Chapter 8

Biomonitoring of Trace Metals in the Coastal Waters Using Bivalve Molluscs

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http://dx.doi.org/10.5772/intechopen.76938

Abstract

Several environmental contaminants including toxic trace metals are being discharged into the coastal environment causing serious threat to marine organisms and posing public health risk. Marine bivalves (mussel, oyster, and clam) have been successfully used as sentinel organisms for monitoring contaminant levels, including trace metals, in coastal waters around the globe. Chemical analyses measure the contaminants present in the biota but do not necessarily reveal potential biological effects. Therefore, the need to detect and assess the effects of contaminants, especially at low concentrations, has led to the development of molecular markers of contaminant effects called biomarkers. Owing to their short time of response, biomarkers in marine bivalves are used as early warning signals of biological effects caused by environmental pollutants. Research into the development and application of accurate biomarker-based monitoring tools for the environmental contaminants has been intensified in several developed countries.

Keywords: bivalves, bioaccumulation, biomarkers, trace metals, mussel watch

1. Introduction

Marine pollution is a major problem that has negative effects on the ocean's ecosystems. Economic developments and urbanization are taking place at an accelerated rate in the coastal zones across the world, putting enormous pressures on coastal waters and marine habitats. Incidents of coastal and marine water pollution have increased throughout the world, mainly due to discharges from rivers, increased surface run-off, drainage from expanding port areas, oil spills, discharges from shipping activities, and domestic and industrial effluent discharges.

Most of the world's wastes around 20 billion tons per year end up in the sea, often without any preliminary processing.

Trace metals are introduced into the coastal waters through natural process and anthropogenic activities. The natural process includes river discharge, rock weathering, wind-generated dust from arid and semi-arid regions of the continents, and hydrothermal circulation at mid-ocean ridges. The anthropogenic sources of metals include agriculture, fossil fuel extraction, refining and burning, chemical production, and intentional and accidental discharges. Trace levels of trace metals naturally occur in the marine environment, and many of them at low concentrations are essential for marine life. However, if their concentrations exceed the natural levels, it will cause a serious threat to marine life. Monitoring and assessment programs are routinely conducted in the coastal waters for planning and implementing mitigation measures to control trace metal pollution. Historically as one of the simple and widely used monitoring techniques, sampling, and analysis of seawater and sediment are being employed for estimating the levels of contaminants including trace metals in coastal waters. Instead of using water or sediment samples, tissue concentrations of contaminants in marine organisms, especially bivalves, are being used as a reliable method for assessing the coastal water quality since 1960s [1–4].

Most of the marine bivalves such as mussels, oysters, and clams are commercially important groups, and several of them are being used for coastal farming around the globe and as popular seafood. Since late 1960s and early 1970s, bivalves such as mussels were used for biomonitoring trace metals in coastal waters [3, 5]. In biomonitoring, tissue burden of trace metals in marine organisms are analyzed, and the biological responses of organisms are measured to assess changes in the environmental quality caused by toxic contaminants [6–8]. This chapter will attempt to provide an overview of the basic concept, methods and the present status of the biomonitoring of trace metals in the coastal waters using bivalve molluscs.

2. Why bivalves

Generally, bivalves are suspension feeders or deposit feeders, or even utilize both feeding methods. They feed on microscopic algae, bacteria, and detritus through filter feeding process. They draw water from the posterior ventral side through the inhalant siphon, and the water passes through the gills and gets expelled through the exhalent siphon. In this process, they filter large quantities of seawater, and the water filtering capacity of typical natural mussel beds has been calculated as 7–12 m^3, m^{-1}, h^{-1} [9, 10]. One single adult blue mussel pumps around 50 ml of seawater per minute during active feeding [11]. As bivalves filter large quantiles of seawater, their tissues absorb some of the contaminants present in water and food particles. Bivalves accumulate trace metals from the surrounding aquatic medium across the cellular membrane (dissolved source) and from the food materials (dietary source) [12].

