International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: SJIF: 5.995
Available Online at www.journalijcar.org
Volume 6; Issue 12; December 2017; Page No. 7914-7918
DOI: http://dx.doi.org/10.24327/ijcar.2017.7918.1252

Research Article

# COMPARATIVE VARIATIONS IN PROTEIN CONTENTS OF THREE FRESHWATER SNAILS *BELLAMYA BENGALENSIS, MELLANOIDES TUBERCULATA* AND *LYMNAEA ACUMINATA* COLLECTED FROM DEDARGAON RESERVOIR OF DHULE DISTRICT (M. S.) INDIA, ON EXPOSURE TO HEAVY METALS

**Waykar B. B and Petare R. K***

Department of Zoology, Dr. B. A. M. University, Aurangabad, (M. S. India)

**ARTICLE INFO**

***Article History:***
Received 16th September, 2017
Received in revised form 25th October, 2017
Accepted 23rd November, 2017
Published online 28th December, 2017

***Key words:***

Protein content, Dedargaon, Snails, Heavy metals, *Bellamya bengalensis, Mellanoides tuberculata, Lymnaea cuminata*

**ABSTRACT**

Protein metabolism in the body of organism is disturbed by various toxicants like heavy metals, pesticides etc.After chronic exposure to zinc, copper, cadmium and lead for 10 and 20 days, the changes in protein contents were observed in whole soft body tissue of three experimental snail species, *Bellamya bengalensis, Mellanoides tuberculata* and *Lymnaea acuminate* collected from Dedargaon reservoir of Dhule district of Maharashtra, India. The results indicates that the protein contents in whole soft body tissues of three experimental snail species were decreased as compared to those of control snails. Among all treated heavy metals, Cd caused more decrease in protein as compared to Zn, Cu and Pb in three snail species. The protein decreased was more after 20 days as compared to 10 days of exposure. A maximum depletion in protein contents were observed in whole soft tissue of *Lymnaea acuminata* in response to accumulated levels of Zn, Cu and Pb while *Mellanoides tuberculata* for Cdas compare to studied snail species. Therefore it is concluded that, snail species *Lymnaea cuminata* is the sentinel organism for biochemical study of heavy metals Zn, Cu and Pb, and *Mellanoides tuberculata* for Cd in freshwater ecosystem.



## INTRODUCTION

The increasing levels of heavy metal concentration in the environment are of great concern. Due to their property of bioaccumulation, persistent in nature and potential of higher toxicity, heavy metal bioaccumulation in food chain is highly dangerous to human health (Censi *et al.,* 2006). Accumulated heavy metals induce generation of reactive oxygen species (ROS) that causes oxidative stress (Regoli, 2000). The ROS attack unsaturated fatty acids of the cell membrane which leads to formation of lipid peroxidation (Viarengo, 1989). The oxidative stress leads to damage of biomolecules like proteins, lipids, nucleic acids and carbohydrates (Kaoud and El-Dahshan, 2010).

The heavymetal causes biochemical responses within individual organisms, even at low concentrations, before these effects are observed at higher levels of organization (Sarkar *et al.,* 2006). Heavy metal causesinhibition of enzymes, genetic damage, metabolic disorders, and also alters the physiological and biochemical processes of animals (Waykar, 2000). Heavy metals mainly react with protein and interferes the physiological activities (Gulbhile and Zambare, 2013).

**Corresponding author:* **Petare R. K**
Department of Zoology, Dr. B. A. M. University, Aurangabad, (M. S. India)

Biochemical responses in aquatic organisms have been used in several monitoring programs to study the anthropogenic pollution (Cajaraville *et al.,* 2000).

