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

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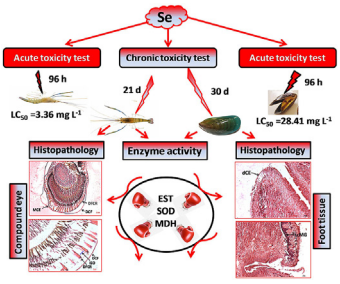
HIGHLIGHTS

- 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



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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

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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 2×10^5 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

groups viz., (i) control (untreated); (ii) 0.08 mg L^{-1} (iii) 0.11 mg^{-1}, (iv) 0.16 mg L^{-1}, (v) 0.22 mg $L^{-1}$and (vi) 0.31 mg L^{-1} of Se and 20 numbers each in duplicate. This long-term customized continuous flow through test was performed for two times. The above concentrations selected for 21-d chronic exposure was based on 96-h LC_{50} derived in the present study.

Similarly, the green mussel were divided into six groups viz., (i) control (untreated); (ii) 20.0 mg L^{-1} (iii) 24.0 mg L^{-1} (iv) 28.0 mg L^{-1} (v) 34.0 mg $L^{-1}$and (vi) 41.0 mg $L^{-1}$of Se and 10 numbers each in duplicate for the 96-h acute definitive test. This short-term continuous flow through test was repeated for three times. For the chronic 30-d exposure study, the green mussel were divided into six groups viz., (i) control (untreated); (ii) 1.5 mg L^{-1} (iii) 2.5 mg L^{-1}, (iv) 4.4 mg L^{-1}, (v) 7.4 mg $L^{-1}$and (vi) 12.5 mg $L^{-1}$of Se and 10 numbers each in duplicate. This long-term definitive test was repeated for two times. The above concentrations selected for 30-d chronic exposure was based on 96 h LC_{50} derived in the current study.

During the acute toxicity tests the PL of shrimp and green mussel were starved and during chronic toxicity tests the PL of shrimp were fed shrimp feed and green mussel were fed microalgae twice a day and the uneaten feed/fecal matters were removed by siphoning at regular intervals. All other environmental conditions were maintained same during all the experiments as those used during acclimation. Upon completion of exposure, live PL of shrimp and green mussel were removed from each respective experimental chamber and stored separately in sealed polycarbonate containers at −80 °C for enzyme activities analysis.

2.5. Determination of metal concentration in seawater from test chambers

Dissolved Se concentration was measured at 24-h intervals (i.e. 24, 48, 72 and 96 h) for acute definitive toxicity tests, 7-d intervals for the PL of shrimp (i.e. 1st, 7th, 14th and 21st day) and 10-d intervals for the green mussel (i.e. 1st, 10th, 20th and 30th day) respectively during chronic definitive tests. Total dissolved Se in acidified experimental seawater was measured using a vapor generation Accessory (VGA) in flame atomic absoption spectrophotometer. Precision and accuracy of the Se analysis including blank and Se standard reference (1000 mg L^{-1}, Merck, Germany) were run between the samples and Se concentrations were measured in the Atomic Absorption Spectrometry (Varian SpectrAA Model220FS) for quantification of dissolved Se concentration.

2.6. Enzyme preparation and native polyacrylamide gel electrophoresis (PAGE)

Enzyme activities analysis was carried out at the end of 21-d exposure for PL of shrimp and 30-d exposure for green mussel. The whole body tissues samples were homogenized using mortar and pestle in an ice bath with liquid nitrogen followed by ice-cold phosphate buffered saline (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.4). After homogenization, the crude lysates were clarified by centrifugation at 12,000 rpm for 10 min at 4 °C. The process was repeated twice to obtain clear supernatants, which served as intracellular enzyme sources. The protein content was estimated by the method described by Bradford (1976) using bovine serum albumin as the protein standard.

The native PAGE electrophoresis was done for the separation of diverse isoforms of esterase (EST), malate dehydrogenase (MDH) and superoxide dismutase (SOD). The activity staining of gels for EST and MDH was done by method of Wendel and Weeden (1989) and SOD by Beauchamp and Fridovoch (1971) as described previously (Nagarjuna and Mohan, 2017) and their images were captured by a CCD camera. The isoenzyme profiles were analyzed by software provided with the gel documentation system (Make: Biovis Gel, 2012F, India).

2.7. Histopathological studies

To carry out the histopathological observations, the compound eye of PL of shrimp and foot of green mussel were dissected freshly at the end of 21-d and 30-d chronic exposure and fixed in Bouin's solution respectively. After fixation, washed in running tap water, the tissues were dehydrated through a graded alcohol series, cleared in xylene and embedded in paraffin wax (58–60 °C). The 5 μm tissues section were taken stained with hematoxylin and eosin followed by DPX mount and observed under light microscope (Make: Carl Zeiss, Model: Axiolab) to note the cellular anomalies.

2.8. Statistical analysis

The acute and chronic definitive toxicity test values were calculated based on the measured dissolved Se concentration. The 96 h LC_{50} and 95% confidence limits for the PL of shrimp and green mussel were calculated based on the mortality endpoint by using Probit Analysis Software (Finney, 1971). The data of long-term survival endpoint of chronic 21-d and 30-d exposure for the PL of shrimp and green mussel were analyzed with analysis of variance followed by Dunnett's (1964) test to calculate NOEC and LOEC values. The chronic value for each test was the geometric mean of the NOEC and LOEC values from the survival response endpoint.

3. Results

3.1. Seawater quality

In the present study, the water quality parameters like temperature, dissolved oxygen, pH and salinity in experimental seawater were in the following order: 25 ± 2 °C, 4.9 ± 0.7 mg L^{-1}, 7.9 ± 0.7 and 29.5 ± 0.3 psμ respectively. The background concentration of Se was also monitored and the values were in the range of 1.47 ± 0.17 μg L^{-1} measured in the seawater used for the experiments.