Historically, bivalve molluscs are considered as valuable marine organisms for environmental monitoring and used as biomonitors of chemical pollution of coastal waters [3, 5, 13]. Bivalves are widely distributed from the North Pole to the South Pole, sessile in nature, and easy to sample and available in a suitable size for chemical analysis. Bivalves are also resistant to a wide

http://dx.doi.org/10.5772/intechopen.76938

Figure 1. Common marine bivalves and their habitats from the Indian coast. (A) Intertidal rocky area showing green mussel beds from the south west coast of India; (B) green mussel *Perna viridis*; (C) enlarged view of green mussels; (D) oyster bed consisting of *Crassostrea madrasensis* and *Saccostrea cucullata* exposed during low tide; and (E) enlarged view of *C. madrasensis and S. cucullata;* (E) clam bed consisting of *Meretrix casta* and (F) enlarged view of the clam *Paphia malabarica*.

range of contaminants and may thrive even in highly polluted environments [3, 14]. These qualities make them a group of candidate species for biomonitoring programs across the globe. As filter feeders, they bioaccumulate various contaminants and their tissue concentrations provide a time-integrated picture of contaminants in the environment [15, 16]. It has been reported that bivalves accumulate trace metals in their tissues at levels up to 100–100,000 times higher than the concentrations observed in the seawater in which they live [5, 17]. Therefore, several chemical contaminants, including trace metals, present at undetectable levels in seawater can be detected in bivalve tissues. Different species of clams, mussels, and oysters have widespread distribution across the continents (**Figure 1**), and many of those species have been successfully used for monitoring the concentrations of contaminants in the marine environment [5].

3. Metal bioaccumulation in bivalves

Cobalt, copper, chromium, iron, magnesium, manganese, molybdenum, nickel, selenium, and zinc are essential metals that are required for various biochemical and physiological functions of animals [18] while other metals such as aluminum, antinomy, arsenic, barium, cadmium, gold, lead, lithium, mercury, nickel, platinum, silver, strontium, tin, titanium, and vanadium have no established biological functions and are considered as non-essential metals [19]. However, the essential metals will be harmful to the organisms if their concentrations exceed the natural levels. The expert's group of International Council for the Exploration of the Sea (ICES) and Oslo and Paris Conventions (OSPAR) highlighted the trace metals such as arsenic, cadmium, chromium, copper, mercury, nickel, lead, and zinc in the marine environment as key substances of concern [20].

Bivalves accumulate both essential and non-essential metals in their soft tissues above the background levels in seawater or sediments, and this process is called bioaccumulation. Bioaccumulation is a good integrative indicator of the chemical exposures of marine organisms such as bivalves in polluted waters [21]. Trace metals cannot be metabolized by organisms, and hence bioaccumulation of trace metals is of particular value as an exposure indicator. However, metal bioaccumulation can be complex. The bioaccumulation levels in mollusks differ among metals in the same bivalve species and among species [13, 21–23] due to the biological role of different metals and to specific strategies of accumulation [23]. In addition, the metal bioaccumulation in bivalves depends on the marine environmental factors (temperature, pH, salinity, co-occurrence of metals, etc.) and the biological conditions (age, sex, sexual maturity stage, etc.) of the species [24, 25].

The gill tissue of bivalves constitutes a key interface for the uptake of dissolved metal ions from water followed by the mantle tissue, and the uptake of metals bound to particulate material is achieved via the digestive tract, in particular, via the digestive gland [23]. Generally, in bivalves, maximum concentrations of metals have been reported in the digestive gland and/or gill tissue followed by mantle and muscle tissue [26, 27]. The bioaccumulation of trace metals in bivalve tissues is dependent on different metabolic processes occurring within specific cell types in target tissues. Metallothioneins (MTs), the low-molecular-weight proteins present in organisms including bivalves are involved in the intracellular regulation of metals such as Cu, Zn, and Cd [28]. Epithelial cells of gill and mantle can synthesize MT and sequester metals into the lysosomes for further transport in circulating hemocytes [29].

4. Bivalves as sentinel organisms

Sentinel organisms accumulate contaminants in their tissues without any harmful effects and can be measured in a sensitive manner the amount of contaminants that are biologically available [30]. Several comprehensive reviews have been published on the use of bivalve molluscs as sentinel organisms and as biomonitors of metal pollution [5, 12, 20, 31–35]. These reviews and studies provide an in-depth discussion on metal bioaccumulation and metal bioavailability, highlighting the historical usage of bivalves in environmental studies.