Protein is most abundant biological macromolecules and is extremely versatile in many physiological functions like homeostasis, detoxification of toxicants etc. Protein metabolism in the body of organism is disturbed by various toxicants like heavy metals, pesticides etc. The secretion or suppression of the proteins is greatly influenced by metal pollution (Kohler *et al.,* 2001). Many researchers reported that accumulated heavy metal causes biochemical alterations in organism (Deshmukh, 2013; Rahane, 2014). David *et al.,* (2003) studied nickel induced changes in some aspects of protein metabolism in the tissues of *Pila globosa.* Singh *et al.,* (2010) studied the effect of deltamethrin on protein, amino acid and nucleic acids levels in foot and nervous tissue of *Lymnaea acuminata.*Mahajan (2012) studied changes in protein content in *Bellamya dissimilis* on chronic exposure to copper sulphate. Shinde (2013), Bhangale and Mahajan (2015) reported that protein content gradually decreases with increase in exposure time. Hence, the estimation of protein is one of the most important parameter in toxicity testing to evaluate metal pollution in the organism.

Proteins form the architecture of the cell. The structural proteins are used as energy source under stressful conditions. In stress conditions, the snails need more energy to detoxify the toxicants and to overcome the induced stress. As snails have a limited amount of carbohydrates, the next alternative source of energy to meet the increased energy demand is proteins.

***Importance of laboratory study***

Determination of toxicity of pollutants and organism's responses in laboratory condition is essential for correct and accurate conclusion in relation to natural sites. Laboratory study helps todetermine the most appropriate sentinel snail species among available experimental species to monitor the heavy metal pollution in environment. It is important to develop a database under laboratory condition, which might be used for future biomonitoring of heavy metal pollution in freshwater ecosystem. It is very important to understand dynamic process of bioaccumulation in protecting the organisms from adverse effect of chemical exposure in the laboratory. Aquatic organisms have ability to absorb metals from the surrounding environment and to accumulate them within their bodies thousands time higher than their concentrations in the environment (Podgurskaya *et al.,* 2004).

***Objectives of the Study***

1. To study the effect of heavy metals Protein contents in soft body tissues of freshwater snails *Bellamya bengalensis Mellanoides tuberculata* and *Lymnaea accuminata* from Dedargaon reservoir of Dhule district under laboratory conditions.
2. To develop a biochemical database using various freshwater snail species which might be used in finding the most appropriate sentinel snail species for metal pollution monitoring programme in the freshwater ecosystem.

## MATERIALS AND METHODS

The total protein content from varioustissues whole soft body tissues of control and experimental snails exposed to chronic concentration of heavy metals for 10 and 20 days. The snails species *Bellamya bengalensis Mellanoides tuberculata* and *Lymnaea accuminata* were collected from Dedargaon reservoir of Dhule district, Maharashtra state, India. After collection, animals were brought to laboratory and were cleaned and acclimatized in aquarium containing dechlorinated tap water for 10 days. During acclimatization and experiment, the animals were fed with fresh water algae and water of aquarium was changed after every 24 h. After acclimatization, the active, medium, uniform sized and healthy snails of each species were selected by measuring their shell length and divided into five groups as below.

1st group was maintained as control.
2$^{nd}$ group was exposed to chronic concentration 0.1990 ppm ($LC_{50/10}$) of Zn up to 20 days.
3$^{rd}$group was exposed to chronic concentration 0.5451 ppm ($LC_{50/10}$) of Cu up to 20 days.
4$^{th}$ group was exposed to chronic concentration 1.7345 ppm ($LC_{50/10}$) of Cd up to 20 days.
5$^{th}$ group was exposed to chronic concentration 06.753 ppm ($LC_{50/10}$) of Pb up to 20 days.

