Marine Environmental Research 121 (2016) 9–19

Contents lists available at ScienceDirect

Marine Environmental Research

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

Environmental monitoring of pesticide exposure and effects on mangrove aquatic organisms of Mozambique

Joachim Sturve [a, *], Perpetua Scarlet [b], Maja Halling [c], Jenny Kreuger [d], Adriano Macia [b]

[a] Dept. of Biological and Environmental Sciences, University of Gothenburg, Gothenburg, Sweden
[b] Departamento de Ciências Biológicas, Faculdade de Ciências, Universidade Eduardo Mondlane, C.P. 257, Maputo, Mozambique
[c] EnviroPlanning AB, Lilla Bommen 5 C, 411 04, Gothenburg, Sweden
[d] Swedish University of Agricultural Sciences, Department of Aquatic Sciences and Assessment, Sweden

A R T I C L E I N F O

Article history:
Received 29 October 2015
Received in revised form
27 April 2016
Accepted 5 May 2016
Available online 23 June 2016

Keywords:
Organophosphates
Acetylcholineesterase
Fish
Invertebrates
Mangrove
Monitoring

A B S T R A C T

The use of pesticides in Mozambique is increasing along with the development of agriculture in the country. Mangroves along the coastlines are ecologically important areas and vital nursing grounds for many aquatic species, several of which are of high economic value in Mozambique. Barred mudskipper (*Periophthalmus argentilineatus*), Jarbua fish (*Terapon jarbua*), Indian white prawn (*Penaeus indicus*) and the clam *Meretrix meretrix* were collected at three mangrove sites in the Maputo Bay area. This was complemented with samplings of the freshwater fish Mozambique tilapia (*Oreochromis mossambicus*), which was collected from three sampling sites along rivers in the surroundings of Maputo and from three sites along the Olifants and Limpopo River. Acetylcholinesterase (AChE) activity, which is an established biomarker for organophosphates and carbamate pesticides, was measured in brain and liver tissue in fish, and hepatopancreas tissue in prawn and clam. Butyrylcholinesterase (BChE) activity was also analyzed. Freshwater samples for pesticide analyses were collected in order to get an initial understanding of the classes and levels of pesticides present in aquatic systems in Mozambique. In addition to field samplings two 48-h exposure experiments were also conducted where the Indian white prawn and Barred mudskipper were exposed to malathion, and Mozambique tilapia exposed to malathion and diazinon. Field results show a significant decrease in AChE activity in fish from four of the sampling sites suggesting that pesticides present in water could be one stressor potentially affecting aquatic organisms negatively. The 48 h exposure experiment results showed a clear dose-response relationship of AChE activity in mudskipper and tilapia suggesting these species as suitable as sentinel species in environmental studies.

1. Introduction

The use of pesticides in Mozambique is increasing along with the development of agriculture activities in the country. Several of the rivers originating from areas in southern Africa with extensive agriculture empty into Maputo bay and along the Mozambican coastline and may contribute to an increased chemical burden in the country (Chemane et al., 1997; Chilundo et al., 2008). A large number of these rivers debouch in mangrove areas that are ecologically important, known as vital nursing grounds for fish, prawns and other aquatic species (Nagelkerken et al., 2008; Robertson and Duke, 1987). The majority of the fish species living in the mangrove are of marine origin and pollution may therefore not only harm residential species but also species living in other costal ecosystems that spend part of their life in the mangrove (Nagelkerken et al., 2008). Disturbances can arise both at an individual and population level due to early life stage exposures (Kruitwagen et al., 2006). Therefore, species residing or visiting these habitats may be potential indicators of the health status of these ecosystems. Many of the vast regions of agricultural areas worldwide are located close to main river basins, increasing the risk of pollution from pesticides and fertilizers used within catchments areas (Mmochi and Francis, 2003).

The most commonly used insecticides worldwide are organophosphorus (OP), carbamate and synthetic pyrethroid substances. OP insecticide substances have a wide range of applications from agriculture to household (Grube et al., 2011). OPs are relatively non-persistent in the environment making them suitable as insecticides,

* Corresponding author.
E-mail address: joachim.sturve@bioenv.gu.se (J. Sturve).

http://dx.doi.org/10.1016/j.marenvres.2016.05.005
0141-1136/© 2016 Published by Elsevier Ltd.

but due to a low target specificity combined with relatively high toxicity they may pose a threat to many non-target organisms, such as fish, insects and crustaceans (Fulton and Key, 2001). OP usage also poses a threat to humans due to malpractice during usage or unintentional exposure through OP contaminated food. Nweke and Sanders (2009) review the effects of modern environmental health hazards (MEHHs) in Africa and state that pesticides including OPs may have implications in disease burden and public health. London (2009) suggests that malpractice of pesticide use in Africa should be placed in a human rights context. Information concerning quantities of pesticides used in the southern part of Africa is sparse, but in South Africa over 3000 pesticides are registered for use (Dabrowski, 2015).

The toxic mode of action of OPs is due to their ability to inhibit acetylcholinesterase (AChE) activity that catalyses the hydrolysis of the neurotransmitter acetylcholine (ACh) (Bocquené and Galgani, 1998; Galgani et al., 1992). An inhibition of AChE can lead to muscle spasms and ultimately death as a result of an overload of ACh in the synaptic cleft, which causes the neuron to incessantly transmit nerve signals resulting in tetany (Bocquené and Galgani, 1998; Fulton and Key, 2001). Sublethal exposures can lead to behavioral and physiological disturbances due to effects on cholinergic nerves in the central nerve system (London et al., 2005). AChE activity can easily be measured and its inhibition is frequently used as a biomarker for OP exposure both in humans and in aquatic species (van der Oost et al., 2003; Jordaan et al., 2013). In addition to AChE, inhibition of butyrylcholinesterase (BChE) has been proposed as a biomarker for OPs. BChE is a non-specific cholinesterase and butyrylcholine is a synthetic molecule not naturally present in the body and is used to distinguish between the two cholinesterase forms. BChE specific function, if it has one, is not yet well understood (Darvesh et al., 2003).