Most of the bivalves such as clams, mussels, and oysters, fulfill the criteria required for a typical sentinel organisms and being successfully used as spatial and temporal trend indicators of contaminants in monitoring program from several parts of the world [3, 7, 12, 14–16, 36–39]. The tissue concentrations of various toxic trace metals in wild mussel species from various regions worldwide are summarized in **Table 1**. The tissue concentrations ranged from low to high values depending upon the environmental status of the study area.

Country	Mussel Species	Ag	Al	As	Cd	Cr	Co	Cu	Fe	Hg	Ni	Pb	Zn	Ti	Se	V	Sr	Ba	Mn	Ref.
San Francisco Bay, USA	*Mytilus edulis* mg/kg dry wt				6.9	4.05							92		4.6					[97, 98]
Claisebrook Cove, Western Australia	*Xenostrobus sp.* mg/kg wet wt		12–61	0.46–0.75	0.21–0.27	0.05–0.17	0.06–0.16	1.7–2.2		<0.01	0.22–85	0.08–0.52	6–9.6		0.34–0.57				3.3–28	[99]
South Island New Zealand	*Perna canaliculatus* mg/kg dry wt			5.35–27.48	0.14–1.67			2.19–18.25			0.08–5.83	0.13–1.53	45.31–147.18							[100]
Offshore South China Sea	*Bathymodiolus platifrons* mg/kg dry wt	2.6–25.13	2.16–6.73	5.89–10.03	0.78–4.35	0.81–1.72	0.1–0.45	5.53–42.31	14.28–56.07		0.42–1.25	4.41–4.8	33.76–79.04				17.98–45.78	2.69–4.06	4.5–8.01	[101]
East coast of China	*Perna viridis* mg/kg dry wt	0.01–0.14		12.64–20.95	0.48–5.31	1.51–10.93		1.45–28.55	96.62–1002		1.3–4.78	0.44–2.93	66.05–231							[102]
East Adriatic Sea, Croatia	*Mytilus galloprov-incialis* mg/kg dry wt			4–30		1–2.9		3.7–11.1	53.4–719		0.8–5	2–7	59.1–273						2–13	[103]
Adriatic Sea (Montenegro coasts)	*Mytilus gallopro-vincialis* mg/kg dry wt							4.6–17.2	128–603				132–345						7.3–85.0	[104]
Tyrrhenian Sea (Gulf of Gaeta)	*Mytilus galloprov-incialis* mg/kg dry wt							5.5–11.5					123–180							[105]
Marmara Sea (NW coasts)	*Mytilus galloprovin-cialis* mg/kg dry wt							6.7–9.5	120–415				208–320						4.5–11.7	[106]

Country	Mussel Species	Ag	Al	As	Cd	Cr	Co	Cu	Fe	Hg	Ni	Pb	Zn	Ti	Se	V	Sr	Ba	Mn	Ref.
Aegean Sea	*Mytilus galloprovincialis mg/kg dry wt*							3.5–5.3	48.6–49.9				17.8–28.5						2.6–4.7	[107]
N Atlantic (Spanish Gallician coasts)	*Mytilus galloprovincialis mg/kg dry wt*							3.9–9.7					159–351							[108]
Island of Gossa (W coast of Norway)	*Mytilus galloprovincialis mg/kg dry wt*							1.3–1.8	11.0–11.7				13.3–15.2							[109]
Spain Cantabrian Coast	*Mytilus galloprovincialis* mg/kg *dry weight*			14.6–31.5	0.4–2.3	2.6–5.7	0.4–69.3	9.1–34.8			1.5–15.4	1.1–13.3	202.7–300.8		5.8–8.7	1.7–7.1			5.6–55.3	[110]
N Aegean Sea (Strait of Canakkale)	*Mytilus galloprovincialis mg/kg dry wt*							0.7–12.9	24.3–82.0				43.8–133.5						0.4–4.8	[111]
Trinidad	*Perna viridis* mg/kg *wet weight*				0.01–0.61	0.06–0.2		1.02–1.98		0.03–0.07	0.3–0.75		11.3–40.37							[112]
Venezuela	*Perna viridis* mg/kg *wet weight*				0.02–0.05	0.12–0.16		1.42–3.43		0.02–0.08	0.22–1.3		8.75–16.38							[112]
Italy Tyrrhenian coastal areas	*Mytilus galloprovincialis* mg/kg *dry weight*				0.33–0.49	0.46–1.31		5.51–11.5				1.67–2.49	123–180							[105]
Black Sea (Turkish coasts)	*Mytilus galloprovincialis mg/kg dry wt*							11.7–23.3					312–396						46.9–73.0	[113]

