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Brachyuran Crabs as a Biomonitoring tool: A Conceptual Framework for Chemical Pollution Assessment

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Abstract

Marine ecosystem is very complex in terms of biological and ecological structure and marine organisms are integral part of this structure. In the recent era marine ecosystem has become largest dumping site for effluents of industries and it is creating serious problems at ecosystem level. So the understanding of different direct and indirect sources of pollution and its effect on marine organism population and ecosystem have got lot of ecological importance for conservation. Brachyuran crabs are highly diverse organisms comprising 700 genera and 5000 species. Crabs play important role in the maintenance of ecosystem and also in the marine food web demonstrating prey- predator relationship. The present study provides a protocol or framework to find out the effect of pollution on marine ecosystem using brachyuran crab as a bio indicator species. The present framework was divided into two different aspects viz ecological assessment and toxicological assessment and different methods have been suggested to assess the effect of pollution on brachyuran crabs for both aspects. To check the effectiveness of the framework, pilot study has been done and the results revealed that pollutants do affect different levels of ecosystem and the effect can be traced using brachyuran crab as a bioindicator species.

Key words: Brachyuran crabs, pollution, ecotoxicology conceptual framework, bio indicator, Gujarat coast.

Introduction

The marine pollution is mainly contributed by industrial, domestic and agricultural wastes[1]. Effluents, particularly from the industries containing hazardous heavy metals and organic toxicants have posed threat to the marine life. Several heavy metals accumulate within the tissues of the crustaceans at a concentration higher than that is present in the water column and sediment[2], and such biomagnification results into environmental toxicity. Thus for conserving the inhabiting marine species and to prevent the marine environment from further devastation, certain authentic and sensitive monitoring methodologies must be constructed[3].

There are certain monitoring systems that include the chemical analysis of the abiotic factors such as sediment and water which prove to be accurate enough; but these may not provide the actual ecological state for many reasons[4]. One, the number and range of the pollutant may exceed the capability of the available chemical test. Second, if the pollutants act synergistically, it may become difficult to measure them directly. And finally, the natural availability of the suspected factors that also contribute to the environmental damage cannot be confirmed by chemical analysis. These problems are overcome by bioindicator systems where the physical and physiological responses of organisms that we select are used for monitoring the environment[3]. Bioindicators are the organisms or group of organisms whose interactions are observed to predict the situation of particular system. The bioindicator species for their growth and survival require specific kind of set of environmental variables and changes in such kind of variables, directly affect the number, reproduction and behavior of the species, which implies that changes in the variables are not suitable for the species[5].

The organism to be used as bioindicator has to fulfill many criteria that include ease in sampling, temporal and spatial abundance of the organism and the range in which the biological responses can be detected [4]. There are many species of bivalves and gastropod molluscs that are used as bioindicators[6,7]. There are many crustacean species also that are used as bioindicators to test the toxicological impacts. The parameters taken into account include percent survival, growth rate of populations, number of adults reaching reproductive age, number of larvae released by females and numbers of progeny surviving.

Amongst different crustacean invertebrates, crabs are highly diverse group comprising 5000 species and 700 genera. The species distributed on diverse habitat play important role in the maintenance of the ecosystem. So any problematic variable which affects the crabs can have major effect on the habitat and ecosystem[8-11]. Studies have been done to assess the health of the particular ecosystem affected by different kinds of pollutions using brachyuran crabs as an indicator species[12-17].

In Gujarat, various mega industries have been set up in the Gulfs of Kutch and Khambhat as well as along the Saurashtra coast[18] and there is an immense anthropogenic pressure that affects the marine life. The Common Effluent Treatment Plant (CETP) discharges the treated polluted effluent directly into the sea waters. The CETPs deal about 2000 SSI units in Maharashtra and 2500 SSI units in Gujarat states respectively. The effluent released contains high amount of heavy metals and organics. Heavy metal from the effluent eventually biomagnifies in the estuarine system and gets deposited in sediments and benthic animal tissues. Recently attempts have been made to investigate and develop pollution bioindicator systems[19]. We carried out some basic ecotoxicological studies in gulf of Khambhat and part of coastal Saurashtra, particularly analyzing the abiotic components. During these studies we realized that macro faunal bioindicators are yet to be explored and studied in Gujarat. In present paper we discuss the basic frame work essential for such faunal indicator studies. Since we have carried out pilot studies on brachyuran crabs, the flagship species of estuarine and inter tidal areas that manipulate and modify the abiotic and biotic status of these communities, in present paper we project brachyuran crabs as prospective bioindicators of coastal pollution.