For each metal and each snail species $LC_{50}$ values for 96 h were determined by Probit analysis method (Finney, 1971). The average values of $LC_{50}$ for all three snails species for each metal were calculated and average concentration was diluted ten times to expose each species of snails for each metal, so that every species received equal dose to study comparative bioaccumulation potential. In nature all animals in the same habitat are exposed to same concentration and hence to develop sentinel model average of LC50 values were used. *Bellamya bengalensis Mellanoides tuberculata* and *Lymnaea accuminata*

Medium sized 10 animals from *Bellamya bengalensis,20 animals from Mellanoides tuberculata* and 30 animals from *Mellanoides tuberculata* were selected from each of experimental and control groups were dissected after 10 and 20 days of exposure period and their whole soft body tissues were dried in oven at 70°-80°C. After oven drying dry weight of tissue was measured and blended into powder. Dry weight of each animal was used to calculate the metal concentration per unit body weight (µg/g) and metal body burden (µg/individual).

Protein content of the tissues was estimated by method of Lowry's (Lowry *et al.*, 1951). 10mg of dry powder of each tissue was homogenized in small amount of 10% TCA and the homogenate was diluted to 10 ml by 10% TCA. Then it was centrifuged at 3000 rpm for 15 minutes. The protein precipitate at the bottom of centrifuged tubes was dissolved in 10 ml 1.0 N NaOH solution. 0.1 ml of this solution of each powder was taken in three test tubes containing 0.9 ml distilled water and 4.0 ml freshly prepared Lowry's 'C' was added. After adding 0.5 ml Folin's phenol reagent, the test tubes were incubated in dark for 30 minutes at 37$^{0}$C. The optical density of blue colour developed was read at 530nm on a double beam Spectrophotometer (Elico BL 200). The blank was prepared in same way without dissolved protein precipitate.

Each observation was verified by taking three replicates. Results are expressed as mean (±) standard deviation (SD). Difference among the means of control and treatment was analysed by Students''t' test. The results are given in the form of tables and graphs with percent change over control and the results of test of significance ($P < 0.05$).

## OBSERVATIONS AND RESULTS

In the present research, changes in proteincontent studied comparatively in three experimental freshwater snails species, *Bellamya bengalensis, Mellanoides tuberculata* and *Lymnaea accuminata* after exposure to chronic concentrations of zinc (0.1990 ppm), copper (0.5451 ppm), cadmium (1.7345 ppm) and lead (6.753 ppm) for 10 and 20 days along with control animals. The results are summarized in table no. 1 and figure no. 1(a) and 1(b). Significant variations ($P<0.05, P<0.01, P<0.001$) were observed in the mean values.

***Changes in protein contents***

The results indicates that the protein contents in whole soft body tissues of three experimental snail species were decreased as compared to those of control snails. Among all treated heavy metals, Cd caused more decrease in protein as compared to Zn, Cu and Pb in three snail species. The protein decreased was more after 20 days as compared to 10 days of exposure. A maximum depletion in protein contents were observed in whole soft tissue of *Lymnaea acuminata* in response to accumulated levels of Zn, Cu and Pb while *Mellanoides tuberculata* for Cdas compare to studied snail species.

**Table 1** Profile of protein contents in whole soft body tissues of freshwater snails, after chronic exposure to different heavy metals (Values are in mg/ 100 mg of dry weight)

| Sr. No. | Snail Species | Treatment | 10 Days | 20 Days |
|---|---|---|---|---|
| 1 | *Bellamya bengalensis* | Control | 51.6±1.89 | 50.34±1.95 |
| | | Zn | 48.99±2.16 (-5.23) | 47.01±2.02 (-6.63) |
| | | Cu | 47.60±2.50 (-7.91) | 45.16±2.40 (-10.30) |
| | | Pb | 45.95±2.27 (-11.10) | 42.77±1.80 (-15.06) |
| | | Cd | 44.64±1.92 (-15.78) | 42.1±2.11 (-19.59) |
| | P-Value | | 0.00000005** | 0.0038* |
| 2 | *Mellanoides tuberculata* | Control | 41.36±2.83 | 39.97±2.14 |
| | | Zn | 40.44±1.63 (-2.23) | 38.50±1.42 (-3.69) |
| | | Cu | 39.88±2.09 (-3.58) | 37.60±1.39 (-5.95) |
| | | Pb | 38.47±1.62 (-6.98) | 35.31±1.90 (-11.67) |
| | | Cd | 34.77±1.07 (-18.97) | 30.70±1.78 (-23.21) |
| | P-Value | | 0.08638406 NS | 0.0027** |
| 3 | *Lymnaea acuminata* | Control | 45.42±1.64 | 44.51±1.38 |
| | | Zn | 42.87±1.04 (-5.63) | 39.30±0.56 (-11.70) |
| | | Cu | 40.61±1.48 (-10.60) | 37.62±1.38 (-15.47) |
| | | Pb | 39.20±2.56 (-13.71) | 35.51±1.45 (-20.23) |
| | | Cd | 39.03±1.36 (-16.38) | 34.51±1.40 (-22.47) |
| | P-Value | | 0.00377131** | 0.0000** |