3.2. Acute and chronic toxicity studies

Test concentrations of dissolved Se were measured in all the test chambers at 24 h intervals (i.e. 24, 48, 72 and 96 h) for acute toxicity test, 7-d intervals for the PL of shrimp (i.e.1st, 7th, 14th and 21st day) and 10-d intervals for the green mussel (i.e.1st, 10th, 20th and 30th day) during chronic toxicity tests respectively. The nominal and measured concentrations of Se were measured to ascertain the recovery of metal and its dissolved concentration in the test chamber throughout the exposure period (Tables 1 and 2). The recovery of Se concentration in the experimental seawater was measured and it ranged from 73.0 to 100.0% for the PL of shrimp and 83.0–98.0% for green mussel during acute exposure and the same was ranged from 84.0 to 98.0% for the PL of shrimp and 88.0–98.0% for green mussel during chronic exposure (Tables 1 and 2). The average 96 h Median Lethal Concentration (LC_{50}) values and 95% confidence intervals derived for the PL of shrimp and green mussel were found to be 3.36 ± 0.86 (2.28–5.63) and 28.41 ± 2.64 (23.89–35.96) mg L^{-1} Se respectively, based on the mean values of three repetitive continuous flow through experiments (Table 3). The survival percentage, NOEC, LOEC and chronic values of PL of shrimp and green mussel exposed to Se for 21-d and 30-d respectively, are given in Table 4. The survival of PL of shrimp and green mussel decreased with increased exposure levels of Se

Table 1
Nominal and measured concentration of Se (mg L^{-1}) in seawater test medium during acute toxicity test on PL of *P. monodon* and *P. viridis*.

Nominal Concentration	Measured concentration (mg L^{-1})					
	Acute 1	Percentage Recovery	Acute 2	Percentage Recovery	Acute 3	Percentage Recovery
P.monodon						
Control						
1.0	0.96 ± 0.074	96.3	0.91 ± 0.16	91.3	1.01 ± 0.18	101.3
1.8	1.66 ± 0.05	92.4	1.54 ± 0.17	85.4	1.61 ± 0.10	89.6
3.2	3.09 ± 0.15	96.5	2.84 ± 0.39	88.7	3.26 ± 0.30	102.0
5.8	5.34 ± 0.21	92.0	5.04 ± 0.47	86.9	4.28 ± 0.44	73.7
10.5	9.95 ± 0.45	94.8	9.60 ± 0.68	91.4	10.10 ± 1.00	96.2
P. viridis						
Control						
20	18.28 ± 0.81	91.39	18.66 ± 0.87	93.3	18.94 ± 0.37	94.7
24	21.38 ± 0.64	89.07	21.31 ± 0.71	88.8	23.60 ± 1.76	98.3
28	23.75 ± 2.54	84.82	23.25 ± 4.37	83.0	25.39 ± 0.71	90.7
34	31.08 ± 2.71	91.40	32.69 ± 2.66	96.1	32.78 ± 0.57	96.4
41	38.45 ± 2.60	93.77	39.08 ± 3.28	95.3	40.71 ± 0.81	99.3

Table 2
Nominal and measured concentration of Se (mg L^{-1}) in seawater test medium during chronic toxicity test on PL of *P. monodon* and *P. viridis*.

Chronic test	Nominal concentration	Measured Concentration (mg L^{-1})				Average	Percentage Recovery
		1-d	7-d	14-d	21-d		
P. monodon							
Test 1	Control (μg/L)	2.08	0.94	0.54	0.99	1.14 ± 0.66	
	0.08	0.08	0.09	0.07	0.08	0.08 ± 0.01	96.9
	0.11	0.10	0.08	0.08	0.11	0.09 ± 0.02	84.1
	0.16	0.15	0.15	0.14	0.17	0.15 ± 0.01	94.5
	0.22	0.22	0.17	0.19	0.18	0.19 ± 0.02	84.7
	0.31	0.34	0.26	0.26	0.29	0.29 ± 0.04	92.7
Test 2	Control (μg/L)	1.98	0.94	2.53	2.64	2.02 ± 0.77	
	0.08	0.08	0.08	0.07	0.07	0.07 ± 0.00	92.2
	0.11	0.10	0.11	0.11	0.11	0.10 ± 0.00	94.3
	0.16	0.17	0.16	0.12	0.19	0.16 ± 0.03	98.4
	0.22	0.23	0.17	0.22	0.21	0.21 ± 0.03	93.8
	0.31	0.26	0.28	0.28	0.29	0.28 ± 0.01	88.7
Chronic test	Nominal concentration	Measured Concentration (mg L^{-1})				Average	Percentage Recovery
		1-d	10-d	20-d	30-d		
P. viridis							
Test 1	Control (μg/L)	1.25	1.27	0.36	0.71	0.89 ± 0.44	
	1.5	1.56	1.53	1.54	1.30	1.48 ± 0.12	98.7
	2.5	2.53	2.39	2.29	2.09	2.32 ± 0.19	93.0
	4.4	4.53	4.34	4.11	4.20	4.29 ± 0.18	97.6
	7.4	7.85	7.46	6.81	6.86	7.24 ± 0.50	97.9
	12.5	11.92	11.19	11.17	11.57	11.46 ± 0.36	91.7
Test 2	Control (μg/L)	BDL	1.06	0.41	0.46	0.64 ± 0.36	
	1.5	1.44	1.46	1.37	1.06	1.33 ± 0.19	88.6
	2.5	2.23	2.27	2.16	2.22	2.22 ± 0.04	88.8
	4.4	4.02	4.23	3.82	3.79	3.96 ± 0.20	90.0
	7.4	6.53	6.85	6.92	6.32	6.65 ± 0.28	89.9
	12.5	11.05	11.33	11.19	11.56	11.28 ± 0.22	90.3

Table 3
Median Lethal concentration (96 h LC_{50}) and 95% confidence limits of Se (mg L^{-1}) for the PL of *P. monodon* and *P. viridis*.