The Concept: The benthic invertebrates show peculiar habitat and substratum preference and thus are indicator of sediment quality. Similarly, water quality also regulates the diversity, abundance and life processes of invertebrates. Macro invertebrates are keystone species in the benthic environment because they play critical role in different functions of the community. Therefore, the diversity, distribution, density, behavior, reproduction, larval settlement and population dynamics of macro invertebrates of the benthic zone can denote whether or not the community is stressed[20].

Why use crabs?

Crabs are the vital component of the marine food chain. Most of the crabs feed on either detritus or the organic matter thus controlling the ecological functioning. In inter tidal area, these organisms work as a super creatures adapting to the harshness of the environment. On the coastal intertidal areas, they are acted upon by variety of environmental factors like desiccation stress, temperature, predation and change in salinity etc. Moreover, the daily tidal fluctuations imply flux in water quality and sediment depositions. These are probable causes of broad scale community pattern variations spatially and temporally. The sediment deposition, grain composition and variations on a diurnal, seasonal and annual basis define the micro habitat conditions[21]. On inter tidal area, these variations are noted vertically, horizontally and laterally. Tidal fluctuation and changes in water quality influence diversity and distribution of pelagic larval stages of benthic forms[22]. Tidal variations induce site selection and abundance of benthic forms along inter tidal area[23].

Crabs play important role in the maintenance, modification and regulation of the benthic environment by influencing both the abiotic and biotic components. They are abundant and serve both as the predator and the prey and hence are located at different trophic levels in the ecosystem[9]. The density of the crabs in mangrove ecosystems can go up to a level of 80-90 crabs per sq. m[4]. Many species of crabs are burrowing in nature and with their burrowing activity they frequently alter the surface characteristics and drive the nutrient cycling[25]. In mangrove ecosystem, burrowing activity of crabs increase the porosity of the soil which on the other hand increase the regeneration of mangrove seedlings[24]. The feeding activity and pellet formation by different species of feedler crabs also changes the substratum characteristics and content of organic matter. The bioturbatory processes by crabs on the intertidal area results into variety of biogenic structures and markings. These structures and processes control variety of processes which are important for health of an ecosystem. Even a routine activity of burrowing facilitates series of chemical and physical alterations in the original sediment quality. The facilitation of oxygen to the subsurface depth allows microbial diversity and sediment oxic-anoxic zoning. The feeding activity by scraping the upper organic rich layer of the sediments regulates the organic content and the algal covering and on the contrary byproducts from gut enriches the sediments with few minerals. The multifaceted role of crab in the ecosystem is presented in figure 1.

How do crabs fit as bio indicator?

Wide range of studies is available on macro invertebrates as an indicator species of aquatic habitat but amongst them specifically, brachyuran crabs are an effective indicator of different changes in both abiotic and biotic factors. Studies have been done with references to changes in different parameters and its effect on crab. Shirley et al.[26] studied that species of oyster reef crabs live in strict range of salinity and any change in salinity due to presence of any kind of pollutant directly affect the population of the crabs. Pandya[25] has also studied the population distribution of brachyuran crab *Uca lactea annulipes* in Mahi estuary, the results of the study revealed that population density of the particular species was very less at polluted site as compare to the non polluted site. The morphology and physiology of the species also shows the direct effect of pollution. Bergey and Weis[14] studied the morphology of fiddler crab *Uca pugnax* and stated that body size of male and female were significantly greater in sites which were polluted as compared to the sites that were non polluted and on the other hand the population of crab in polluted area was low when compared with the non polluted. The burrowing crabs are mostly filter feeder and they feed on the substratum available. Beltrame et al.[17] have used the brachyuran crab *Neohelice granulate* as an indicator species to study the presence of any heavy metals in estuaries of Atlantic coast of Arghentina. Al-Mohanna and Subrahmanyam[27] used *Portunus pelagicus* as pollution indicator from the Gulf of Kuwait wherein an associated problem of heavy metals along with the oil spill of Gulf War was found. Turoczy et al.[28] have given reports of the crab *Pseudocarcinus gigas* showing higher cadmium concentrations from Australia. Results of all these studies suggest that brachyuran crabs have potential to be used as a bioindicator species.