( ±) indicates standard deviations.
(-) values indicates percent change over control.
*P<0.05, **P<0.01, ***P<0.001, NS- Non significant.

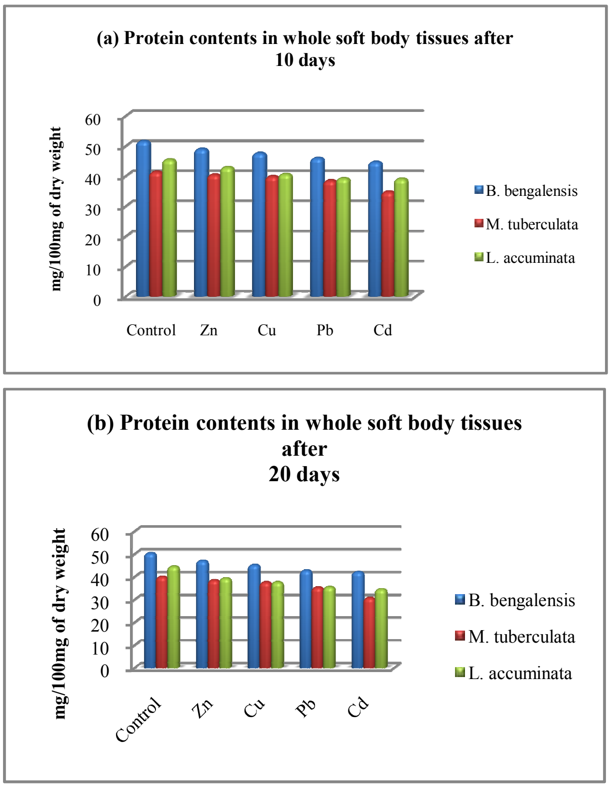


**Figure 1** Profile of protein contents in whole soft body tissues of freshwater snails, after chronic exposure to different heavy metals (Values are in mg/100mg of dry weight).

Overall results showed that the protein contents were depleted more after 20 days as compared to 10 days of exposure due to increase in metal concentrations in all three snail species. It was also observed that, Cd caused more decrease in protein contents as compared to Zn, Cu and Pb in three snail species. The study indicates that maximum depletion in protein contents were observed in whole soft tissue of *Lymnaeaacuminata* in response to bioaccumulation of Zn, Cu and Pb while *Mellanoidestuberculata* for Cdas compare to studied snail species. Thus, proteins levels in the tissues after exposure to heavy metals can be considered as the indices for stress.

## DISCUSSION

Animals when exposed to toxicants (xenobiotics) shows biochemical changes. Biochemical study of animals in laboratory condition is an important diagnostic tool in the assessment of risk and hazards of potential animal or human exposure (Krishna and Ramchandran, 2009). Change in proteins, ascorbic acid, DNA and RNA levels in the soft body tissues of aquatic organisms after exposure to metals can be considered as indices of stress.