Marine organisms	Acute toxicity test	LC_{50} mg L^{-1}	95% confidence interval
P. monodon	Test 1	2.572	1.83–3.49
	Test 2	3.247	2.21–5.02
	Test 3	4.273	2.81–8.39
	Mean SD	3.36 ± 0.86	2.28–5.63
P. viridis	Test 1	30.66	26.74–39.4
	Test 2	25.5	20.43–31.36
	Test 3	29.08	24.49–37.11
	Mean SD	28.41 ± 2.64	23.89–35.96

during chronic toxicity test. A minimum 58% survival of PL of shrimp was observed at the highest test concentration of Se (0.29 mg L^{-1}) in Chronic I test and 53% survival at 0.28 mg L^{-1} Se observed in Chronic II test after 21-d exposure. The average chronic value of 0.10 ± 0.03 mg L^{-1} was derived based on the geometric mean of the NOEC (0.08 ± 0.01) and LOEC (0.13 ± 0.04) respectively. Similarly, a minimum 40% survival of green mussel was observed at the highest concentration of Se (11.46 mg L^{-1}) in chronic I test and 50% survival at 11.28 mg L^{-1} Se observed in chronic II test after 30-d exposure. The average chronic value of 2.27 ± 0.06 mg L^{-1} Se was derived based on the geometric mean of the NOEC (4.13 ± 0.23) and LOEC (3.06 ± 0.13) respectively.

3.3. Enzyme activities

Induction of diverse isoforms of EST, SOD and MDH activities by native PAGE electrophoresis were studied in the PL of shrimp

Table 4
Nominal and measured concentration, percentage of survival, NOEC, LOEC, and. Chronic values of Se (mg L^{-1}) for the PL of *P. monodon* and *P. viridis*.

Chronic toxicity test	Nominal conc.	Mean measured conc.	Percentage survival	NOEC	LOEC	Chronic value
P. monodon						
Test 1	Control	1.14 ± 0.66[a]	90	0.09	0.15	0.12
	0.08	0.08 ± 0.01	90			
	0.11	0.09 ± 0.02	88			
	0.16	0.15 ± 0.01	78			
	0.22	0.19 ± 0.02	68			
	0.31	0.29 ± 0.04	58			
Test 2	Control	2.02 ± 0.77[a]	93	0.07	0.1	0.08
	0.08	0.07 ± 0.00	90			
	0.11	0.10 ± 0.00	78			
	0.16	0.16 ± 0.03	68			
	0.22	0.21 ± 0.03	60			
	0.31	0.28 ± 0.01	53			
Mean ± S.D.				0.08 ± 0.01	0.13 ± 0.04	0.10 ± 0.03
P. viridis						
Test 1	Control	0.89 ± 0.44[a]	90	2.31	4.29	3.15
	1.5	1.48 ± 0.12	85			
	2.5	2.32 ± 0.19	80			
	4.4	4.29 ± 0.18	65			
	7.4	7.24 ± 0.50	55			
	12.5	11.46 ± 0.36	40			
Test 2	Control	0.64 ± 0.36[a]	100	2.22	3.96	2.96
	1.5	1.33 ± 0.19	100			
	2.5	2.22 ± 0.04	95			
	4.4	3.96 ± 0.20	80			
	7.4	6.65 ± 0.28	65			
	12.5	11.28 ± 0.22	50			
Mean ± S.D.				2.27 ± 0.06	4.13 ± 0.23	3.06 ± 0.13

[a] Values are expressed in terms of μg L^{-1}.

exposed to Se for 21-d. Three isoforms of EST activities were observed in the PL of shrimp. Isoform I, II and III (Rm 0.044; 0.13; 0.203) of EST activities were increased at test concentration of 0.16, 0.21 and 0.28 mg L^{-1} Se compared to control (Fig. 1a). One isoform (Rm 0.267) of SOD showed increased activity at NOEC (0.07), LOEC (0.10) and at other higher concentrations like 0.16, 0.21 and 0.28 mg L^{-1} Se compared to control (Fig. 1b) and MDH activity (Rm 0.108) was increased at 0.21 and 0.28 mg L^{-1} Se concentrations compared to control (Fig. 1c) observed in shrimp.

Sublethal toxicity of Se for 30-d exposure was studied in the green mussel has caused an induction of diverse isoenzymes of EST, SOD and MDH activities by native PAGE electrophoresis. Two isoforms of EST activities were observed in the green mussel. Isoform I (Rm 0.205) of esterase activity was increased at NOEC (2.32) and at other higher concentrations such as 4.29, 7.24 and 11.46 mg L^{-1} Se compared to control. The isoform II (Rm 0.395) of esterase activity was increased at 7.24 and 11.46 mg L^{-1} Se concentration compared to control (Fig. 2a). One isoform of SOD (Rm 0.216) was decreased at NOEC (2.32) and followed by an increase in test concentrations of 4.29, 7.24 and 11.46 mg L^{-1} Se compared to control (Fig. 2b). One isoform of MDH activity (0.248) increased at 11.46 mg L^{-1} Se concentration compared to control (Fig. 2c) observed in green mussel.

3.4. Histological studies

The histology of untreated ommatidia of compound eye of the PL of shrimp observed normal rhabdom, cone cell and corneal cells attached with cornea facet (Fig. 3a and b). Deformation and fusion

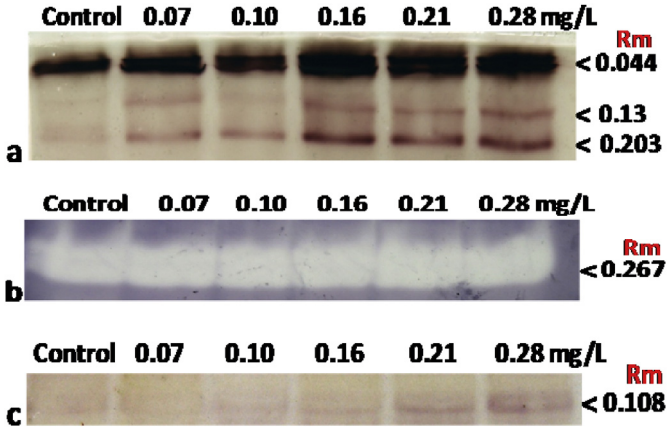

Fig. 1. Native PAGE electrophoresis of esterase (a), superoxide dismutase (b), and malate dehydrogenase (c) in the PL of *P. monodon* exposed to Se for 21-d. ('Rm' denotes Relative mobility).