Country	Mussel Species	Ag	Al	As	Cd	Cr	Co	Cu	Fe	Hg	Ni	Pb	Zn	Ti	Se	V	Sr	Ba	Mn	Ref.
Turkey Eastern Aegean Sea	*Mytilus galloprovincialis* mg/kg *dry weight*				0.24–0.49	0.32–7.27		2.44–5.49		0.11–0.15		0.84–2.41	75.9–201							[114]
Italy Venice Lagoon	*Mytilus galloprovincialis* mg/kg *dry weight*				1.16–6.59	0.16–2.75		3.55–10.8				1.08–4.27	135–400							[115]
Brazil	*Mytella guyanensis* mg/kg *dry weight*		778–2458	1.44–23.1	Bdl–1.42	Bdl–3.13	Bdl–611	6.03–102	Bdl–1820	Bdl–0.35		Bdl–19.4	50.8–141		Bdl–49.6	Bdl–6.93	35.5–95.8	Bdl–88.7	30.7–3520	[116]
India	*Perna viridis* mg/kg *wet weight*				0.24–3.49	Bdl–0.46		Bdl–1.84	Bdl–235.6		Bdl–2.89	Bdl–1.95	Bdl–17.36						1.91–8.77	[15]

Table 1. Selected trace metal concentrations in the soft tissue of wild mussel species from various regions worldwide.

4.1. Mussel watch programs

Mussels and other marine bivalves are widely used as sentinel organisms in "mussel watch" programs for indicating levels of pollutants in the coastal marine environment due to their ability to bioaccumulate organic or toxic elements [40]. Under mussel watch program, environmental contaminants (trace metals, hydrocarbons, pesticides, etc.) accumulated in the soft tissue of natural, cultured, or deployed bivalves (clams, mussels, and oysters) collected from a set of defined geographical locations over a time-span of several years are systematically and repeatedly measured for assessing and comparing the coastal water quality [5, 40–42]. A prominent example is the US Mussel Watch Program originally started in 1976 [3, 43] and established as the Mussel Watch component of National Oceanic and Atmospheric Administration's (NOAA) National Status and Trends (NST) program during 1986–2012 [44, 45, 46]. In spite of the criticisms and limitations [47], the US mussel watch results made valuable contributions to our understanding of trace metal contamination and its biogeochemistry in coastal ecosystems [5].

Project phase and year	Study areas	Bivalve species	List of contaminants	References
IMW Phase I (Initial Implementation): 1991–1993	South America, Central America, Mexico and Caribbean	Blue mussels (*Mytilus* sp.) 134 stations Oysters (*Crassostrea* sp.)–18 stations Other bivalves–24 stations	Total Polychlorinated biphenyls (PCBs), total Chlordane (CHLs), and total HCHs	[5, 117]
IMW Phase II 1997–1999	Asia Pacific Region (Japan, South Korea, Russia, China, the Philippines, Vietnam, Malaysia, Cambodia, Thailand, Indonesia and India)	Blue mussel, (*M. edulis*), and the green mussel (*Perna viridis*).	Total PCBs, dichloro diphenyl trichloroethane and its metabolites (DDTs), CHLs, hexachlorocyclohexane isomers (HCHs) and hexachlorobenzene (HCB), polychlorinated dibenzo-p-dioxins and furans (PCDDs/Fs), coplanar PCBs (Co-PCBs), Butyltins (BTs) and some heavy metals	[38, 118–121]
IMW Pilot Study—Black Sea. 1996–1997	Six Black Sea Countries (Bulgaria, Georgia, Romania, Russia, Turkey and Ukraine).	Blue mussels (*M. galloprovincialis*)- 5–13 sites	PAHs, PCBs, DDTs	[122]
Western Mediterranean Basin and the International Mediterranean Commission (CISEM) Mussel Watch program. 2002–2006	The coasts of the Western Mediterranean Basin (Spain, France, Italy, North Tunisia, Algeria and Morocco)	Caged mussels (*Mytilus* sp.) deployed at 122 sites	Heavy metals, chlorinated pesticides and PCBs and PAHs	[123–125]

Table 2. Details of the International Mussel Watch (IMW) program conducted from various parts of the globe [5].