Most of the toxicants interact with enzymes, metabolites or other cellular components of the organisms. They affect the integrated functions like survival, growth, reproduction and behaviour of the organism (Abou *et al.,* 2005). It is well known that long term toxicant stress adversely affects overall metabolism in the cell. Protein, lipid and nucleic acid chains are sensitive to oxidative stress and susceptible to oxidizing reactions. Heavy metals have affinity for metal sensitive "thiol'' group. These block functional group of proteins, remove the essential metal, lead to changes, denature enzymes and disturb cell integrity (Hall, 2000).

Many researchers reported that heavy metals stress leads to alterations in protein, ascorbic acid, DNA and RNA in molluscs (Nawale, 2008; Andhale and Zambre, 2011; Shinde, 2013; Deshmukh, 2013).

In the present investigation, after chronic exposure to the heavy metals Zn, Cu, Cd and Pb for 10 and 20 days a significant decrease in protein content was observed in whole soft body tissue of experimental freshwater snail species *Bellamya bengalensis, Mellanoides tuberculata and Lymnaea acuminata* as compared to snails maintained as control. The results of ANOVA test indicated that the variations between the mean values of protein contents were statistically significant ($P<0.05$, $P<0.01$, $P<0.001$) in three experimental snails species.

Among the tested heavy metals, Cd caused more depletion in protein content in three experimental freshwater snail species as compared to Zn, Cu and Pb. Highest depletion of protein content was observed in whole soft body tissues of *Lymnaea acuminate* in response to accumulated levels of Zn, Cu and Pb, while in *Mellanoides tuberculata* for Cd.

The result clearly demonstrated that, there was progressive decrease in the protein content as exposure period was increased. Similar results were obtained by (Satyaparameshwar *et al.,* 2006; Shide, 2013; Deshmukh, 2013).

Heavy metals are inducers of reactive oxygen species (ROS). Generation of ROS can lead to decrease in capacities of the antioxidants system causing enormous oxidative damage to proteins, lipids and DNA (Livingstone 2001). The decrease in protein content of tissue indicates enhancement of proteolysis to meet the high energy demands under heavy metal stress. Lomte and Alam, (1982) recorded decrease in protein content in various body tissues of snails, *B. bengalensis* after malathion treatment. Khan *et al.* (2001) reported decreased level of protein in gastropod *Babylonia spirata* due to increased utilization of protein under the cadmium stress. Radwan *et al.*, (2008) observed a marked reduction in the level and distribution of proteins after 7 and 14 days in digestive gland of land snail, *Eobania vermiculata***.** According to Kulkarni *et al.,* (2005), the decrease in protein content may be due to possible utilization of protein for metabolic purposes and enhanced proteolysis to meet the higher energy demand under toxicant stress. The depletion of protein may be due to more utilization of protein in stressful environmental conditions and attributed to the inhibition of protein synthesis (Joshi and Kulkarni, 2011). Shinde (2013) and Deshmukh (2013) observed decrease in protein in different tissues of experimental bivalves after chronic exposure to heavy metals for 10 and 20 days as compare to control bivalves.

## CONCLUSION

There was significant decrease in proteins, ascorbic acid, and DNA and RNA contents in whole soft body tissues of three experimental freshwater snail species after chronic exposure to heavy metals Zn, Cu, Cd and Pbfor 10 and 20 days.

1. There was progressive decrease in the proteins, contents of three experimental freshwater snail species as exposure period was increased.
2. Highest depletion in proteins contents was observed in whole soft body tissues of *Lymnaea acuminata* after exposure and bioaccumulation of heavy metals Zn, Cu and Pb, while in *Mellanoides tuberculata* for heavy metal Cd as compare to other snails' species.
3. Among the tested heavy metals, Cd caused highest decrease in proteins contents in whole soft body tissues of three experimental freshwater snail species.
4. It is concluded that, snail species *Lymnaea cuminata* is the sentinel organism for biochemical study of heavy metals Zn, Cu and Pb, and *Mellanoide stuberculata* for Cd in freshwater ecosystem.