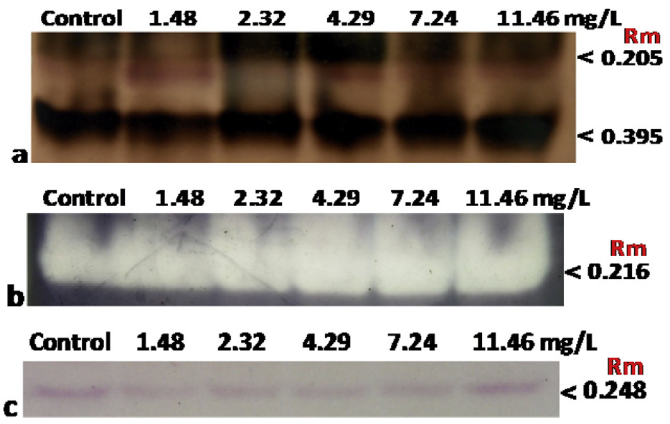

Fig. 2. Native PAGE electrophoresis of esterase (a), superoxide dismutase (b), and malate dehydrogenase (c) in *P. viridis* exposed to Se for 30-d. ('Rm' denotes Relative mobility).

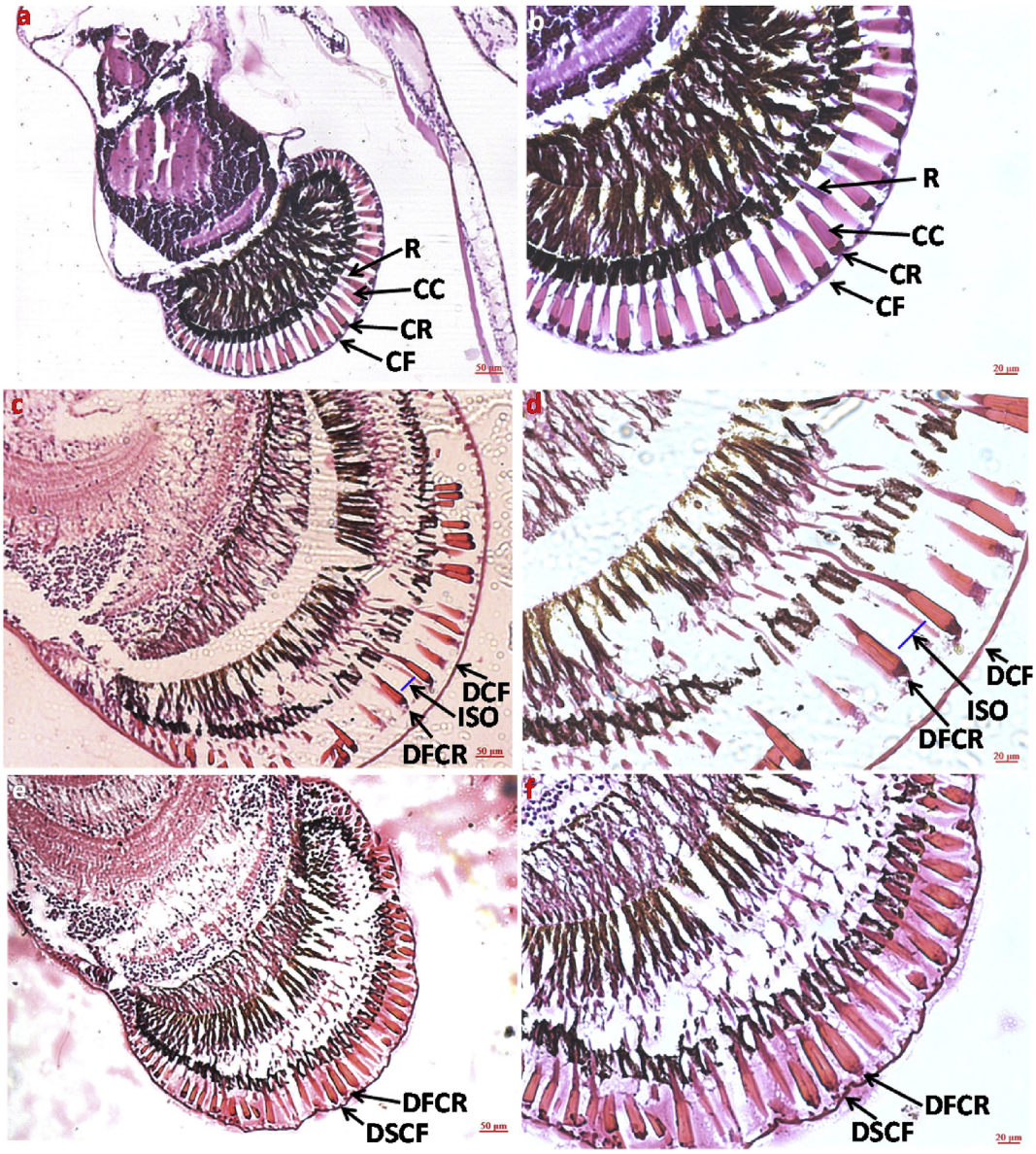

Fig. 3. Histopathological section shows the compound eye of PL of *P. monodon* exposed to different test concentration of Se (mg L^{-1}) for 21-d and stained with heamatoxylin and eosin. c-l were exposed to 0.08, 0.09, 0.15, 0.19 and 0.29 mg L^{-1} Se respectively. (a) and (b) Untreated ommatidia of compound eye of PL of shrimp shows rhabdom (R), cone cell (CC) and corneal cells (CR) attached with cornea facet (CF); (c) and (d) deformation and fusion of corneal cells (DFCR) and detached from the cornea facet (DCF) and increased space between ommatidia (ISO); (e) and (f) deformation and fusion of corneal cells (DFCR) and detached from the cornea facet (DCF) were observed in the compound eye of PL of shrimp (a, c, e 100X and b, d, fl 200X). (g) and (h) deformation and fusion of corneal cells (DFCR) and detached from the cornea facet (DCF) and malformation of compound eye (MCE); (i) and (j) corneal cells detached from the corneal facet (CRDCF), ruptured cornea facet (RCF) and increased space between ommatidia (ISO); (k) and (l) deformed and compressed corneal cells (DCCR) attached with corneal facet, degeneration of corneal cell (dCR) and ommantida (dO) were observed in the compound eye of PL of shrimp (g, i, k 100X and h, j, l 200X).