Brachyuran crabs have number of advantages as compared to other macro benthic species to be used as a bioindicators. i. Crabs prefer specific kind of substratum to live and if the substratum is polluted by any kind of pollution then it will have effect on crab population so by studying the crab population one can make out the health of the substratum, e.g. rock crabs[29]. ii. The burrowing crabs are mostly filter feeder or detrivore while crabs of rocky shores are mostly herbivore or carnivore. If the pollutant is present in the substratum, plant and animal body which is food item then it may have effect on feeding behavior of crab. iii. Crabs not only show evidence of environmental features such as salinity and sediment types and content, but they also respond to anthropogenic factors, making them key bioindicators of human impact on benthic communities[15]. iv. Crab also shows the effect of heavy metal toxicity on the reproductive cycle and different kinds of behavior[17].

Proposed Studies for Pollution Assessment in Bioindicator Species

The marine organisms when exposed to the toxicants for a longer duration of time accumulate the toxicants in their tissues. The process of metal accumulation in the tissues and organs is species-dependent and hence we find different organisms in the same environment having different concentrations of metals in them[30,31,32]. Thus there is a need to determine the toxicant concentration in the animal tissues, in this case, crabs. There are different methodologies that are carried out experimentally for the determination of such toxicants (figure 2).

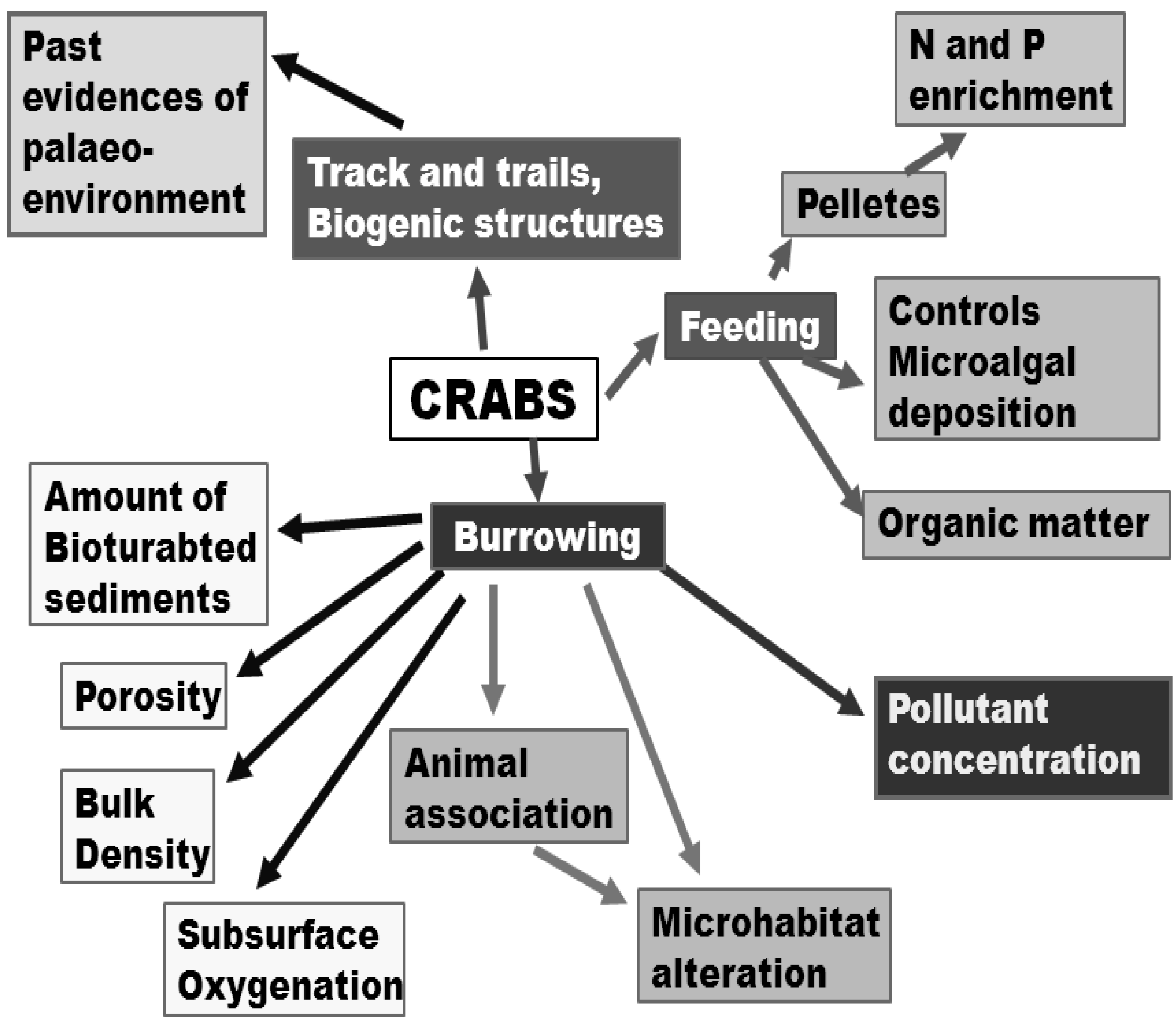

Figure-1
Role of brachyuran crabs in the ecosystem

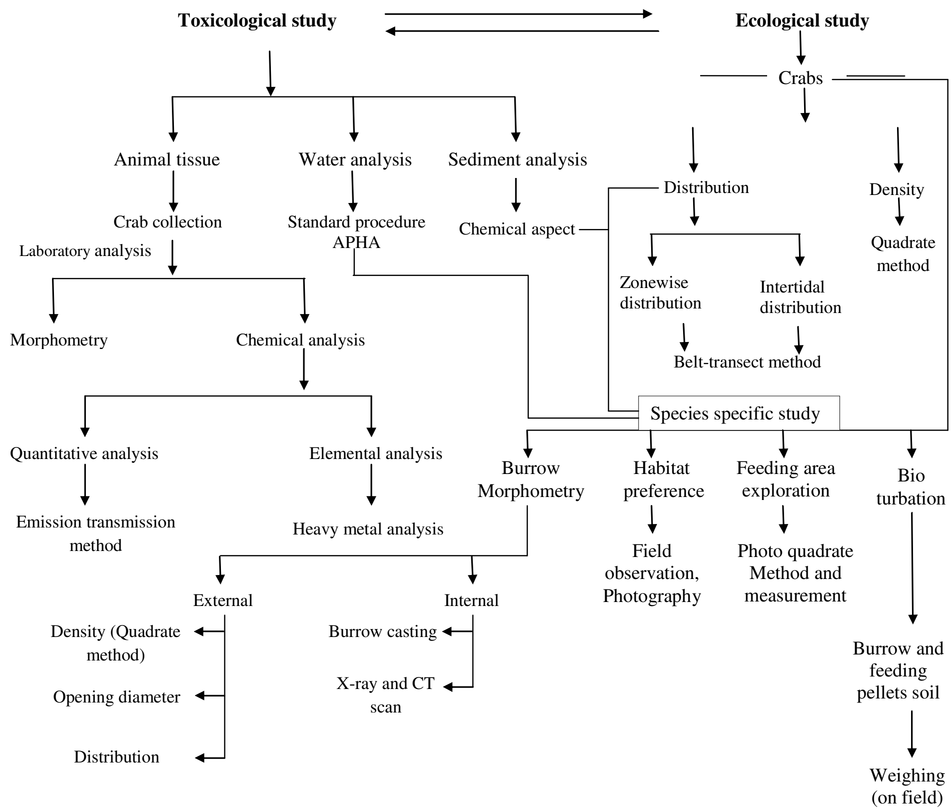

Figure-2
Framework of the proposed ecotoxicological studies using crab as pollution indicator

Collection of the specimen and taxonomic identification: The crab specimen be collected from the study area by hand, rinsed to remove sediment particles, placed in specimen jars, labeled and kept in ice for transportation[33,34]. Identification and further analysis be done in the laboratory. Specimen having similar size belonging to each species be categorized for analyzing metals within the tissues[35]. Morphometric analysis including weighing, measurements of carapace size etc. and length of the chelipede (using vernier calipers) should be done[34]. The crab samples should be dried at 105-110°C in an oven[33-36]. The percentage dry matter of the samples can be calculated by using the formula[33]:

Percent dry matter = (W3-W1) / (W2-W1) X 100

Assessment of the Water Quality

Water quality is an important factor that is responsible for the toxicant accumulation in the marine organisms. Water samples should be collected using Nikson bottle from the surface. A two liter capacity bottle can be used for sampling and for physical and chemical analysis. The heavy metals in the samples be analyzed by the methods described below for toxicant analysis in crabs.

