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ROPME MUSSEL WATCH PROGRAMME 2014

Technical Report: No. 5

REGIONAL DISTRIBUTION OF BIOTOXINS IN ROCK AND PEARL OYSTERS

Prepared by:

MESL/IAEA
Monaco, December 2015

For:

REGIONAL ORGANIZATION FOR THE PROTECTION OF THE MARINE ENVIRONMENT

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1. INTRODUCTION

Under the ROPME Mussel Watch Programme 2014, a survey was undertaken in February-July 2014 to screen for a series of natural and anthropogenic contaminants in oyster samples collected in key coastal areas of Bahrain, I.R. Iran, Iraq, Oman, Saudi Arabia and the United Arab Emirates (UAE). This report summarizes the results of the analysis of the natural biotoxins saxitoxins and brevetoxins associated to harmful algal blooms.

2. SAMPLING METHODOLOGY

Oyster samples from selected locations along the coast of Kingdom of Bahrain (K.Bh), Islamic Republic of Iran (I.R. Iran), Iraq, Sultanate of Oman (Oman), Kingdom of Saudi Arabia (KSA) and the United Arab Emirates (UAE) were collected in February - July 2014, as part of the ROPME Mussel Watch Programme. The sampling stations, locations and types of samples collected are given on the Figures 1 and 2 and in Table 1.

Oyster samples were freeze dried and consisted of 7 Pearl Oysters samples, identified as *Pinctada radiate* and 16 Rock Oyster samples identified as *Saccostrea cucullata*. The number of individual oyster in each sample was only specified for UAE (n=20 per site).

No information was available on the sample composition (whole or part of the organisms) and wet weigh. Overall the dried Pearl and Rocky oyster samples received were clear and homogenous except that from Oman which appeared burnt during drying process (See Figure 3). No information was provided on the presence or absence of concurrent harmful algal bloom during the sampling period.

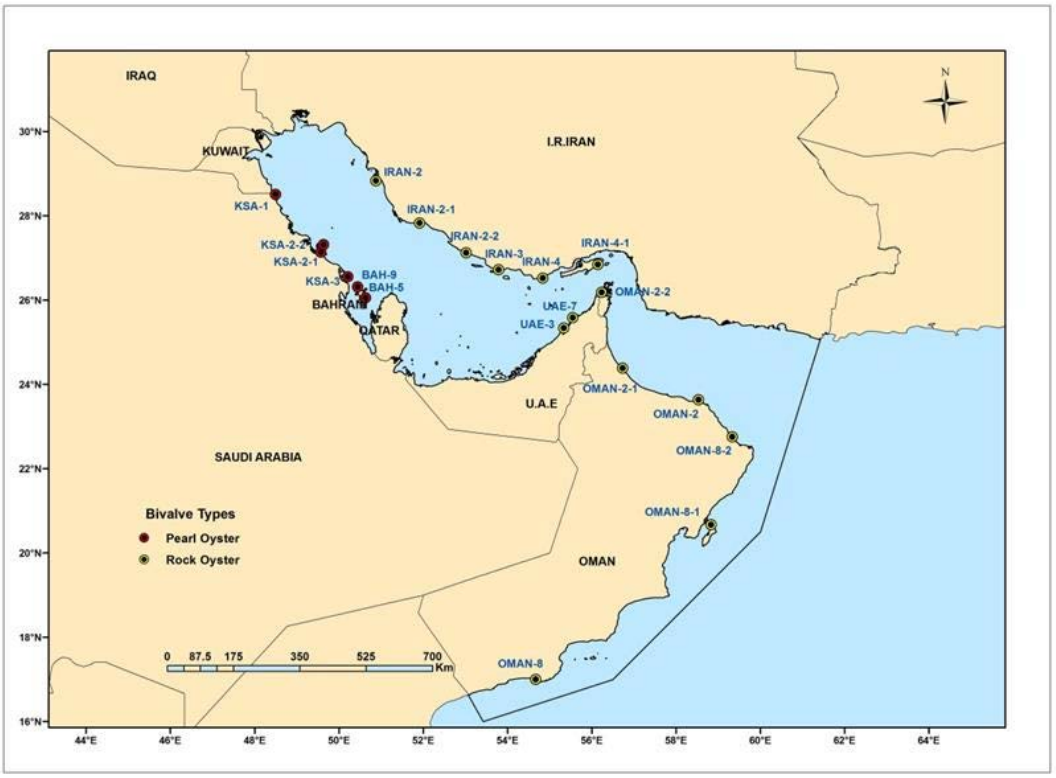

Figure 1. Map of sampling station (by code number)

