AJAB

Asian J Agric & Biol. 2019; Special Issue:17-24.

Original Article

Proteomic analysis and assessment of heavy metals in hepatopancreas of mud crabs from Setiu and Kuala Sepetang

Nur Syafinaz Mohd Razali[1], Nakisah Mat Amin[1], Wan Bayani Wan Omar[1], Mhd Ikhwanuddin[2], Nurul Huda Abd Kadir[1*]

[1]Faculty of Science & Marine Environment, Universiti Malaysia Terengganu, 21030 Terengganu, Malaysia
[2]Institute of Tropical Aquaculture, Universiti Malaysia Terengganu, 21030 Terengganu, Malaysia

Received:
January 20, 2019
Accepted:
October 30, 2019
Published:
December 05, 2019

**Corresponding author email:*
nurulhuda@umt.edu.my

Abstract

Mud crabs are commonly found in Southeast Asia and are in high demand for its nutrients. However, studies reported that estuary areas are easily contaminated with pollutants due to the anthropogenic activities. Hepatopancreas is one of important tissues that involve in food digestions and detoxifications. Glutathione (GSH) and glutathione-S-transferase (GST) play roles in metabolisms of detoxification of xenobiotics. Therefore, this study focuses on the analysis of trace metals in hepatopancreas of mud crabs from Kuala Sepetang (highly contaminated area) and Setiu Wetlands (low contaminated area) and their protein response. Trace metals in hepatopancreas were analyzed by using Inductive Couple Plasma–Mass Spectrometer (ICP-MS). Proteins of hepatopancreas were extracted and GSH activity was measured. Next, protein expressions were observed in 1D electrophoresis and Western blotting. Our results shows that the concentrations of trace metals in hepatopancreas were highly significant in Kuala Sepetang compared with Setiu Wetlands. However, there is no significant difference in GSH activity in both locations. From 1D electrophoresis, the intensity of the protein band at ~60-75 kDa were dense in Kuala Sepetang compared with Setiu with values 15620.23±7829.83 and 3687.83±2933.69 respectively. Based on Western blot result, this protein band was related to GST as the response towards the level of trace metals abundance in the area. This proved that mud crabs in Sepetang location were stressed in polluted areas.

Keywords: Trace metals, Mud crabs, Hepatopancreas, Proteins

How to cite this:

Razali NSM, Amin NM, Omar WBW, Ikhwanuddin M and Kadir NHA, 2019. Proteomic analysis and assestment of heavy metals in hepatopancreas of mud crabs from Setiu and Kuala Sepetang. Asian J. Agric. Biol. Special Issue: 17-24.

Introduction

Scylla sp also known as mud crabs is belonging to Portunidae family. There are four species of Scylla which are *Scylla serrata*, *Scylla olivacea*, *Scylla paramamosain*, and *Scylla tranquebarica*. Recently, mud crabs were highly demanding by local fishermen due to their unique taste and high fleshy meats contents. They can be distinguished by observing the morphological of claws and the

carapace lobe of mud crabs. Genus of Scylla is commonly distributed in the muddy area and coastal mangrove area (Fazha et al., 2017). *S. serrata* is the largest genus of *Scylla* that can be found in tropical and subtropical of Indo-West-Pacific region. However, this species known as non-indigenous species and the highest abundance species is *S. olivacea* in Malaysia (Ikhwanuddin et al., 2010).

Setiu Wetlands is located at Eastcoast region of Peninsular Malaysia in the state of Terengganu while Kuala Sepetang is located at Northern region of Peninsular Malaysia in the state of Perak. Both locations are unique since it consist of eastuary, wetlands, river and mangrove areas. Due to this geographical structure, it has diverse biodiversity. However, eastuary areas are easily contaminated with trace metals elements such as Ni, Zn, Cu, Pb, Li and Cr from anthropogenic activities (mining, boating, aquaculture, agriculture activities and household waste from rural areas) (Leong et al., 2017). These factors will influence the populations and the health of aquatic organisms including mud crabs. Research update showed the number and the size of the mud crabs were caught in the wild were decline (Ikhwanuddin et al., 2010). According to Vince Cruz et al. (2015), morphological deformities and divergent sexual morphology were observed in mud crabs as the result of excessive uptake of trace metals.