**Acknowledgement**

The authors gratefully acknowledge Department of Zoology, Dr. B. A. Marathwada University, Aurangabad (M.S.) for providing laboratory facility for carrying out this work.

## References

Abou El-Niga EH, Khalid El-Moselhy and Hamed MA (2005): Toxicity of cadmium and copper and their effect on some biochemical parameters of the imarine fish Mugilseheli. *Egyptian Journal 0f Aquatic Research*, 31(2): 601-71.

Andhale, A.V. and Zambare, S. P. (2011): Effect of nickel induced biochemical alterations in fresh water bivalve, Lammellidens marginalis, *Journal of Ecobiotechnology*, 3(11): 18-24.

Bhangale B. S. and Mahajan P. R., (2015): Biochemical changes in various Organs of Lamellidens consobrinus due to Toxicity stress. *International Research Journal of Biological Sciences*, (4), 11.

Cajaraville, M. P., Bebianno, M. J., Blasco, J., Porte, C., Sarasquete, C., Viarengo, A. (2000): The use of biomarkers to assess the impact of pollution in coastal environments of the Iberian Peninsula: a practical approach, *Sci. Total. Environ*., 247(2-3): 259-311.

Censi, P., Spoto, S. E., Saiano, F., Sprovieri, M. and Mazzola, S. (2006): Heavy metals in coastal water system. A case study from the North Western Gulf of Thailand. *Chemosphere*, 64: 1167-1176.

David, C. P. (2003): Establishing the Impact of Acid Mine Drainage through Metal Bioaccumulation and Texa Richness of Benthic Insects in a tropical Asian Stream (The Philippines). *Environmental Toxicology and Chemistry*, (22) 12:2952-2959.

Deshmukh, G. M. (2013): Biomonitoring of heavy metal pollution of jayakwadi reservoir at Paithan by using bivalves as bioindicators. Ph. D. thesis submitted to Dr. B. A. M. University, Aurangabad, (M.S.) India.

GulbhileShamsundar, D. and ZambareSureshchandra, P. (2013): Curative Role Of Caffeine On Mercury Induced Alterations Of Protein Levels In The, Lamellidenscorrianus (LEA). *International Journal of Development Research*. (3), 06:001-004.

Hall, J. L., (2000): Cellular mechanisms for heavy metal detoxification and tolerance. J.Exp. Botany, 53: 1-11.

Joshi P. P. and Kulkarni G. K., (2011):Cypermethrin and fenvalerate induced protein alterations in freshwater crab Barytelphusacunicularis (Westwood). *Recent Research in Science and Technology,* (12): 07-10.

Kaoud, H. A., El-Dahshan, A. R. (2010):Bioaccumulation and histopathological alterations of the heavy metals in Oreochromisniloticus fish. *Nat. Sci*. 8(4):147-156.

Khan A. K., Shaikh A.M. and Ansari N.T., (2001): Cadmium chloride toxicity in protein level from body parts and whole body of marine edible gastropod, Babylonia spirata Uttar Pradesh *J. Zool*., (3):203-206.

Kohler, T., Van Delden, C., Curty, L. K., Hamzehpour, M. M., and Pechere, J. C., (2001): Overexpression of the MexEF-OprN multidrug efflux system affects cell-to-cell signaling in *Pseudomonas aeruginosa*. *J Bacteriol* 183: 5213-5222.

Krishna H., and Ramachandran A. V., (2009): Biochemical alterations induced by acute exposure to a combination of chlorpyrifos and lead in winstar rats*. Biology and Medicine,* 1(2): 1-6.

Kulkarni A. N., Kamble S. M., and Keshavan R, (2005): Studies on impact of Hildan on biochemical constituents in the freshwater mussel, Lamellidens corrianus. *J. Aqua. Biol,* 20 (1): 101-104.

Livingstone, D. R. (2001): Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. *Mar Pollut Bull*, 42:656-666.

