the lesser South African harbours. It handles container ships with the containers carrying mainly agricultural products and automotive parts. The port also acts as a spill over point for container ships when Durban Harbour and Cape Town Harbour are too congested. The port also contains a large open area car loading terminal. The fishing industry makes extensive use of the port. Finally the port has a large manganese ore terminal, and handles petroleum

* Corresponding author. Tel.: +27 182992385.
E-mail address: victor.wepener@nwu.ac.za (V. Wepener).

http://dx.doi.org/10.1016/j.marpolbul.2014.03.032
0025-326X/

products imported from other ports in South Africa (Ports and ships, nd). The aim of this paper is to assess spatial and temporal bioaccumulation of metals in resident and transplanted mussels in three South African harbours. The three harbours used for the spatial assessments were Richards Bay Harbour, Port Elizabeth Harbour and Cape Town Harbour. Richards Bay Harbour was used for temporal assessment with data to form two different sampling events.

2. Materials and Methods

2.1. Temporal assessment

Control brown mussels (*Perna perna*) for transplantation were collected from Sheffield Beach, KwaZulu-Natal, South Africa during 2006 and 2009 (Fig. 1). Mussels between 40 and 50 mm were sampled between March and April in both 2006 and 2009 in order to prevent using spawning mussels during the transplantation studies (McQuaid, 2005). Mussels were suspended in duplicate polyethylene cages ($n = 15$ per cage) from navigational buoys at a depth of 1 m in Richards Bay Harbour. Following a deployment period of 6 weeks both transplanted and resident mussels, which were attached to the same buoy, were collected.

2.2. Spatial assessment

Control mussels for the spatial transplantation were collected from the Tsitsikamma National Park in the Eastern Cape Province of South Africa. While no baseline data is available on metal levels at the Tsitsikamma Nature Reserve the site is expected to be near pristine as it is situated in a marine protected area and thus not impacted by anthropogenic activities (Degger et al., 2011). Mussels were transplanted to Richards Bay, Port Elizabeth and Cape Town harbours. The same procedures described for the temporal assessment were applied.

2.3. Metal analyses

Following collection at the different sites all mussels were kept on ice and returned to the laboratory for dissection (Degger et al., 2011). In addition mussels collected from the original control source sites were also prepared for metal analyses. The byssal threads were removed, the tissue rinsed in ultrapure MilliQ water and the whole mussel was then dried in a drying oven at 60 °C for at least 48 h to attain constant weight. The dried mussel tissue was weighed and underwent standard microwave digestion using an Ethos Advanced touch Control Microwave station. Acid digestion took place using 30% Suprapure hydrogen peroxide and 70% Suprapure nitric acid (1:7 v/v). The samples were then diluted to a known volume with 1% HNO_3 (AR). The digested samples were then analysed using a Thermo ICP-OES and Thermo ICP-MS. The samples were analysed for aluminium (Al), arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), manganese (Mn), iron (Fe), nickel (Ni), lead (Pb), strontium (Sr) and zinc (Zn). All metal concentrations were then expressed in µg/g dry weight.

Indium was added to the samples as an internal standard to correct for interferences from high dissolved salts in the different matrices. For quality control purposes certified reference materials (NRCDOLT3 Dogfish liver tissue; CRM 278 mussel tissue Community Bureau of reference, Geel, Belgium) were also analysed. Recoveries are indicated in Table 1 and recoveries were between 90% and 105% of the certified values.

Analysis of variance was conducted using SPSS version 18. Data were tested for normality and homogeneity of variance prior to the application of *post-hoc* comparisons using Kolmogarov–Smirnov and Levene's tests (Zar, 1996) respectively. Where appropriate the Scheffé test for homogenous data and Dunnett's – T3 test for non-homogenous data were used for *post-hoc* comparisons. Statistical significance was regarded at a level of $P < 0.05$. Discriminant functional analysis (DFA) was conducted on the transplanted and resident mussel data to plot metal uptake between the resident and transplanted mussels.

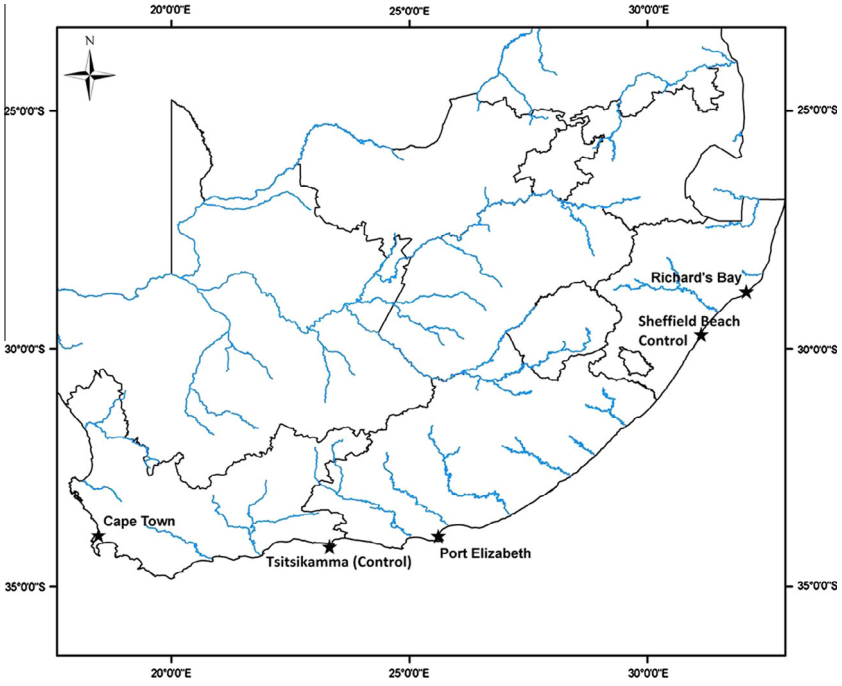

Fig. 1. Position of sampling sites along the South African coastline. Sheffield Beach served as source location for transplanted mussels in the temporal assessment in Richards Bay Harbour. Tsitsikamma was the source of the transplanted mussels for the spatial assessment in Richards Bay, Port Elizabeth and Cape Town harbours.

Please cite this article in press as: Greenfield, R., et al. The usefulness of transplantation studies in monitoring of metals in the marine environment: South African experience. Mar. Pollut. Bull. (2014), http://dx.doi.org/10.1016/j.marpolbul.2014.03.032