of corneal cells and detached from the cornea facet and increased space between ommatidia were observed in the compound eye of the PL of shrimp exposed to 0.08 and 0.09 mg L^{-1} Se (Fig. 3c–f). Malformation of compound eye, deformation and fusion of corneal cells and detached from the cornea facet were observed in the compound eye of the PL of shrimp exposed to 0.15 mg L^{-1} Se (Fig. 3g and h). Corneal cells detached from the corneal facet, ruptured cornea facet and increased space between ommatidia were observed in the compound eye of the PL of shrimp exposed to 0.19 mg L^{-1} Se (Fig. 3i and j). Deformed and compressed corneal cells attached with corneal facet, degeneration of corneal cells and ommantida were observed in the compound eye of the PL of shrimp exposed to 0.29 mg L^{-1} Se (Fig. 3k and l).

Histology of untreated foot of green mussel showed ciliated epithelium lining, mucous gland and enzyme gland (Fig. 4a). The effect of various test concentrations on the foot tissues varied between different types of glands as follows: Shrinkage of mucous gland at 1.33 mg L^{-1} Se (Fig. 4b); degenerative changes in phenol gland cells at 2.22 mg L^{-1} Se (Fig. 4c); detached enzyme gland from sub epithelial cells at 3.96 mg L^{-1} Se (Fig. 4d); degenerative changes in ciliated epithelium lining at 6.65 mg L^{-1} Se (Fig. 4e); shrinkage and clumping of mucous gland at 11.28 mg L^{-1} Se were observed in the foot of green mussel (Fig. 4f).

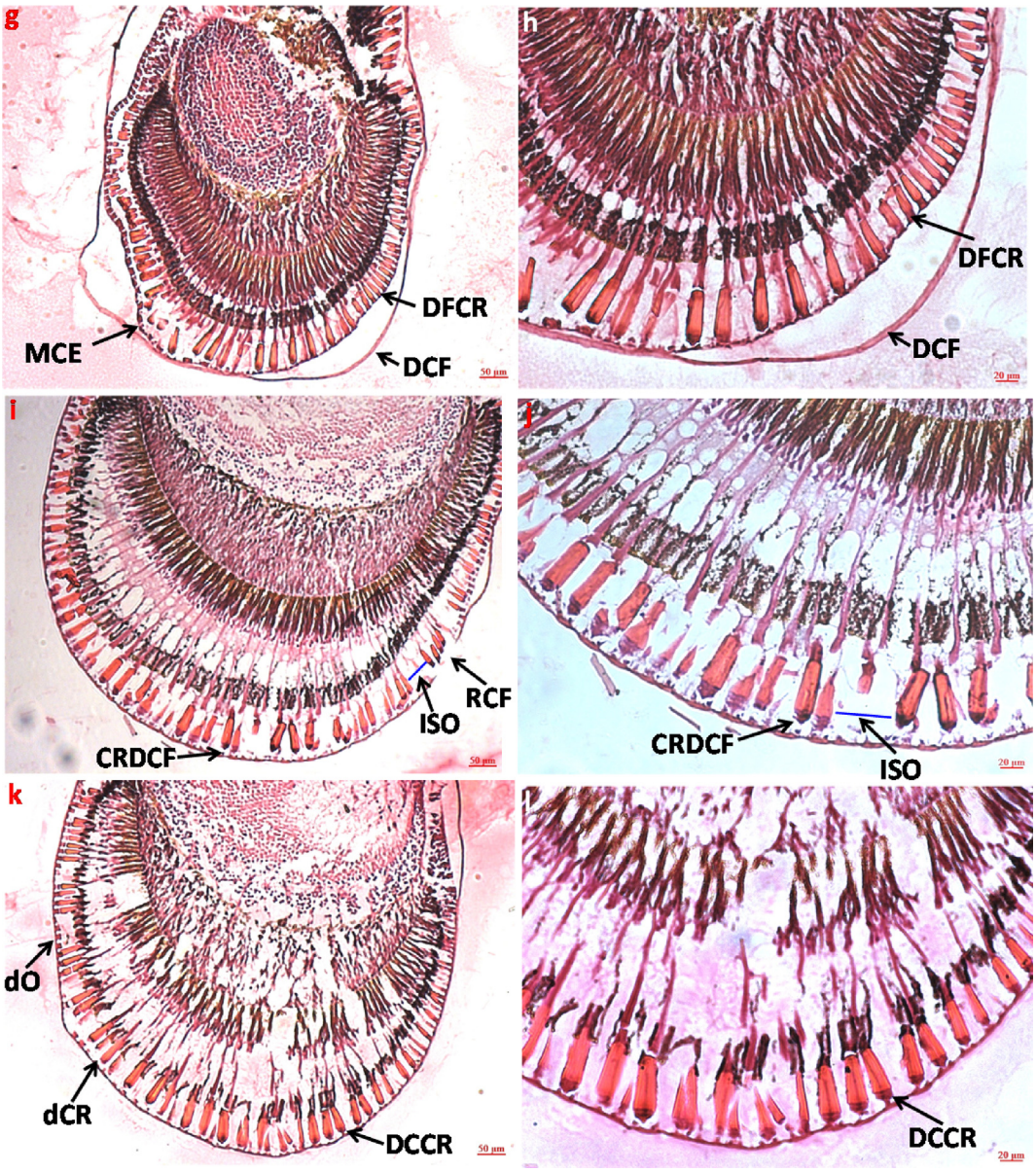

Fig. 3. (*continued*).