In order for aquatic organisms to survive in this toxic environments, mud crabs will alter their physiological and biochemical activities. Hepatopancreas was chosen for the present study because it easily accumulates trace metals. It also plays important roles in food digestion and regulation of metabolism (Kovačević et al., 2008). Glutathione (GSH) is tripeptide antioxidant consist of glutamyl, cysteinyl and glycine that widely distributed in all living organism. GSH will activate the glutathione-S-transferase (GST) and other stress proteins for detoxification of xenobiotics from body of mud crab (Singaram et al., 2013) Therefore, the aims of this study is to evaluate the antioxidant activities of GSH, GST and other stressed protein expressions under high and low contaminated of trace metals areas.

Material and Methods

Sampling site and mud crabs samples

Wild mud crabs were collected at Setiu Wetlands and Kuala Sepetang during low tides. Ten matured male wild mud crabs *S. olivacea* were collected randomly from Setiu Wetlands and Kuala Sepetang using a 'bintuh'. Mud crabs were transported to laboratory and stored at -80° C prior the analysis. Then, mud crabs were cleaned with distilled water and dissected to collect the hepatopancreas for determination of concentration of trace metals and proteomic works.

Determination of trace metals in hepatopancreas

Trace metals concentrations were determined by following protocol from Azlisham et al. (2009) with slight modifications. Dry weight samples were obtained after freeze dried using freeze dryer and grounded using mortal and pestle. Samples were weighed (0.2 g) to digest with 2 mL of 37% hydrochloric acid (HCl). The mixture of the samples and HCl were heated until it dried. Then, 10 mL of 20% nitric acid (HNO_3) were added into the mixtures prior diluted with 50 mL of deionized distilled water. Lastly, samples were analyzed by using Inductive-couple plasma- mass spectrometry (ICP-MS).

Protein extractions

One g of each tissue was homogenized in 5 mL of 50 mM Tris-HCl buffer (pH 7.8) containing 1 mM EDTA, 1mM DTT, 0.5 mM sucrose, 150 mM KCL and 1 mM PMSF (Paital and Chainy, 2010). The mixture of samples and buffer was centrifuged at 1000 x g for 10 min at 4 °C. Then, supernatant obtained was collected and centrifuged again at 10000 x g for 10 min at 4 °C before was stored at -80°C for further analysis. The absorbance of protein content of hepatopancreas of mud crabs was measured using Bradford's method (1976) at 595 nm using a spectrophotometer (Spectro UV-VIS RS).

Glutathione assay

This assay was followed the method of (Hissin and Hilf, 1976). Extracted proteins samples (40 µl) were added with 160 µl of 6.5% 5-sulfosalisylic (SSA). Next, samples were incubated on ice for 20 mins before centrifuged at 14,000 rpm, for 5 min at 4 °C. After centrifugation process, supernatants were collected and diluted until 10,000 fold with phosphate buffer (pH 7.4). Diluted samples (100 ul) were added on 96-well microplate reader for determination of GSH. Standard of GSH is prepared in the range 0 µM – 4000 µM on the same microplate. After that, 33 ul of 1X OPA as fluorescence agent was added in each well that contains GSH standard and samples. The 96-well microplate was incubated at room temperature for 30 mins under dark conditions. Then, microplate were measured at excitation of 320 nm and emissions at 460 nm by using fluorescence microplate reader (Tecan Infinite F50).

1D Electrophoresis

Extracted proteins samples (25 µg) were mixed 1:1 with 2X loading buffer (0.15 M Tris-HCl, 1.2% (w/v) SDS, 60% (v/v) glycerol, 15% (v/v) 2-mercaptoethanol, and 0.09% (w/v) bromophenol blue) (Porte *et al*., 2001). The sample mixtures were heated for 5 min at 90 °C prior the electrophoresis. Protein samples were loaded into the 10% of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) at 90 V for 1 and half hour.

Western blot analysis

Western blotting analyses were carried out by transfer 1D SDS gel to PVDF membrane by using Turbo-blot Biorad (Fujii et al., 2001). During blocking process of the membrane, 5% of skim milk was used for 2 hours. Then, the membrane was incubate with polyclonal primary antibody of rabbit GST (1:10000, Santa Cruz: A5800) for 12 hours at 4 °C. After incubation periods, membrane was washed three times with Tris-buffered saline-Tween 20 (TBST) before incubated with anti-mouse IgG secondary antibody (1:2000, Santa Cruz) for 4 hours at 4 °C. Membrane wash washed again with TBST triplicate. The bounded protein on membrane were conjugated with horseradish peroxidase (HRP) substrate for visualization of the specific protein band. Then, image of the membrane was visualized by using Gel-Doc BioRad.