Later, the contaminant monitoring programs similar to mussel watch were implemented throughout the world either for monitoring long-term spatial and temporal pollution trends covering large marine region containing multiple monitoring stations and several anthropogenic contamination sources [36–38, 48–51] or for monitoring and solving local pollution problems covering a small geographical areas [7, 8, 15, 32, 52–58].

The mussel watch program initiated in USA has led to the formation of the International Mussel Watch (IMW) Projects [5]. It was initiated by the International Oceanographic Commission (IOC) in collaboration with the United Nations Environment Program (UNEP) and the US NOAA. **Table 2** summarizes the details of the international mussel watch program conducted from different geographical locations. Recently, the advantages and limitations of the mussel watch concept were discussed 40 years after its inception [5].

5. Biomarkers of exposure in bivalves

Chemical analyses of bivalve tissue samples measure the contaminants present but do not necessarily reveal potential biological effects on bivalves. Therefore, biomarkers were developed to assess the health status of the marine organisms, especially bivalves. Biomarkers are the early warning signals about the health status of bivalves exposed to toxic contaminants, because a toxic effect or response will be apparent at the molecular or cellular level before it is noticeable at higher biological levels. The concept of biomarker is borrowed from medical science, which describes a measurable indicator such as blood cholesterol profile connected to relevant clinical endpoints like atherosclerosis and heart attack. The biochemical biomarkers (acetylcholinesterase inhibition for exposure to neurotoxic compounds, cytochrome P450 for detoxification of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs), and the different methods to detect genotoxicity), which are used in marine environmental monitoring are still used in humans [59–61].

During the last decade, several biomarkers sensitive to contaminant exposure and/or impact have been developed as tools for use in marine environmental monitoring and impact assessment [7, 8, 62]. During the same time, various monitoring agencies began to focus on locating the source of contamination and fates as well as the impact as contaminants are usually discharged into the coastal waters, especially estuaries, where effects have been most significantly detected. The European Union's Water Framework Directive (WFD) also stressed the requirement of monitoring programs to assess the achievement of good chemical and ecological status for all water bodies by 2015 [63]. In the past 30–40 years, numerous biomarkers have been developed on bivalve mollusks, especially mussels (see **Table 3**) with the objective to apply them for environmental biomonitoring. Biomarkers based on responses at physiological level, cellular/tissue level, and molecular level of bivalve molluscs are developed and recommended as tools for studying the effects of contaminants on field and laboratory exposed bivalves, especially mussels [6, 64–66]. Research into the development and application of accurate biomarker-based monitoring tools for the environmental contaminants has been intensified in several developed countries, and they are using several biomarkers based in marine bivalves to monitor the environmental quality of coastal and estuarine waters [20].

Group	Biomarker name	Description	References
Bivalve Physiology	Body Condition Index (BCI)	Assessment of tissue weight in comparison with shell cavity volume or shell length	[7, 59, 126]
	Stress on stress response (SOS)	Assessment of survival rate during aerial exposure	[71]
	Scope for growth (SFG)	Measurement of physiological energy balance	[59, 76]
Metal-binding cysteine-rich proteins	Metallothioneins (MTs)	Measurement of metal binding proteins in tissue samples. Compensatory mechanism during exposure to heavy metals (Cd, Fe, Hg, Zn, As)	[28]
Cellular Responses	Lysosomal membrane stability (LMS); lipofuscin and neutral lipids accumulation	Assessment of the condition of lysosomes and the related cell injury	[7, 8, 61]
DNA integrity markers	Micronuclei	Assessment of toxic impact on chromosomes	[91, 92, 127]
	DNA adducts	DNA damage assessment	[91, 92, 128]
	Comet assay	Single cell DNA damage assessment	[91, 92, 128]

Table 3. List of biomarkers routinely used for monitoring the coastal waters quality using marine bivalves.