Assessment of the Sediment Quality

Sediment samples should be collected by using a polyethylene corer, by following standardized methodologies. Samples can be digested in the microwave vessels, acid cleaned; with a standardized mixture of hydrofluoric acid and nitric acid[37]. The heavy metals in the samples can be analyzed by the methods described below for toxicant analysis in crabs.

Assessment of Toxicants in Crabs

Microwave acid digestion of dried samples should be done by using a standardized chemical combination of concentrated nitric acid and hydrogen peroxide[33,36,38,39]. There are various analytical methods that can be applied for determination of concentration: i. The heavy metal content of the crabs collected can be determined by using Energy Dispersive X-Ray Fluorescence (EDXRT) Technique[34]. ii. For most heavy metals; analysis can be done by using Inductively Coupled Plasma – Mass Spectrometer (ICP-MS) instrument[33]. iii. Particularly for Fe, techniques including Inductively Coupled Plasma – Emission Spectrometer (ICP-ES) can be used[33]. iv. An Open tube block digestion method for determining Arsenic can be used. This includes digestion of the dried specimen sample in a concentrated mixture of nitric acid and perchloric acid. Analysis can be done by using VG/ICP-MS; where VG is Vapour Generation[33]. v. In wet digestion method, to determine the heavy metals concentrations: Cd, Cu, Zn, Pb; analysis can be done by using an atomic absorption spectrophotometer GBC-AVANTA[36,40]. vi. For Hg metal, analysis can be done by Atomic absorption spectrophotometer (Perkin Elmer)[39].

Quantitative analysis: Quantitative analysis of the pollutants in the samples should be done by using the Emission Transmission (E-T) method, which is already having a number of quantification methods, previously applied[41-46].

Studies of Physiological Indicators in Crabs: The physiological indicators include metabolites and enzymes associated with important functions like energy production. The metals and other pollutants are reported to induce oxidative stress as one of the mechanism of action. We propose to study Succinate dehydrogenase[47], acid phosphatase[48] cholesterol[49], superoxide dismutase[50], glutathione peroxidase[51], glutathione-s-transferase[52], oxidative degradation of lipid[53], reduce glutathione[54], ascorbic acid[55] and steroidogenic parameters in various tissues of crabs.

Studies of Behavioral Indicators in Crabs: The activities of crabs exhibit distinct behavioral processes. The burrowing, bioturbatory, feeding, territoriality, inter and intra specific interactions, mating displays and reproduction[8,9], larval settlement and juvenile establishment etc activities and processes are integral life processes of crabs and also significant behavioral responses to the pollution. These activities will be observed in polluted and reference non polluted regions and compared for selected crab species which will serve as indicators. The burrowing and bioturbatory activities should be studied through C T Scan X-ray studies of cores and resin burrow casts. All other activities will be studied in situ by visual observations and video recordings.

Studies carried out so far: Studies were carried out in the polluted region of Gulf of Khambhat, as well as part of Saurashtra coast along Junagadh district, Gujarat where the crab species were considered as bioindicators.

Study area: Gujarat, the leading industrialized state, has longest coast line among Indian states and extensive industrialization is further proposed. The Gulf of Khambhat is very specific in studying sedimentology as well as water quality because of its geomorphology and hydrodynamics. During low tide vast area remain open for extended period. A channel brings the industrial effluent in this region from the industrial area of Vadodara. The general and ecotoxicological hazards of such industrial effluent and its components have been studied in our laboratory[56-60]. The habitat characterization and distribution of brachyuran crab have been studied in the Gulf of Khambhat estuaries that demonstrate differences in crab distribution and diversity in the polluted and non polluted regions[61-63]. Saurashtra region is located in the south west part of the state and has been attractive location for industrial and associated infrastructure development. The coastal zone has a cliffed shoreline and a flat coastal plain dotted with milioite limestone. The Saurashtra intertidal region is primarily rocky in nature having upper margins of the sandy shore. This intertidal area is narrow in width, has lesser slope gradient, high tidal currents and very rich in marine biodiversity[64,67]. The present study area is selected on the basis of presence of different kinds of industries, pollution level, habitat type and marine fauna. Our recent studies demonstrated occurrence of several brachyuran and anomuran crab species in the widely diverse habitats of Gulf of Kachchh and on Saurashtra coast[65-68].