Statistical analysis

Data in each assay were collected and analyzed by using statistical analysis of T-test.

Results and Discussion

Concentration of trace metals in hepatopancreas

The level concentration of trace metals in hepatopancreas of mud crabs was presented in Table 1. From the present study showed that the concentrations of Ni, Zn, Pb, Li and Cr in hepatopancreas from Kuala Sepetang was higher significantly than in Setiu Wetlands ($p<0.05$). Due to their geographical structure, most of the residents in both locations rely on fishing activities, aquaculture, farming activities as their economical source. However, Kuala Sepetang showed the increasing number of charcoal industry and shrimp aquaculture activities year by year (Hazmi, 2018). On the other hand, charcoal industry in Setiu Wetlands was under constraint activities (Jusoff and Bin Hj Taha, 2014). This activities easily affect water quality in Kuala Sepetang that caused the decline populations fish and crustaceans (Leong et al., 2017). Cu, Zn and Ni are released in ash waste during combustions process of charcoal (Chugh and Behum, 2014). Other worst reasons, Kuala Sepetang has large fishing village community near the river have incorrect method for disposable of their daily rubbish (Leong et al., 2017). In other study, oil spilling from boating activity and mining activities will lead to the contamination of Pb in the ecosystems (Tchounwou et al., 2012). In addition, our previous studies showed that concentrations of metals was significantly correlated with metal accumulation in mud crabs, tissues from Setiu Wetlands (Amin et al., 2018). Furthermore, protein expression of the mud crabs hemolymph and hepatopancreas differed according to the changes of the environmental conditions including heavy metals contents (Razali et al., 2017).

According to Bo et al. (2015), edible snails of *Belalamya sp.* was highly contaminated with heavy metals in mixed area (industrial and commercial) compared with agriculture and residential area in

Taihu Lake region, China. This is also supported by (Amin et al., 2018) no significant difference on concentrations of trace metal in mud crabs from agriculture and aquaculture sites. Concentrations of trace metals (Cu, Ni, Fe, Co, Mn, Cr and Zn) in *Mastacemblus armatus* were high at the Power Plant site (Javed and Usmani, 2013). This showed that, heavy metals mostly deposited in aquatic organisms that near with industrial and commercial activities.

Table-1: The concentrations of trace metals deposited in hepatopancreas of mud crabs from Setiu Wetlands and Kuala Sepetang.

Locations/trace metals	Setiu wetlands	Kuala Sepetang
Ni	BD	1.02±0.10*
Cu	37.74±9.50	25.23±7.98
Zn	41.46±3.66	103.49±23.48*
Pb	BD	0.61±0.24*
Li	0.08±0.01	0.20±0.03*
Cr	0.24±0.03	2.98±0.22*

BD: below detection limit *significant difference using T-test $p<0.05$.

However, all waste from anthropogenic activities will drained out to the river and affect the health of aquatic organism. Some of trace metals will dissolved in water and accumulated in sediments (Azlisham et al., 2009). Trace metals might be deposited in the body of mud crabs during their moulting stages. This is because, mud crabs will absorb water 85% one day after ecdysis (Thi et al., 2014). Moreover, trace metals can be accumulated in aquatic organisms through biomagnifications. Mud crabs feed cockles, small fish, squid as their feeding behavior (Viswanathan and Raffi, 2015). Besides that, mud crabs have burrowing behavior that tends to the accumulated of polluted sediments into their body (Kamaruzzaman et al., 2012).

Proteins expressions in hepatopancreas

GSH is non protein thiol act as cofactor for other enzymes such as GST, glutathione peroxidase (GPx) and glutatahione reductase (GR). Based on present study, there was no significant difference in level of GSH in hepatopancreas form both locations ($p<0.05$) (Fig 2). This result was contrary with other previous study by Słupik et al. (1999), it found the level of GSH was low (5.41 µg/mg protein) in snails from polluted area in Poland. Moreover, the concentrations of GSH in fish *Cryprinus carpio* was also lower in Sitilce site (high polluted) which is 1.48±0.10 µmol/g protein compared to Samsat site (low polluted) which is 3.98±0.14 µmol/g protein (Karadag et al., 2014). However, other study showed that the level of GSH can also increasing in fish at the polluted area (Stoliar and Lushchak, 2012). At the same time, the level of GSH was not detected significantly difference from polluted and non-polluted area. Reported by El-Gazzar et al. (2014), there was no significant difference in level of GSH in gills of fish after exposure with Cd.