5.1. Physiological biomarkers

The biological indicators of health in bivalves such as Body Condition Index (BCI), stress on stress response (SOS), and scope for growth (SCF) have been recommended as broad markers of stress caused by either environmental changes or contaminants [59, 64, 67–70]. The stress on stress response is a simple test, which measures the mortality rate (time to kill 50% of the sample) of bivalves when exposed to air [70, 71]. The SOS test examines whether stress caused by environmental changes or contaminants have altered the capacity of bivalves to survive under adverse conditions such as aerial exposure. The body condition index (ratio between soft tissue dry/wet weights to its overall size) is a general indicator of favorable growth conditions as well as the overall biological status. The body condition index is routinely used in aquaculture and environmental monitoring studies to assess the health condition of mussels [7, 25, 72].

The growth, reproduction, and survival of bivalves depend on the availability of sufficient energy reserve in their body. Exposure to contaminants negatively affects the energy balance of bivalves due to the high-energy demand for maintaining homeostasis at the expense of growth, storage, defense, and reproduction [73]. Fitness of an individual organism can be measured in terms of Scope for Growth (SfG), which is the measurement of physiological energy balance and it ranges from optimal (positive values) to stressed conditions (negative values) when the organism is exposed to contaminants or unfavorable environmental conditions [74, 75]. The SFG has been widely used in field monitoring studies [76, 77]. The SFG and the growth rates of mussels were drastically reduced when mussels from uncontaminated sites were transplanted along known pollution gradients or placed in the most contaminated areas [78, 79].

5.2. Cellular biomarkers

The digestive gland cells in bivalves play a key role in digestive and absorptive processes and also in the detoxification and excretion of contaminants [80]. The lysosomal system in the digestive cells was identified as the main target site for the toxic effects of most of the environmental contaminants including trace metals [81]. Lysosomal responses to cell injury due to contaminant exposure or stress caused by environmental changes fall into three categories: (1) changes in lysosomal contents, (2) changes in fusion events, and (3) changes in membrane permeability [81].

Changes in lysosomal membrane permeability of bivalves can be measured using the lysosomal membrane stability (LMS) test [82–84]. The LMS test can be conducted by using two different methodologies: (i) a cytochemical method using cryostat sections of digestive gland tissue and (ii) an in vivo cytochemical method using hemolymph cells. Biomarkers such as LMS, accumulation of lipofuscin and neutral lipids in bivalves were successfully used for coastal pollution monitoring studies [7, 8, 69, 70, 82–84]. Subsequently, different regional conventions have recommended the use of LMS as a general stress biomarker of chemical pollution within the framework of the pollution biomonitoring programs [67, 68, 85]. The proposed integrated assessment approach of contaminants and their effects in the NE Atlantic Baltic Sea Action Plan and in the Mediterranean Ecosystem Approach (EcAp) have included the LMS in mussels as one of the core biomarkers [86–88].

It has been demonstrated that metallothioneins (inducible low molecular, sulfhydryl proteins) levels in the digestive cells of bivalves will be induced after exposure to trace metals such as Cd, Cu, and Zn [89]. The induction of metallothioneins (MT) in bivalves has been proposed as biomarkers of trace metal stress, and it has been recommended to use in coastal pollution monitoring studies [67, 68, 85, 90].

5.3. Biomarkers of genotoxicity

A wide variety of chemical contaminants capable of directly or indirectly damaging the DNA of marine organisms are being discharged into the marine environment. These genotoxic chemicals are capable of inducing some changes in the molecular and cellular levels of marine bivalves [91, 92]. Two well-known tests, micronucleus assay and comet assay, are being widely used to assess the genotoxic effects of environmental contaminants on marine bivalves [91, 92]. The micronucleus assay is used to detect the structural and numerical chromosomal changes while the comet assay (single-cell gel electrophoresis) is used to detect DNA strand breaks in marine bivalves.

6. Coastal pollution monitoring using biomarkers a case study

The biomarkers in marine bivalves based on sub-lethal effects of contaminants are ecologically relevant and can be used to give subtle signals of response to contaminants before damage becomes irreversible. The water quality in European coastal sites was classified ranging from class 1 (clean areas) to class 5 (highly polluted areas), based on global biomarker index for

Baltic mussels [93]. The Marine Strategy Framework Directive (Directive 2008/56/EC) since 2008 emphasized on the importance of assessing key biological responses for evaluating the health of organisms and linking the observed changes to potential contaminant effects [94].