4. Discussion

The average 96-h Median Lethal Concentration (LC_{50}) values and 95% confidence limits derived for the PL of *P. monodon* and *P. viridis* were found to be 3.36 ± 0.86 (2.28–5.63) and 28.41 ± 2.64 (23.89–35.96) mg L^{-1} of Se respectively, based on the mean values of three repetitive customized continuous flow through experiments in the present study. There was no mortality of PL of shrimp and green mussel initially with treatment of different concentrations of Se, however, the mortality of organisms was increased with increased exposure time and concentration of the metal. A similar result was also found by Bambang et al. (1995) when nauplii and zoeae are the most sensitive and juveniles of *P. japonicus* is the most tolerant to cadmium. The sensitivity of an organism to a toxic agent can vary depending on its size, age and stage of development (Duquesne et al., 2004; Dutra et al., 2016), because several enzymes may have differential activities during embryonic and growth development as reported by Barbieri et al. (2002). Early life stages of aquatic organisms are generally more sensitive to metal toxicity than older stage or adults (McKim, 1977; Wang, 1987; Usman et al., 2013; Duquesne et al., 2004; Dutra et al., 2016). Previous authors reported that the 96 h LC_{50} of Se was 1.74 mg L^{-1} for *Acartia clause* (USEPA, 1980); 2.11 mg L^{-1} for *Acartia clause* (Lussier, 1986); 0.839 mg L^{-1} for *Acartia tonso* (Lussier, 1986); 0.8 mg L^{-1} for *Acartia tonso* (USEPA, 1980); 1.5 for mg L^{-1} *Mysidopsis bahia* (opossum shrimp) (Ward et al., 1981); 0.21 mg L^{-1} for *Mysidopsis bahia* (mysid) (USEPA, 1978; Ward et al., 1981) and 0.4 mg L^{-1} for *Crassostrea virginica*, (Fowler et al., 1981). The findings of the previous authors used different species which are in various stages of development, hence the results from the present study could not be compared, further unfortunately no report was available for the PL of shrimp justifying the present study. The percentage survival of the PL of shrimp and green mussel decreased with increased Se concentration for 21-d and 30-d exposure respectively. The NOEC (0.08 ± 0.01 mg L^{-1}), LOEC (0.13 ± 0.04 mg L^{-1}) and chronic value of 0.10 ± 0.03 mg L^{-1} for the PL of shrimp and NOEC (2.27 ± 0.06 mg L^{-1}), LOEC (4.13 ± 0.23 mg L^{-1}), and chronic value of 3.06 ± 0.13 mg L^{-1} Se were derived for green mussel. The above