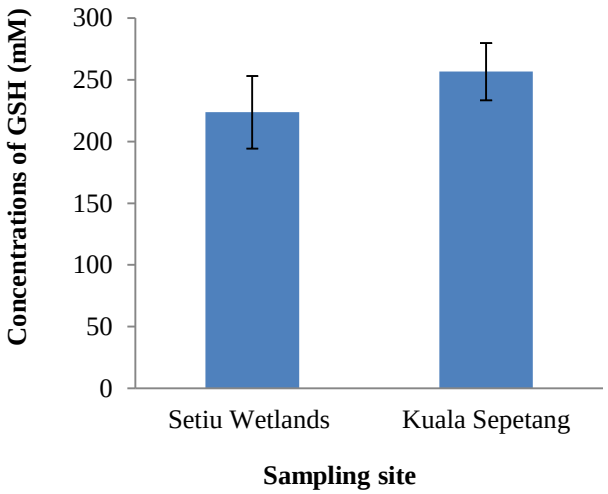


Figure-1: The concentrations of GSH in hepatopancreas of mud crabs

In general, tissues will produce reactive oxygen species (ROS) such as hydrogen peroxide, superoxide ions, hydroxical radical and hydroxical anion in order to eliminate foreign substances that enter the body of mud crabs (Lushchak, 2011). The high level of ROS will elevate the level of oxidative stress and cause the decreasing level of GSH as it has been utilized that can be converted into oxidized glutathione and inefficient GSH regeneration during exposure of pollutants (Yildirim et al., 2011). However, other study concluded that the level of GSH will increased as the level of pollutants was increase (Mahboob et al., 2014).

The polyclonal GST were expressed in hepatopancreas of mud crabs from both locations at ~70 and ~60 kDa (figure 2). Briefly, GST in eukaryotes can be observed at the range of ~26 kDa molecular weight (Kitteringham et al., 2003). However, molecular weight of GST is depends on their types of subunits (Gabel et al., 2002). Based on our result, the intensity band of GST were high in Kuala Sepetang (15620.23±7829.83) compared to Setiu Wetlands (3687.83±2933.69). This proved that GSH has been utilized to activate GST proteins to protect the cells from damage after effect of pollutants. Previous study also found the intensity of GST were high in clam *Ruditapes decussatus* after exposure of xenobiotics (4,4'DDE and methoxychlor) (Girard et al., 2004) . GSH is a substrate for GST activity in all organisms including mud crabs. In the presence of GST protein, GSH will act as conjugator with xenobiotics by transferring the hydrogen ion and detoxification may occurs (Muposhi et al., 2015). This stipulate that GST is the response of biomolecules signaling for protection of cells against the oxidative stress (Mahboob et al., 2014).