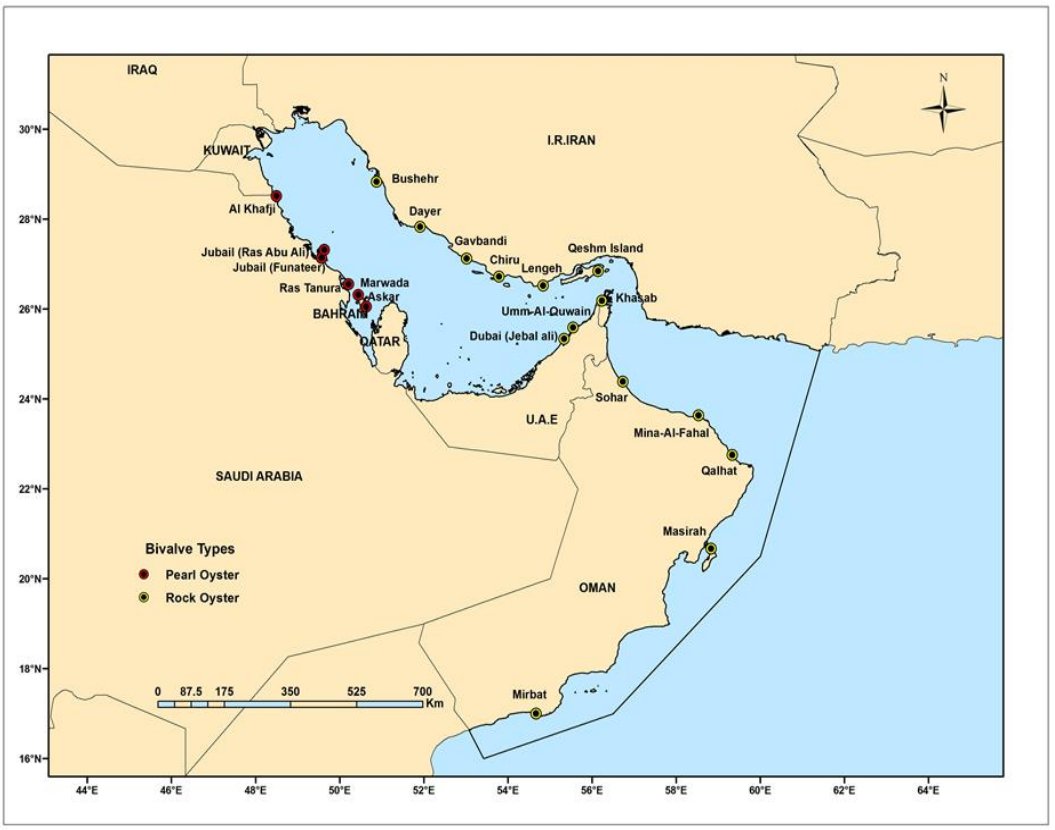

Figure 2. Map of sampling station (by name of location)

Table 1. Biota sampling sites

Country	Date	Site Name	Code	Station	Latitude	Longitude	Remarks	
K.Bh	2014-02-18	Askar *	Bah-5	1	26°3' N	50°37' E	Pearl Oyster	
	2014-02-17	Marwada	Bah-9	1	26°18' N	51°26' E	Pearl Oyster	
I.R.Iran	2014-02-17	Bushehr *	IRAN-2	1	28°49' N	50°52' E	Rock Oyster	
	2014-02-19	Dayer	IRAN-2-1	1	27°49' N	51°54' E	Rock Oyster	
	2014-02-20	Gavbandi	IRAN-2-2	1	27°7' N	53°1' E	Rock Oyster	
	2014-02-21	Chiru (new site)		1	26°43' N	53°47' E	Rock Oyster	
	2014-02-22	Lengeh *	IRAN-4	1	26°31' N	54°50' E	Rock Oyster	
	2014-02-24	Qeshm Island	IRAN-4-1	1	26°50' N	56°8' E	Rock Oyster	
Iraq	?/07/2014	Shat Al Arab		1			Pearl Oyster	10 Km offshore
OMAN	2014-04-01	Mirbat *	OMAN-8	1	17°0' N	54°40' E	Rock Oyster	
	2014-03-14	Masirah	OMAN-8-1	1	20°40' N	58°50' E	Rock Oyster	
	2014-02-08	Qalhat	OMAN-8-2	1	22°45' N	59°20' E	Rock Oyster	
	2014-02-06	Mina Al Fahal *	OMAN-2	1	23°37' N	58°31' E	Rock Oyster	
	2014-02-10	Sohar	OMAN-2-1	1	24°23' N	56°44' E	Rock Oyster	
	2014-04-08	Khasab	OMAN-2-2	1	26°11' N	56°14' E	Rock Oyster	
KSA	2014-03-06	Ras Tanura *	KSA-3	1	26°33' N	50°12' E	Pearl Oyster	
	2014-03-08	Jubail	KSA-2-1	1	27°8' N	49°34' E	Pearl Oyster	pleasance harbour (Fanateer)
	2014-03-09	KSA-2	KSA-2-2	2	27°18' N	49°38' E	Pearl Oyster	30 km N. Jubail (Ras abu Ali)
	2014-03-09	Al Khafji	KSA-1	1	28°30' N	48°29' E	Pearl Oyster	little No. of oysters
UAE	2014-02-13	Umm Al-Quwain *	UAE-7-1	1	25°35' N	55°33' E	Rock Oyster	
		UAE-7	UAE-7-1	2			Rock Oyster	
			UAE-7-1	3			Rock Oyster	
			UAE-7-2	1			Pearl Oyster	50 m offshore of R. Oyster stations
			UAE-7-2	2			Pearl Oyster	
			UAE-7-2	3			Pearl Oyster	
	2014-02-18	Dubai (Jebal Ali)	UAE-3	1	25°20' N	55°20' E	Rock Oyster	