The studies conducted prior to 1990s from Puget Sound, Washington, reported high concentrations of toxic metals, polycyclic aromatic hydrocarbons (PAHs) and PCBs in sediments and toxicant-induced, adverse effects in benthic fish samples collected from the urban associated sites [95]. As an example of how biomarker-based indices can be integrated into environmental monitoring of Puget Sound, biomonitoring study using mussels was conducted in 1992 [7]. Blue mussels (*Mytilus edulis*) were collected from their natural beds from nine sites in Puget Sound (**Figure 2**). Sites included the minimally contaminated reference areas of Oak Bay, Coupeville, and Double Bluff, in central and north Puget Sound, and Saltwater Park of south Puget Sound. Urban sites that were sampled for mussels included Eagle Harbor, Seacrest and Four Mile Rock in Elliott Bay, City Waterway in Commencement Bay, and Sinclair Inlet.

Relatively high tissue concentrations of contaminants including toxic trace metals were observed in mussels tissue samples from the urban-associated sites compared to the minimally

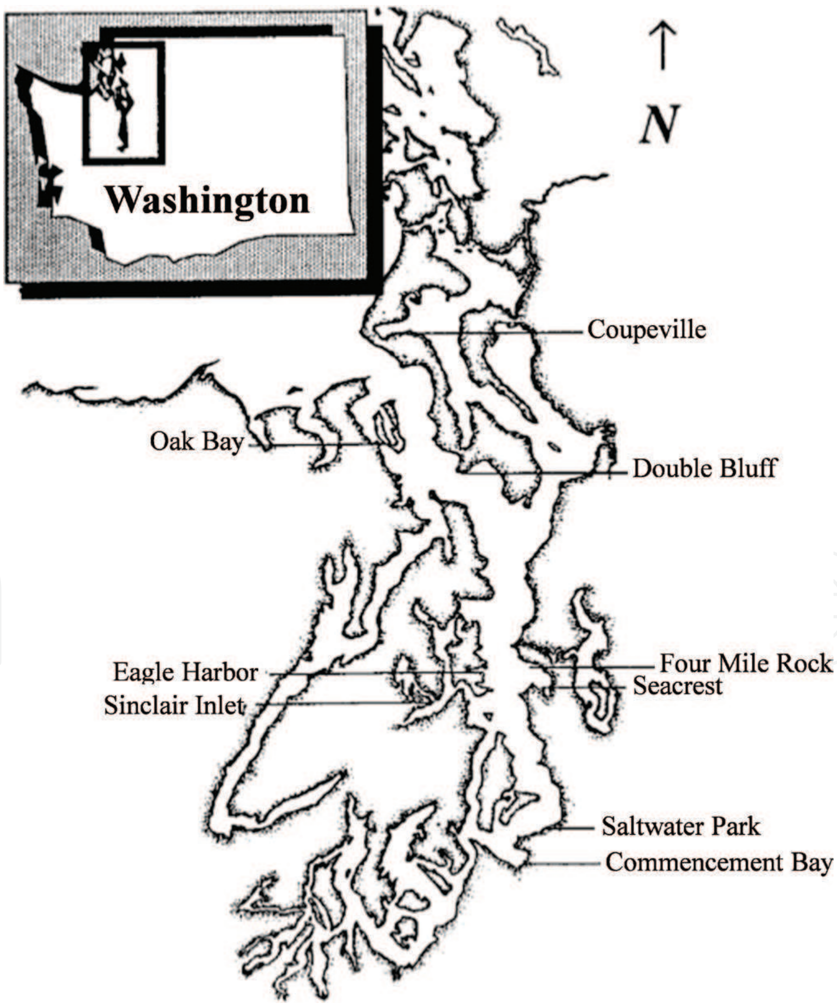

Figure 2. Map showing the mussel sampling sites in Puget Sound, Washington [7].