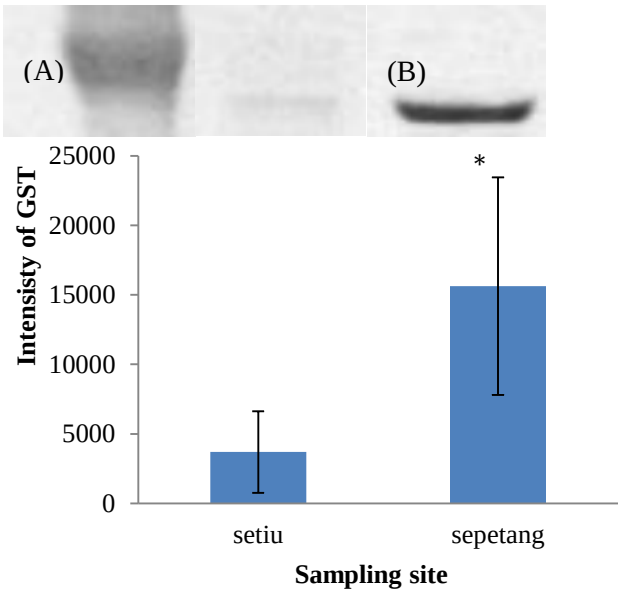
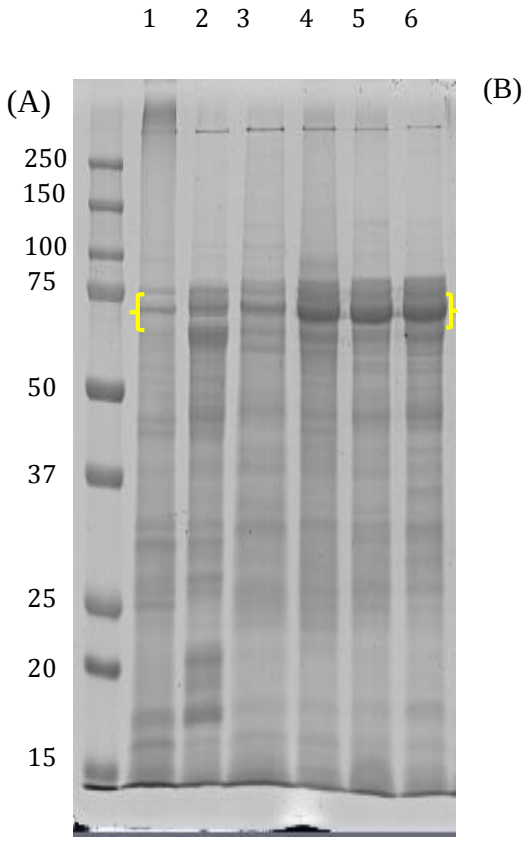


Figure-2: (A) Western blot for detection of glutathione-S-transferase (GST).

Lane 1 indicates proteins of hepatopancreas of mud crabs from Setiu Wetlands while lane 2 from Kuala Sepetang. (B) Intensity of GST protein bands from both locations. * indicates significant difference at $p< 0.05$ by using independent T-test.



Locations	Number of protein bands	Molecular weight (kDa)
Setiu Wetlands	18	108.9, 94.7, 76.4, 70.0, 63.4, 59.7, 55.7, 52.5, 49.6, 45.5, 43.6, 38.3, 32.1, 30.5, 26.5, 25.4, 17.4, & 16.3
Kuala Sepetang	18	132.0, 100.0, 75.0, 68.6, 64.4, 59.2, 54.7, 50.0, 46.3, 41.0, 38.6, 35.7, 31.9, 29.0, 26.7, 25.2, 17.8, & 16.0

Figure 3: (A) SDS-PAGE gel showing proteins profile in hepatopancreas of mud crabs. Lane 1, 2 and 3 for hepatopancreas of mud crabs from Setiu Wetlands while lane 4, 5 & 6 from Kuala Sepetang. (B) The number of protein bands and their molecular weight in hepatopancreas from both locations.

Other proteins expressed also can get involve with oxidative stress and detoxification process. From 1D SDS gel in figure 3, there was ~18 bands protein from hepatopancreas from both locations. At ~65-70 kDa, the density of this band was high in Kuala Sepetang compared to Setiu Wetlands. This protein might be related with heat-shock protein group. Heat-shock protein (HSPs) are present in all organisms. HSPs will be expressed during oxidative stress and involved in metabolism, growth, differentiation, programmed cell death, and fertilization (Yang et al., 2013). In recent study, the expressions of HSP 70 was high in Tilapia fish at the area that contaminated with trace metals of Cd and Zn (Muposhi et al., 2015). This is also reported by Hamer et al. (2004), HSP 70 were increased in mussel gills at the polluted area in Rovinj area. They also concluded that HSPs can reach maximum level after 3 days, a week or a month or persistent after exposure of trace metals.

Conclusion

High anthropogenic activities lead to the contaminations of trace metals in hepatopancreas of mud crabs from Kuala Sepetang. From our findings, GSH and GST protein were involved in detoxification of xenobiotics in hepatopancreas of mud crabs and can be good bioindicator in polluted area.

Acknowledgement

This research was funded by a grant from the Ministry of Higher Education (NRGS 53131 and Trans-FRGS 59291). Laboratory works were carried out at the Central Laboratory Facility, Universiti Malaysia Terengganu.

Contribution of Authors

Razali NSM: Data collection and manuscript writing
Kadir NHA: Data interpretation and manuscript writing
Omar WBW: Designed research methodology
Ikhwanuddin M: Manuscript final reading and approval
Amin NM: Conceived idea and manuscript final approval

Disclaimer: None.
Conflict of Interest: None.
Source of Funding: This research was funded by a grant from the Ministry of Higher Education (NRGS 53131 and Trans-FRGS 59291).