Liu, F. and Jan, K.Y. (2000): DNA damage in arsenate and cadmium treated bovine aortic endothelial cells. *Free Radic. Biol. Med*. 28: 55-63.

Lomte V. S. and Alam S. M., (1982): Changes in biochemical components of the prosobranch Bellamya (Viviparus) bengalensis on exposure to malathion. Proc. Symp. Physiol. Resp. Anim. Pollutants.

Lowry, O. M., Rosenbrough, N. J., Farr, A. C., Randall, R. F. (1951):Protein estimation with Folin Phenol reagent. *J. Biol. Chem*., 193: 265-275.

Mahajan, P. R., (2012): Cure of heavy metal ($CuSO_4$) induced alterations in an experimental model, *Bellamya dissimilis* by caffeine (1, 3, 7 - Trimethylxanthine). *Indian Journal of Fundamental and Applied Life Sciences* Vol.( 2), 4:34-39.

Nawale, S. P. (2008): Synergistic effect of Caffeine (1, 3, 7-Trymethylxanthine) and ascorbic acid on heavy metal induced alterations in an experimental model, Lamellidenscorrianus (Lea). Ph. D. Thesis, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad (M.S.) India.

Podgurskaya O. V., Kavun V. Ya, and Lukyanova O. N., (2004): Accumulation and Distribution of Heavy Metals in Organs of Mussel Crenomytllus grajaims and Modiolus modiolus from upwelling regions of the Okhotsk Sea and Sea of Japan. *Biologiamorya*, 30(3): 219-226.

Radwan M. A., Essawy A. E., Abdelmeguied N. E., Hamed S. S., and Ahmed A. E., (2008): Biochemical and histochemical on the digestive gland of Eobania vermiculata snails treated with carbamate pesticides. *Pestic.Biochem. Physiol*. 90:154-167.

Regoli, F. (2000): Total oxyradical scavenging capacity (TOSC) in polluted and translocated mussels: a predictive biomarker of oxidative stress. *AquatToxicol*, (50):351-361.

Sarkar, A., Ray, D., Shrivastava, Amulya, N. and Sarker, S. (2006): Molecular Biomarkers: Their Significance and application in Marine Pollution Monitoring. *Ecotoxicology*, (15): 333-340.

Satyaparameshwar, K., Reddy, T.R. and Kumar, N.V. (2006): Effect of chromium onprotein metabolism of freshwater mussel, Lamellidens marginalis. *J.Environ. Biol.,* (27): 401-403.

Shinde S. M. (2013): Assessment of the bivalves to develop the biomarkers of the heavy metal pollution in freshwater. Ph.D. Thesis, Dr. B. A. M. University Aurangabad (M.S.), India.

Singh, A. N., Shukla, A. K., Jagannadham, M. V. and Dubey, V. K. (2010): Purification of a novel cysteine protease, procerain B, from Calotropisprocera with distinct characteristics compared to procerain. *Process Biochemistry.*, (45):399-406.

Viarengo, A., Pertica, M., Canesi, L., Accomando, R., Mancinelli, G. and Orunesu, M. (1989): Lipid peroxidation and level of antioxidant compounds (GSH, vitamin E) in the digestive glands of mussels of three different age groups exposed to anaerobic and aerobic conditions. *Mar Environ Res*, (28): 291-295.

Waykar, B. B. (2000): Effect of pesticides on some physiological activities of fresh water bivalve, Parreysia cylindrica. Ph. D. Thesis, Marathwada University, Aurangabad, India.

**How to cite this article:**

Waykar B. B and Petare R. K (2017) 'Comparative variationsin protein contents of three freshwater snails bellamya bengalensis, mellanoides tuberculata and lymnaea acuminata collected from dedargaon reservoir of dhule district (m. S.) India, on exposure to heavy metals', *International Journal of Current Advanced Research,* 06(12), pp. 7914-7918.
DOI: http://dx.doi.org/10.24327/ijcar.2017.7918.1252

*******