Chemosphere 199 (2018) 340–350

Contents lists available at ScienceDirect

Chemosphere

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

# Effect of selenium on *Penaeus monodon* and *Perna viridis:* Enzyme activities and histopathological responses

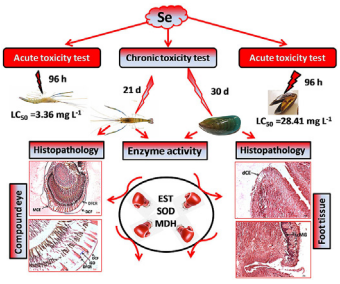
Avula Nagarjuna, Panneerselvam Karthikeyan, Dhandapani Mohan**, Shambanagouda Rudragouda Marigoudar*

*Integrated Coastal and Marine Area Management, Government of India, Ministry of Earth Sciences, NIOT Campus, Chennai, Tamil Nadu, India*

## HIGHLIGHTS

- Study reports acute and chronic toxicity of selenium on *Penaeus monodon* and *Perna viridis*.
- Selenium affects key antioxidant enzymes (EST, SOD and MDH) and induces oxidative stress.
- Histopathology evidenced that, selenium affects byssus thread formation in green mussels and vision of shrimp.

## GRAPHICAL ABSTRACT




## ARTICLE INFO

*Article history:*
Received 16 November 2017
Received in revised form
1 February 2018
Accepted 9 February 2018
Available online 10 February 2018

Handling Editor: Jim Lazorchak

*Keywords:*
Penaeus monodon
Perna viridis
Bioassay
Enzyme activity
Histopathology
Selenium

## ABSTRACT

The study was carried out to evaluate enzyme activities and histopathological changes due to the effect of acute and chronic definitive toxicity of selenium (Se) on the post larvae (PL) of giant tiger shrimp (*Penaeus monodon*), and green mussel (*Perna viridis*). The 96-h Median Lethal concentration ($LC_{50}$) for the PL of shrimp was 3.36 mg $L^{-1}$ and the chronic value for the long-term survival endpoint in a 21-d exposure was 0.10 mg $L^{-1}$. The green mussel 96-h $LC_{50}$ was 28.41 mg $L^{-1}$ and the chronic value for the long-term survival endpoint in a 30-d exposure was 3.06 mg $L^{-1}$. Native polyacrylamide gel electrophoresis revealed altered diverse isoforms of esterase, superoxide dismutase and malate dehydrogenase activities in the PL of shrimp and green mussel exposed to sublethal concentration of Se. Cellular anomalies such as deformation and fusion of corneal cells, detachment of corneal cells from cornea facet and increased space between ommatidia were observed in the compound eye of PL of shrimp exposed to Se for 21-d. Shrinkage and clumping of mucous gland, degenerative changes in phenol gland, and ciliated epithelium were observed in the foot of green mussel exposed to Se for 30-d. This study shows that cellular anomalies in the compound eye of PL of *P. monodon* and foot tissues of *P. viridis* described would affect the vision of shrimp and byssus thread formation in green mussel.



## 1. Introduction

Coastal waters are under risk from pollutants due to the increasing anthropogenic activities leaving behind their signatures in water and biota. Pollution from the metals are of greater concern owing to their persistence and biomagnification. Selenium (Se) is

* Corresponding author.
** Corresponding author.
*E-mail addresses:* mohan@icmam.gov.in (D. Mohan), srmarigoudar@icmam.gov.in (S. Rudragouda Marigoudar).

https://doi.org/10.1016/j.chemosphere.2018.02.068
0045-6535/

geogenic indispensable micronutrient involved in cellular functions of all living organisms and present in foodstuffs such as cereals, meat and fish (WHO, 2017; Novoselov et al., 2002; Rayman, 2012; Mangiapane et al., 2014; Schiavon et al., 2017). Se concentration in the surface seawater of Bay of Bengal has been reported between 0.11 and 0.18 μg $L^{-1}$ (Srichandan et al., 2016). Presence of elevated Se concentrations in the coastal waters has been related to industrial activities such as metal mining, coal combustion, oil refining, and agriculture besides release of untreated sewage effluents. Se concentrations are also reported in ground water (49–341 μg $L^{-1}$) and soil (2.3–11.6 mg $kg^{-1}$) (Bajaj et al., 2011; Selvam et al., 2017). Some studies in the industrial and coastal cities of India reported that, the Se concentrations have exceeded the maximum permissible limits of drinking water (WHO, 2017; Selvam et al., 2017; Ramesh et al., 1995; Bajaj et al., 2011).

Se can be toxic when present at high levels in the environment and is referred as double-edged sword (Bajaj et al., 2011). Se can bioaccumulate in aquatic organisms resulting in adverse effects when it exceeds threshold levels (Rigby et al., 2010). Various studies reported its effects on viability of eggs, mortality in hatchlings, morphological deformities, and pathological changes in various tissues of fishes (Hamilton, 2003, 2004; Lemly, 2002). Considering the toxicity, adverse effects and bioavailability of Se in the environment, United States Environmental Protection Agency (USEPA) has prescribed 3.1 μg $L^{-1}$ as chronic water quality criteria for the protection of aquatic organisms in lotic water for 30-d (USEPA, 2016) and 71 mg $L^{-1}$ (continuous exposure) for saltwater (USEPA, 1987). It is also pertinent to note that, such regulatory criteria are not prescribed for the protection of coastal and marine organisms in India. Generally, safety criteria values are based on acute and chronic toxicity values.