Veraval is amongst the largest fish landing sites of India. Veraval is surrounded by different kind of small and large industries like Rayon industry and fish processing units. The place is also known for boat manufacturing and the coastal area receives different kinds of waste from these units. Sutrapada is a small village which is situated 30 km from Veraval. The place harbors a large soda ash manufacture unit and the coastal water receives wide variety of chemical pollutants. Kodinar is situated 45 km south from Vearval and has largest cement manufacture unit in the state while Alang is the world's biggest ship breaking yard. Till now, around 70% of ships are sent to the Alang-Sosiya ship recycling yard[69]. During the breaking or dismantling of the ship the coastal water receives different kinds of wastes including all solids, liquids and gaseous wastes.

The study areas were surveyed during 2010 to 2012 for habitat categorization and crab sampling. The samples were collected for water and sediment analysis and several specific studies like sediment composition, quantitative faunal studies, burrow casting, radiological studies, behavioural studies etc. Water, sediment, crabs and plant samples were collected from different stations along the Mahi estuary from Gulf of Khambhat. Sediment samples were taken up to 15 inch depth. Samples were analyzed for their heavy metal content using Atomic Absorption Spectrophotometer (tables- 1, 2, 3).

Table-1
Pollutant analysis in water samples from Mahi estuary

Stations	C. O. D. mg/l	B. O. D. mg/l	Oil & Grease mg/l	Cd	Co	Cu	Cr	Ni	Pb
Sarod-1	780	230	38	18	7	6	10	16	21
Sarod-2	436	107	26	5	1	3	1	3	6
Kamboi-1	189	86	24	0	0	1	2	4	3
Kamboi-2	123	45	18	3	0	3	0	2	3

Table-2
Pollutant analysis in sediment samples from Mahi estuary

Station	**C. O. D.**	**B. O. D.**	**Oil and Grease**	**Cd**	**Co**	**Cu**	**Cr**	**Ni**	**Pb**
	mg/g	**mg/g**	**mg/g**	**µg/g**	**µg/g**	**µg/g**	**µg/g**	**µg/g**	**µg/g**
Sarod-1	0.36	1	0.02	50	50	18	10	12	80
Sarod-2	0.11	0.5	0.06	11	0	20	9	7	50
Kamboi-1	0.02	0.4	0.08	0	0	15	8	20	15
Kamboi-2	0.01	0.5	0.03	0	0	10	7	6	30

Table-3
Heavy metal analysis in crab, plant, effluent and sediment samples from Mahi estuary

Sample	**Zn**	**Fe**	**Co**	**Cr**	**Ni**	**Pb**	**Cd**
	ppm	**ppm**	**ppm**	**ppm**	**ppm**	**ppm**	**ppm**
Sample-1 crab tissue	0.459	34.421	0.023	1.088	0	0.017	0
Sample-2 crab tissue	0.39	13.489	0.02	4.324	0	0.032	0.134
Sample-3 vegetation	0.317	123.895	0.044	7.919	0.012	0.01	0.015
sample-4 effluent	0.036	0	0.067	0	0	0.026	0
Sample-5 sediment	1.419	882.597	0.316	0.278	4.726	0	11.92

Note: Values for all heavy metals are in ppm, "0" indicates Values below detection limit

Conclusion

The results revealed that the heavy metals which are present in the effluent of industries are accumulated in the ecosystem at different levels like water, sediments, vegetation and animal tissue. The results suggest that crabs are prone to heavy metal accumulation. The sources of the heavy metal accumulation in crabs are water and sediment. The heavy metal accumulation and other pollution ingredients the marine environment do affect the ecology, physiology and behavior of marine organisms and crab could be used as an indicator species to reveal the effect of pollution on biota. The reasons for use of crab as an bio indicator species are: i. Crabs are the most important organisms to be used as bio indicators because of their huge diversity and density, ii. Crabs play important role in maintenance of different marine ecosystems so any factor affecting their health will cause damage to whole ecosystem, iii. Crabs as a prey and predators play important role in marine food web so tracing of pollutant in food chain will be very easy using crabs. iv. Crabs also show peculiar preference to micro habitats. So health of different habitats can be predicted using crabs, v. The toxicological assessment at different levels of biological organization with special reference to crabs strongly increases the sensitivity of pollution assessment in the concerned area.

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