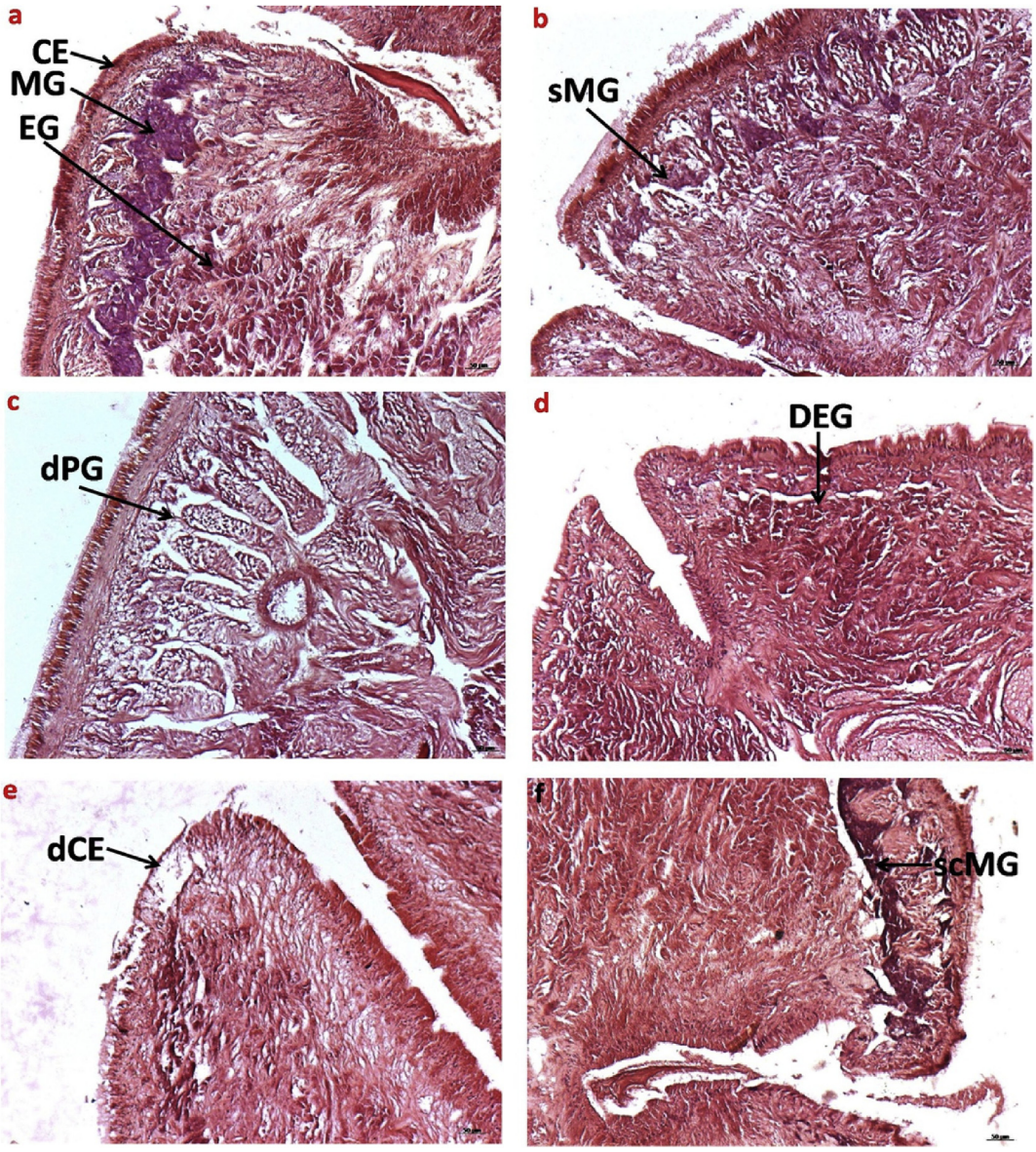

Fig. 4. Histopathological section shows the foot of *P. viridis* exposed to different test concentration of Se (mg L^{-1}) for 30-d and stained with heamatoxylin and eosin. b-f were exposed to 1.33, 2.22, 3.96, 6.65, and 11.28 mg L^{-1} Se respectively. (a) Untreated foot of green mussel shows ciliated epithelium lining (CE), mucous gland (MG) and enzyme gland (EG); (b) shrinkage of mucous gland (sMG); (c) degenerative changes in phenol gland (dPG); (d) detached enzyme gland (DEG) from subepithelial cells; (e) degenerative changes in ciliated epithelium lining (dCE); (f) shrinkage and clumping of mucous gland (scMG) were observed in the foot of green mussel (100X). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

results are in agreement with the earlier report of Hamilton et al. (1990) who also demonstrated a similar situation in *Oncorhynchus tshawytscha* that concentration-response relation between reduced survival of *O. tshawytscha* and increased concentration of dietary Se for 60 and 90-d exposure. Early life stages of fish are generally more sensitive to toxicant stresses because of the lack or underdevelopment of metabolic mechanisms essential for withstanding the toxicant stresses, or interference with metabolic processes that are vital to developing organisms (Rand and Petrocelli, 1985).

The first effects of contaminants usually occur at the cellular or subcellular level (Pickering, 1981; Stephan and Mount, 1973). Esterases catalyze the hydrolysis of various types of exogenous and endogenous esters; preferably they are composed of short chain fatty acids (Bornscheuer, 2002). Esterases represent a diverse group of hydrolases catalyzing the cleavage and formation of ester bonds. The interest in these enzymes also resides in the fact that, they do not require cofactors, usually they are stable and even active in organic solvents (Bornscheuer, 2002). In the present study, three isoforms of EST activities were observed in the PL of shrimp exposed to Se for 21-d. Isoform I, II and III (Rm 0.044; 0.13; 0.203) of EST activities were increased at 0.16, 0.21 and 0.28 mg L^{-1} of Se compared to control. However, two isoforms of EST activities were observed in green mussel exposed to Se for 30-d. Isoform I (Rm 0.205) of EST activity was increased at NOEC (2.32) and at other higher concentrations such as 4.29, 7.24 and 11.46 mg L^{-1} Se compared to control. The isoform II (Rm 0.395) of EST activity was increased at 7.24 and 11.46 mg L^{-1} Se concentration compared to control. Increased EST activities were observed in the PL of shrimp and green mussel exposed to Se for 21-d and 30-d respectively, due to increased ROS levels in the cells and they might have disturbed the integrity of cell membranes. This observation is agreement with

previous studies of Van Hoewyk et al. (2008), Grant et al. (2011) and Schiavon et al. (2012) who also reported that excess Se in the cell causes ROS accumulation. Se at higher doses may become toxic to organisms by acting as pro-oxidant, primarily because of its capacity to replace sulfur in proteins, which then lose their correct folding (Van Hoewyk et al., 2008). Direct enzyme inhibition can be related to very specific sites and can be toxicant specific, as in acethylcholinesterase (AChE), while other systems are less specific and their effect is more general (Mayer et al., 1992). Carboxylesterases are considered as a group of esterases with wide substrate specificity (Walker and Thompson, 1991), and this makes them to play a major role in the detoxification of many different types of xenobiotics such as organophosporous, pyrethroids, phthalate ester plasticizers, oil dispersants and other environmental pollutants that affect fishes and other aquatic organisms (Al-Ghais, 2000; Galloway et al., 2002; Wheelock et al., 2005; Leticia and Gerardo, 2008). The increased AChE activity observed in neurodegeneration is associated with the presence of high concentrations of reactive oxygen and nitrogen species (Melo et al., 2003). Exposure to copper and mercury caused a significant inhibition of AChE activity in estuarine fish, *Pomatoschistus microps* (Vieira et al., 2009). The metals like Cu, Zn, Cd, Hg and detergents inhibit AChE activity in aquatic organisms at both *in vivo* and *in vitro* conditions, but the mechanism of AChE inhibition by metals is still unknown (Frasco et al., 2005; Kopecka-Pilarczyk, 2010).