Biomarkers can be characterized as functional measures of exposure to stressors which are usually expressed at the biochemical, cellular, or tissue level (Tu et al., 2010). Oxidative stress occurs when reactive oxygen species (ROS) overwhelm the cellular defences and damage proteins, cell membranes, and DNA (Kelly et al., 1998). ROS are the by-products of electron transport chains, enzymes and redox cycling (Kelly et al., 1998) and their production may be enhanced by xenobiotics (Winston and Di Giulio, 1991; Slaninova et al., 2009). The first effects of contaminants usually occur at the cellular or subcellular level and they can be good indicators of pollutant toxicity (Pickering, 1981; Stephan and Mount, 1973; Overstreet, 1988). Enzyme-inhibition biomarkers are a good choice because their effects altering entire metabolic pathways can be related to reductions in growth and reproduction in whole populations (Blackstock, 1984). Biochemical changes including the enzyme responses can be captured by means of histology. Histology is an important technique used for assessing the effects of pollutants in vital processes because it identifies early changes in cellular level. Histological biomarkers are sensitive and responsive to environmentally realistic concentrations and preferably exhibit a dose response relationship to levels of pollution (Au, 2004). It is pertinent to note that, effects of Se on activities of enzymes and histopathological studies are scare. In view of this present study was undertaken to study the toxicity of Se on sensitive native marine organisms such as post larvae of *Peneaus monodon* and *Perna viridis* after exposure for 21-d and 30-d respectively. Since, these toxicity values would be useful for the formulation of safety criteria in the region or elsewhere in general. Particularly, *P. monodon* and *P. viridis* are native and share the common marine water ecosystem, even though their habitat are distinct as they inhabit in mud or sand bottom and shallow rocky littoral or sublittoral, respectively. They are important components of marine food chain, sensitive to change in the water quality, amenable to laboratory conditions, and are commercially important. Hence, in the present study these species were considered for the evaluation of toxicity and effect of Se. Tissues such as compound eye of shrimp and foot of green mussel were selected for histopathological studies based on key functions i.e., vision and formation of byssus thread respectively. The present study would be useful for environmental monitoring assessment and also provide data for the development of water quality criteria for environmental protection.

## 2. Materials and methods

### 2.1. Collection and maintenance of experimental organisms

The post larvae of *P. monodon* (PL 11-14d) (Crustacean) were obtained from a commercial prawn hatchery at Anumanthai village (Lat 12.065305; Long 79.883620) located near Marakanam, Kancheepuram district, Tamil Nadu, India. The green mussels, *P. viridis* (30–35 mm length) (Bivalve) were collected from the groins/tetrapods piled over the shoreline area near Puducherry harbor, Puducherry (Lat 11°54′ 24.27" N; Log79°49′ 41.61" E), along the South East coast of India. Post larvae and green mussels were immediately transported to the laboratory and released into separate tanks containing filtered seawater in a temperature controlled room (26 ± 1 °C) in homogenous salinity (30 psμ) and pH (8.0 ± 0.2). The PL were fed with pellet feed (CP9910S 2 MM, CP India Pvt Ltd) and the green mussels were fed laboratory reared microalgae (cell density of $2 \times 10^5$ cells $L^{-1}$ approximately) during maintenance. The uneaten feed/faecal matters were cleaned from the rearing tanks by siphoning and 50% of water exchange was done frequently. A photoperiod of 12 h Light and 12 h dark was maintained in the room during acclimation and toxicity tests.

### 2.2. Seawater quality

Seawater collected from bar-mouth region of Ennore estuary, Chennai, Tamil Nadu, India. The seawater was filtered through sand filter, charcoal filter, 10 μm size filter and then finally passed through UV treatment device (Make: Pentair) to kill pathogenic microbes. Salinity, pH, temperature and dissolved oxygen (DO) were measured at regular intervals during acclimation and the experiments by pre-calibrated Hydrolab water quality probe (Quanta, USA).

### 2.3. Test solution and treatment

Anhydrous sodium selenite ($Na_2SeO3$, Himedia) was used for preparation of 1000 mg $L^{-1}$ stock solution by dissolving 2.19 g in 1 L of ultrapure water. Aliquots of stock solution were diluted for selected exposure concentrations of Se.

### 2.4. Acute and chronic toxicity bioassay tests

Range finding tests (RFT) were conducted for 48 h with five different concentrations of Se to fix the range of concentrations for definitive test. Followed by RFT, definitive bioassay experiments viz., acute and chronic tests were conducted for customized flow through test method by using the programmable dispensing pumps (Model ISMATEC Nos: ISM936D, ISM933, ISM915A). The bioassay tests for acute and chronic toxicity were conducted by following the method of Sprague (1971) and Stephan et al. (1985).

The PL of shrimp were divided into six groups viz., (i) control (untreated); (ii) 1.0 mg $L^{-1}$ (iii) 1.8 mg $L^{-1}$ (iv) 3.2 mg $L^{-1}$ (v) 5.8 mg $L^{-1}$and (vi) 10.5 mg $L^{-1}$of Se and 20 numbers each in duplicate for the 96-h acute definitive test. This short-term customized continuous flow through test was repeated for three times. For the chronic 21-d exposure study, PL of shrimp were divided into six