Several aquatic species found in the Maputo bay area may be used as sentinel species to study OP exposure. The Mozambique tilapia (*Oreochromis mossambicus*) is native to Africa, but is today also found in subtropical and tropical fresh waters all around the world due to aquaculture (Canonico et al., 2005). The species has been used extensively for toxicological studies and is commonly found in the fresh water systems in Mozambique. The Jarbua fish (*Terapon jarbua*) is common in most mangrove areas and like many other fish species the omnivorous Jarbua spends the juvenile life stage in the mangrove and return to the sea to spawn (Nagelkerken et al., 2008). In comparison to many other fish species that only spend specific life stages in the mangrove, the Barred mudskipper (*Periophthalmus argentilineatus*) is stationary in the mangroves. Due to its amphibious properties the mudskipper can dwell in the intertidal zone, an environment with highly variable conditions, without competition from other fish species (Kruitwagen et al., 2007). The mudskipper has been used in previous studies as a sentinel species for monitoring marine pollution (Bu-Olayan and Thomas, 2008; Kruitwagen et al., 2006). Prawns are one of Mozambique's most important exported seafood and together with fish also a main part of the local diet (Chemane et al., 1997). The Indian white prawn (*Penaeus Indicus*) is easy to catch as it seldom buries in the mud and is commonly found in the mangroves (Rönnbäck et al., 2002). The Indian white prawn spawns at sea and at the postlarval stage settles in inshore and estuarine waters such as the mangrove (Rönnbäck et al., 2002). Another important food source for the local community is the clam *Meretrix meretrix*, an invasive species commonly found in the inner parts of Maputo bay. This species has previously been used for the assessment of water quality, such as municipal treatment effluent (Wan et al., 2015).

The aim of the present study was to investigate the impact of pesticides on aquatic freshwater and mangrove species using acetylcholinesterase (AChE) and to some extent butyrylcholinesterase (BChE) inhibition as biomarkers. For that purpose, Jarbua fish, Barred mudskipper, Indian white prawn and the clam Meretrix meretrix, were sampled at presumably polluted and unpolluted mangroves in Maputo bay. Mozambique tilapias (M tilapia) were collected from three sampling sites along rivers in the surroundings of Maputo and from three sites along the Olifant and Limpopo River north of Maputo.

Jarbua fish, Indian white prawn and M tilapia were also exposed to organophosphate in a laboratory study in order to study species-specific AChE inhibition. In order to obtain an initial understanding of the classes and approximate levels of pesticides present in freshwater systems in Mozambique, chemical analysis of water samples were performed.

2. Materials and methods

2.1. Chemicals and reagents

Acetylthiocholine (ACTC) iodine, butyrylthiocholine (BuCTC) 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), malathion and diazinon were obtained from Sigma Aldrich (St Louis, United States). Bovine serum albumin (BSA), Folin- Ciocalteu's phenol reagent, KH_2PO_4, Na_2HPO_4*$12H_2O$, NaH_2PO_4*H_2O, Na2CO3 were obtained from Merck (Darmstadt, Germany). NaOH was obtained from KEBO Lab (Spånga, Sweden). All other chemicals were of analytical grade.

2.2. Experimental setup

2.2.1.. Field sampling

Mangrove sampling sites: Jarbua fish, Barred mudskipper, Indian white prawn and the clam *Meretrix meretrix* were sampled from three locations around Maputo bay (Fig. 1B). The first site was Barrio Luís Cabral located 6 km west of Maputo city centre on north shore of Maputo Bay, were there is a small mangrove stretching out in the vicinity of Barrio Luís Cabral. The mangrove, which receives the water from the Infulene River, is situated within the city harbor and the Espírito Santo estuary. The second site was Bairro dos Pescadores, a local fisherman's village located in an intertidal area 12 km north of Maputo City. The third site was Inhaca Island situated 32 km east of the Maputo coast (Rönnbäck et al., 2002). The island is partly a nature reserve and sparsely populated except for the community centre located on the northwest part of the island. Due to the low population density and the absence of domestic and industrial effluents, the environment on Inhaca Island is less affected by anthropogenic activities compared to the surroundings of Maputo (Omodei Zorini et al., 2004). For the clam *M meretrix*, the site Ilha Xefina outside the Bairro dos Pescadores was used as reference site since that species is not available at Inhaca.

Fresh water sampling sites: M. tilapia were sampled from three sites along two rivers in the Maputo area (Fig. 1C). The Infulene River runs along the western Maputo city border and the river is lined with allotments that support small-scale peri-urban farming and the river water is used for irrigation. The sewage treatment plant (STP) of Maputo city is situated in conjunction with the last part of Infulene River, before the river empties in Maputo bay. The first site is downstream the STP (Infulene DS) and the second site is upstream the STP (Infulene US). The third site is the river Umbeluzi southwest of Maputo city. This site is considered as a reference site since no major anthropogenic activities can be detected in the area.

Three sites along the Limpopo and Olifants rivers were also selected for M. tilapia sampling (Fig. 2B). Lake Massingir that is a man-made water reservoir controlling the water flow of the Olifants River. Olifants River originates in South Africa and flows through large agricultural areas and into the Kruger National Park continuing into Mozambique. The second site was Chirrunduo