References

Amin NM, Omar WBW, Kadir NHA, Razali NSM, Ubaidillah FNAM and Ikhwanuddin M, 2018. Analysis of trace metals (Ni, Ci, and Zn) in water, mud and various tissues of mud crab, Scylla olivacea from Setiu Wetlands, Terengganu, Malaysia. J. Sustain. Sci. Manage. 13(2): 5–14.

Azlisham M, Vedamanikam VJ and Shazilli NAM, 2009. Concentrations of cadmium, manganese, copper, zinc, and lead in the tissues of the oyster (Crassostrea iredalei) obtained from Setiu Lagoon, Terengganu, Malaysia. Toxicol. Environ. Chem. 91(2): 251–258.

Bo L, Wang D, Li T, Li Y, Zhang G, Wang C and Zhang S, 2015. Accumulation and risk assessment of heavy metals in water, sediments, and aquatic organisms in rural rivers in the Taihu Lake region, China. Environ. Sci. Pollut. Res. 22(9): 6721–6731.

Bradford MM, 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248-54.

Chugh YP and Behum PT, 2014. Coal waste management practices in the USA : an overview. Int. J. Coal Sci. Technol. 1(2): 163–176.

El-Gazzar AM, Ashry KE and EL-Sayed YS, 2014. Physiological and Oxidative Stress Biomarkers in the Freshwater *Nile tilapia*, *Oreochromis niloticus* L., Exposed to Sublethal Doses of Cadmium. Alexandria J. Vet. Sci. 40: 29–43.

Fazhan H, Waiho K and Ikhwanuddin M, 2017. Non-indigenous giant mud crab , *Scylla serrata* (Forskål , 1775) (Crustacea : Brachyura : Portunidae) in Malaysian coastal waters : a call for caution. Marine Biodiver. Records. 10 (26): 1-7.

Fujii T, Mori K, Takahashi Y, Taniguchi N, Tonosaki A, Yamashita H and Fujii J, 2001. Immunohistochemical study of glutathione reductase in rat ocular tissues at different developmental stages. Histochem. J. 33(5):

267–272.

Gabel CA, Geoghegan KF, Kamath AV, Jermiin LS, Coggan M, Easteal S and Perregaux DE, 2002. Identification, Characterization, and Crystal Structure of the Omega Class Glutathione Transferases. J. Biol. Chem. 275(32): 24798–24806.

Girard JP, Hoarau P, Roméo M, Gnassia-Barelli M and Garello G, 2004. Effect of three xenobiotic compounds on Glutathione S-Transferase in the clam Ruditapes decussatus. Aquat. Toxicol. 68(1): 87–94.

Hamer B, Hamer DP, Müller WEG and Batel R, 2004. Stress-70 proteins in marine mussel Mytilus galloprovincialis as biomarkers of environmental pollution: A field study. Environ. Int. 30(7): 873–882.

Hazmi I, 2018. Fireflies population and the aquaculture industry (Coleoptera: Lampyridae) of the Sungai Sepetang, Kampung Dew, Perak, Malaysia. 22(2): 217–237.

Hissin PJ and Hilf R, 1976. A fluorometric method for determination of oxidized and reduced glutathione in tissues. Anal. Biochem. 74(1): 214–226.

Ikhwanuddin M, Bachok Z, Hilmi MG, Azmie G and Zakaria MZ, 2010. Species diversity, carapace width-body weight relationship, size distribution and sex ratio of mud crab, genus scylla from Setiu Wetlands of Terengganu. J. Sustain. Sci. Manage. 5(2): 97–109.

Javed M and Usmani N, 2013. Assessment of heavy metal (Cu, Ni, Fe, Co, Mn, Cr, Zn) pollution in effluent dominated rivulet water and their effect on glycogen metabolism and histology of Mastacembelus armatus. SpringerPlus. 2(1): 1–13.

Jusoff K and Bin Hj Taha DHD, 2014. Managing Sustainable Mangrove Forests in Peninsular Malaysia. J. Sustain. Develop. 1(1). DOI:10.5539/jsd.v1n1p88

Kamaruzzaman BY, Akbar John B, Maryam BZ, Jalal KCA and Shahbuddin S, 2012. Bioaccumulation of heavy metals (Cd, Pb, Cu and Zn) in Scylla serrata (Forsskal 1775) collected from Sungai Penor, Pahang, Malaysia. Pertanika J. Trop. Agric. Sci. 35(1): 183–190.