*Location sampled during the ROPME Contaminant Screening Programme (1994-2005) and Mussel Watch Programme 2011

Figure 3. Photos of the samples from each country

3. ANALYTICAL PROCEDURES

The analytical protocols for determination of biotoxins in biota samples are presented in this section.

Sample extraction

Dried samples were first rehydrated using an estimation of 80% water content as per value found in the literature (the actual initial fresh weight not being provided). Aliquot of 0.5g of dried shellfish were hence prepared by adding 2g of water to approximate a fresh shellfish tissue weigh of 2.5g.

Brevetoxin and saxitoxin extraction.

For brevetoxin analysis hydrated samples were extracted with acetone (1:3 w:v) using a probe sonicator. Samples were then centrifuged and the supernatant collected. This step was repeated once and both supernatants were pooled and dried. The dry extract was then portioned twice in methanol 80% and hexane to remove the lipids. The methanol fraction was dried and re-suspended in 100% methanol for toxin analysis.

For saxitoxin analysis, hydrated samples were extracted with 0.1N HCl in a boiling water bath for 5min. The mixture was then centrifuged at 3,000 rpm for 10 min and the supernatant filtered through 0.45 µm nylon filters before toxin analysis.

Toxin analysis

- Saxitoxin analysis with the AOAC Official Method of Analysis (OMA 2011.27) Receptor Binding Assay (Van Dolah *et al.* 2012, IAEA-TEC-DOC-1729)

All filtered extracts were analyzed by receptor binding assay (RBA) using a tritiated dihydrochloride-saxitoxin (from Quotient bioresearch, UK), and quantified by comparison to certified reference solutions of saxitoxin (STX) purchased from National Research Council Canada. Toxicity is therefore reported in saxitoxin equivalents (STX eq.) for sample above the limit of quantification. Brevetoxin analysis by radioligand-receptor binding assay (IAEA TEC-DOC-1729).

Methanol extracts were analyzed by receptor binding assay (RBA) using a tritiated PbTx-3 (from ARC, USA) and quantified by comparison to commercially available standard brevetoxin PbTx-3. Toxicity is therefore reported in PbTx-3 equivalents (PbTx-3 eq.) for sample above the limit of quantification.

4. RESULTS AND DISCUSSION

Results of RBA analyses are given in Table 2. Brevetoxins were below the level of detection in all samples. Saxitoxins were below the detection level in 22 samples and quantifiable in 4 samples. Tissue concentrations ranging from 7.63 μg STX eq./100g, and 13.3 μg STX eq./100g were measured in Pearl oysters from Saudi Arabia and Iraq and Rock Oyster from I.R. Iran (Table 2).

Table 2. Saxitoxines and brevotoxins in oyster samples

No. of sampling sites	Country	Date	Site Name			Bivalve type	Total STX Equiv. µg/100g	Brevetoxins votoxinsBre µg/100g
			Name	Code	Station			
2	K.Bh	2014-02-18	Askar *	Bah-5	1	*Pearl Oyster*	<dl	<dl
		2014-02-17	Marwada	Bah-9	1	*Pearl Oyster*	n/a	n/a
6	I.R. Iran	2014-02-17	Bushehr *	IRAN-2	1	*Rock Oyster*	13.63	<dl
		2014-02-19	Dayer	IRAN-2-1	1	*Rock Oyster*	<dl	<dl
		2014-02-20	Gavbandi	IRAN-2-2	1	*Rock oyster*	<dl	<dl
		2014-02-21	Chiru (new site)		1	*Rock Oyster*	<dl	<dl
		2014-02-22	Lengeh *	IRAN-4	1	*Rock Oyster*	<dl	<dl
		2014-02-24	Qeshm Island	IRAN-4-1	1	*Rock oyster*	<dl	<dl
1	Iraq	?/07/2014	10 Km offshore Shat Al Arab		1	*Pearl oyster*	7.63	<dl