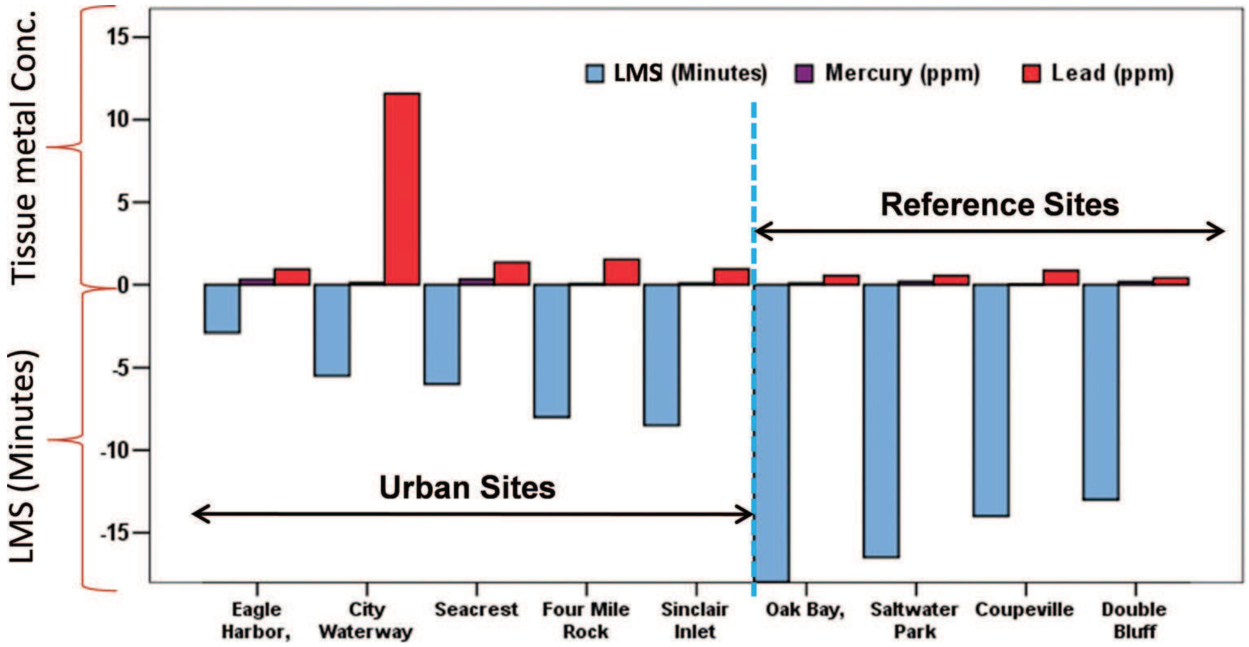

Figure 3. Relationship between lysosomal membrane stability (LMS) and tissue concentration of heavy metals (mercury and lead) of mussels from urban-associated and reference sites in Puget Sound [7].

contaminated (reference) sites (**Figure 3**). Mussels from contaminated sites showed low LMS, enhanced lipofuscin deposition, and increased accumulation of lysosomal and cytoplasmic unsaturated neutral lipids (**Figure 3**). Mussels from the contaminated sites were smaller in size together with lower somatic tissue weight relative to shell length [7]. Highly significant correlations were observed between tissue concentrations of selected toxic elements (measures of anthropogenic exposure) and LMS [7]. The study showed that biomarkers in mussels have the potential to be used as sensitive, accurate, and rapid techniques for assessing the biological impact of environmental contaminants in the coastal waters. The study results were in agreement with the previous study results, which showed an association between metabolites of aromatic compounds in bile and the occurrence of hepatic lesions in English sole (*Parophrys vetulus*) from Puget Sound [96].

7. Conclusion

Commercially and ecologically important marine bivalves (clams, mussels, and oysters) are widely used for monitoring levels of trace metals in the marine environment from several parts of the world. Trace metal monitoring using bivalves has several advantages compared to using seawater or sediment samples for the same purpose. Bivalves such as mussels are having global distribution from the polar to the tropical region and being successfully used for temporal and spatial trend monitoring of trace metals in the coastal waters across the globe. Recently several biomarkers, the biological responses of bivalves to contaminants including trace metals, are being developed and tested to assess the coastal water quality. The biomarkers of stress in bivalves give early warning signal about the presence of toxic trace metals in the marine environment.

Acknowledgements

We thank the Center for Environment and Water, Research Institute, King Fahd University of Petroleum and Minerals, Dhahran, Saudi Arabia, for providing research facilities. We acknowledge the research funding (# T.K. 11-0629) of the King Abdulaziz City for Science and Technology (KACST). We also thank our colleagues and students who helped us to prepare this manuscript.

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