Karadag H, Firat O and Firat O, 2014. Use of oxidative stress biomarkers in cyprinus carpio L. for the evaluation of water pollution in ataturk dam lake (Adiyaman, Turkey). Bull. Environ. Contam. Toxicol. 92(3): 289–293.

Kitteringham NR, Powell H, Jenkins RE, Hamlett J, Lovatt C, Elsby R and Park BK, 2003. Protein expression profiling of glutathione S-transferase pi null mice as a strategy to identify potential markers of resistance to paracetamol-induced toxicity in the liver. Proteomics. 3(2): 191–207.

Kovačević TB, Borković SS, Pavlović SZ, Despotović SG and Saičić ZS, 2008. Glutathione as a suitable biomarker in hepatopancreas, gills and muscle of three freshwater crayfish species. Arch. Biol. Sci. 60(1): 59–66.

Leong R, Ling Z, Andrew T, Huat LL and Sen TS, 2017. Kuala Sepetang Estuary: Current use and Long-Term Sustainability. JSM Environ. Sci. Ecol. 5(3).

Lushchak VI, 2011. Environmentally induced oxidative stress in aquatic animals. Aquat. Toxicol. 101(1): 13–30.

Mahboob S, Balawi HFAA and Ahmed Z, 2014. An evaluation of water pollution in the wadi hanefah , a natural reservoir in Saudi Arabia using oxidative stress indicators in Poecilia latipinna, (January).

Muposhi VK, Utete B, Sithole-Niang 1 and Mukangenyama S, 2015. Active biomonitoring of a subtropical river using glutathione-s-transferase (GST) and heat shock proteins (HSP 70) in oreochromis niloticus as surrogate biomarkers of metal contamination. Water SA. 41(3): 425–431.

Paital B and Chainy GBN, 2010. Antioxidant defenses and oxidative stress parameters in tissues of mud crab (*Scylla serrata*) with reference to changing salinity. Compar. Biochem. Physiol. Part C. 151: 142–151.

Razali NSM, Ubadillah FNAM, Omar WBWO, Kadir NHA and Amin NM, 2017. A Preliminary Proteomic-Based Study Of Wild Mud Crab (Scylla Olivacea) Responses Towards The Suroundings Of Setiu Wetlands Lagoon, Terengganu. Malays. Appl. Biol. 46(4): 157-162.

Singaram G, Harikrishnan T, Chen FY, Bo J and Giesy JP, 2013. Modulation of immune-associated parameters and antioxidant responses in the crab (Scylla serrata) exposed to mercury. Chemosphere. 90(3): 917–928.

Słupik G, Łaszczyca P and Kafel A, 1999. Heavy

Metals, Glutothione S T Poll. 685–688.
Stoliar BO and Lushchak VI, 2012. Environmental Pollution and Oxidative Stress in Fish. Oxidative Stress - Environmental Induction and Dietary Antioxidants. DOI: 10.5772/38094
Tchounwou PB, Yedjou CG, Patlolla AK and Sutton DJ, 2012. Mol. Clin. Environ. Toxicol. 101: 1–30.
Thi N, Nguyen B, Chim L, Lemaire P, Wantiez L, Ea L and Nam V, 2014. Archimer. 434: 499–509.
Vince Cruz CCE, Ramos G and Ablan-Lagman MC, 2015. Heavy metal levels in mud crabs (Scylla spp.) from East Bataan Coast. Environ. Sci. Pollut. Res. 22(8): 6359–6363.
Viswanathan C and Raffi SM, 2015. The natural diet of the mud crab Scylla olivacea (Herbst , 1896) in Pichavaram mangroves, India. Saudi J. Biol. Sci. 22(6): 698–705.
Yang Y, Ye H, Huang H, Li S, Liu X, Zeng X and Gong J, 2013. Expression of Hsp70 in the mud crab, Scylla paramamosain in response to bacterial, osmotic, and thermal stress. Cell Stress Chaperon. 18(4): 475–482.
Yildirim NC, Benzer F and Danabas D, 2011. Evaluation of environmental pollution at Munzur River of Tunceli applying oxidative stress biomarkers in Capoeta trutta (Heckel, 1843). J. Ani. Plant Sci. 21(1): 66–71.