Table 2. Saxitoxines and brevotoxins in oyster samples (Contd…)

6	OMAN	2014-04-01	Mirbat *	OMAN-8	1	*Rock Oyster*	<dl	<dl
		2014-03-14	Masirah	OMAN-8-1	1	*Rock Oyster*	<dl	<dl
		2014-02-08	Qalhat	OMAN-8-2	1	*Rock Oyster*	<dl	<dl
		2014-02-06	Mina Al Fahal *	OMAN-2	1	*Rock Oyster*	<dl	<dl
		2014-02-10	Sohar	OMAN-2-1	1	*Rock Oyster*	<dl	<dl
		2014-04-08	Khasab	OMAN-2-2	1	*Rock oyster*	<dl	<dl
4	KSA	2014-03-06	Ras Tanura *	KSA-3	1	*Pearl Oyater*	<dl	<dl
		2014-03-08	Jubail	KSA-2-1 (Fanateer)	1	*Pearl Oyster*	9.67	<dl
		2014-03-09	KSA-2	KSA-2-2 (Ras abu Ali)	2	*Pearl Oyster*	13.3	<dl
		2014-03-09	Al Khafji	KSA-1	1	*Pearl Oyster*	<dl	<dl
3	UAE	2014-02-13	Umm Al-Quwain *	UAE-7-1	1	*Rock oyster*	<dl	<dl
			UAE-7	UAE-7-1	2	*Rock oyster*	<dl	<dl
				UAE-7-1	3	*Rock oyster*	<dl	<dl
				UAE-7-2	1	*Pearl Oyster*	<dl	?
				UAE-7-2	2	*Pearl Oyster*	<dl	?
				UAE-7-2	3	*Pearl Oyster*	<dl	<dl
		2014-02-18	Dubai (Jebal Ali)	UAE-3	1	*Rock oyster*	<dl	<dl

5. CONCLUSION

In the ROPME Sea Area (RSA), attention has been given over the last 2 decades to harmful algal blooms, providing information on phytoplankton species composition, abundance and biomass, in relation to environmental condition such as eutrophication, pollution, climate change etc. (Dorgham, 2013). The number of reports on harmful algal bloom has been increasing, and their impact on drinking water and seafood safety and security and the environment have become more widespread and persistent. Fish and marine mammals mortalities, aquaculture loss, traditional fishery restriction, and closure of desalination plants, have been attributed to a variety of microalgae, harmful through toxin production or by their biomass. This new regional approach attempts, for the first time, to provide toxicity data through an opportunistic collection of shellfish in 26 sites along the coastline of 6 countries of the RSA.

Two relevant neurotoxins were monitored in Rock and Pearl Oysters, the brevetoxins and the saxitoxins, both produced by phytoplankton species reported in the RSA, and responsible of human neurotoxic shellfish poisoning (NSP) and paralytic shellfish poisoning (PSP), respectively.

The levels of brevetoxins were below the detection limit of the RBA in all samples tested, while the levels of saxitoxins were below the detection limit in all but four (4) sites: Bushehr (IRAN 2), IRAQ, Fanateer (KSA 2.1) and Ras Abu Ali (KSA 2.2). The estimated values, ranging from 7.63 to 13.3 µg STX eq./100g, were below to the 80 µg STX eq./100g regulatory limit applied in most countries (eg. EU or USA) or to the 40 µg STX eq./100g value applied in the Philippines. Yet those values remain of concern considering the fact that no concurrent blooms were reported during the sampling period. As well, the absence of brevetoxin does not preclude the possibility of contamination with these toxins during toxic blooms.

6. RECOMMENDATION

The results of the present survey revealed low saxitoxin like activity in selected Oysters collected in the framework of the ROPME Mussel Watch 2014 campaign. It is recommended to complement these receptor binding assay results by analyzing the samples using high performance liquid chromatography with fluorescent (HPLC-Fl), an analytical method that would provide PSP toxins profile. Future work could also include systematic shellfish sampling concomitant to relevant harmful algal blooms, as well as toxic phytoplankton cyst bed mapping.

7. ACKNOWLEDGEMENTS

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