SOD is an enzyme that catalyzes the dismutation of superoxide anion into oxygen and hydrogen peroxide. One isoform of SOD activity (Rm 0.267) was observed in the PL of shrimp exposed to Se for 21-d. Isoform of SOD activity was increased at higher concentrations (viz., 0.07, 0.10, 0.16, 0.21 and 0.28 mg L^{-1} Se) compared to control leading to detoxification of increased production of superoxide anion in the mitochondria. One isoform of SOD activity (Rm 0.216) was observed in the green mussel exposed to Se for 30-d. The SOD activity was decreased at NOEC (2.32 mg L^{-1} Se) and followed by an increase in other concentrations (viz., 4.29, 7.24 and 11.46 mg L^{-1} Se) compared to control due to continuous production of superoxide anion in the mitochondria resulted initially decreased and followed by stable SOD activity. SOD triggers its activity against stress induced due to metal/pesticide toxicity and neutralized the ROS produced by the cells (Zorov et al., 2014). Mukhopadhyay and Chattopadhyay (2014) reported slightly increased Mn Sod expression and unchanged Cu/Zn Sod expression in the liver of *Danio rerio* exposure to sodium fluoride. Guardiola et al. (2016) reported an increased SOD activity in the liver cells of *Sparus aurata* exposed to methylmercury. Activity of SDH was inhibited in large yellow croaker *Pseudosciaena crocea* exposed to Zinc (Zheng et al., 2017).

MDH involves in citric acid cycle which is a vital cell process for all kinds of functions in the cell (Oh et al., 2002). Oxaloacetate, a metabolite of NAD-dependent MDH activity, plays a key role as an antioxidant, possibly by scavenging hydrogen peroxide (Oh et al., 2002). One isoform of MDH (Rm 0.108) activity was observed in the PL of shrimp exposed to Se for 21-d. The MDH activity was increased at 0.21 and 0.28 mg L^{-1} Se concentration compared to control. Also one isoform of MDH activity was observed in the green mussel exposed to Se for 30-d. The MDH activity (0.248) was increased at 11.46 mg L^{-1} of Se concentration compared to control. Increased MDH activity means increase in total ATP levels in mitochondria leading to increase in total energy production and to overcome energy crisis/demand in the cells due to Se toxicity. This is agreement with the previous study, Nagarjuna and Mohan (2017) reported by increased malate dehydrogenase activity in the juveniles of *Mugil cephalus* exposed to nickel. Further, Zheng et al. (2017) observed that the MDH activity was inhibited in large yellow croaker *P. crocea* exposed to Zinc. Muller (1986) reported that cadmium concentrations in the hepatocyte cells affected the integrity of its mitochondrial membranes concomitantly impairing the hepatocellular energy supply. Cadmium causes severe membrane integrity damage with a consequent loss of membrane-bound enzyme activity which can result in cell death (Younes and Siegers, 1984). In mitochondria the increase in specific enzyme activities of the tricarboxylic acid cycle such as NAD-isocitrate dehydrogenase, succinate dehydrogenase, and malate dehydrogenase suggested that cadmium intoxication response of *S. aurata* is a strong stimulation of the terminal pathway of biological oxidation, probably due to alteration of mitochondrial membrane integrity (Vaglio and Landriscina, 1999).

The characteristics of the typical crustacean compound eye have been reported by Matsuda and Wilder (2010). However, the information is scarce concerning toxicological studies besides their physiological importance of compound eye of the PL of shrimp. In the present study, cellular anomalies such as deformation and fusion of corneal cells and detached from the cornea facet, increased space between ommatidia, malformation of compound eye, ruptured cornea facet, deformed and compressed corneal cells attached with corneal facet, degeneration of corneal cells and ommantida in the compound eye of larvae of tiger shrimp were observed which would affect its primary function viz., to convert light energy into nerve impulses transferred to the ganglion, leading to loss or poor vision. The present study is the first report on Se induced histological changes in the compound eye of PL of shrimp. Most of the previous studies on compound eye of crustacean species addressed adaptive changes during light and dark conditions (Struwe et al., 1975; Frixione et al., 1979; Nicol and Yan, 1982; Hallberg and Elofsson, 1989; Meyer-Rochow, 2001; Matsuda and Wilder, 2010; Sanudin et al., 2014). However, studies addressing the effect of metals and other environmental contaminants on compound eye are absolutely absent and cellular anomalies described in this study is due to changes caused by Se ions diffused from ambient water into soft tissues of compound eye through corneal facet or through circulatory system. However, the mechanism of Se entry and its effect on compound eye is unresolved which needs to be studied further for better understanding.

The foot tissues of green mussel did not receive much attention in toxicological studies besides their physiological function. Foot consists of white gland, phenol gland and enzyme gland cells, which are responsible for the formation of byssus threads in green mussel. Secretions of the gland react with each other in the central groove of the foot to form the byssus (Morton, 1964) as a result of an auto-quinone tanning mechanism (Smyth, 1954). It is interesting to note that each test concentration have influenced the morphological changes in foot tissues which are delineated in the present study. Cellular changes such as shrinkage and clumping of mucous gland, degenerative changes in phenol gland and ciliated epithelium, and detached enzyme gland from sub epithelial cells were observed in the foot of green mussels exposed to Se for 30-d would affect the byssus threads formation. Rajagopal et al. (2003) reported that continuous chlorination has affected the foot activity, byssus thread production, filtration activity and shell valve movement in marine mussels. In the present study, cellular anomalies in the compound eye of the PL of shrimp and foot of green mussel would affect vision of shrimp and byssus threads formation due to accumulation of ROS in cells which disturbed the cellular membrane function and clearly evidenced by histopathological studies.

5. Conclusion

The results of the present study provided the acute and chronic toxicity values of Se and its effect on enzyme activities response and histopathological alterations can be effectively used for

environmental monitoring assessment which may also provide a framework for the development of water quality criteria for environmental protection. The PL of shrimp was more sensitive to Se toxicity than green mussel. This study confirmed that the experimental approach constitutes the identification of changes at the cellular and tissue level that may occur from the effect of Se stress. Prior to toxic effects at the level of the cell membrane, Se has its own effect on metabolic processes in cells as reflected by alterations in activities of enzymes and might be causing ROS accumulation in the cells. The integrity of cellular membranes is of ultimate importance for the maintenance of functional viability of cells. There is a need for effective regulatory measure and proper monitoring in the environment in order to reduce the hazardous effects on target and non-target species.

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