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Seasonal Variations in Protein Content of Whole body Tissues of Three Freshwater Snails from Four Reservoirs of Dhule district of M. S. (India) in Relation to heavy Metal Pollution

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ABSTRACT: Protein metabolism in the body of organism is disturbed by various toxicants like heavy metals, pesticides etc. The total protein contents in soft body tissues of three freshwater snails *Bellamya bengalensis*, *Mellanoides tuberculata* and *Lymnaea acuminata* were estimated seasonally from four reservoirs of Dhule district. The observed results were compared in relation to the heavy metal pollution. The results showed lower protein contents in whole soft body tissues of snail species *Bellamya bengalensis* collected from Dedargaon reservoir followed by Latipada and Malangaon reservoirs and higher at Jamkhedi reservoir. For *Mellanoides tuberculata* lower protein contents were found in Dedargaon reservoir than Latipada reservoir while for *Lymnaea acuminata* lower protein contents were found in Dedargaon than Malangaon reservoir. This indicated that snail species inhabiting at Dedargaon reservoir are more under environmental stress than other three reservoirs, while snail species inhabiting at Jamkhedi reservoir are under less environmental stress. Thus, the order of metal pollution at four reservoirs was Dedargaon > Latipada > Malangaon > Jamkhedi. The results also indicated that protein contents were lowest in summer and highest in monsoon and intermediate in winter season in three snail species at four studied reservoirs.

KEYWORDS: Total protein, Freshwater snails, reservoirs of Dhule district, Seasonal variation, heavy metals.

I. INTRODUCTION

The cumulative levels of heavy metal concentration in the environment are of great concern. When animals are exposed to polluted aquatic environment containing metals, these metals accumulate in various tissues significantly (Fernandes *et al.*, 2008). Accumulated heavy metals induce generation of reactive oxygen species (ROS). The ROS attack unsaturated fatty acids of the cell membrane which leads to formation of lipid peroxidation (Viarengo, 1989). Increased level of lipid peroxidation is responsible for decreased in the levels of GSH and antioxidant enzymes activities that results in oxidative stress (Regoli, 2000). The oxidative stress causes oxidative damage to biomolecules like proteins, lipids, nucleic acids and carbohydrates (Araujo *et al.*, 2006; Kaoud and El-Dahshan, 2010). Heavy metals mainly react with protein and interferes the physiological activities (Gulbhile and Zambare, 2013). Heavy metals are known to induce many changes in biochemical and physiological dysfunctions of the organisms.

The information obtained through conventional metal pollution monitoring system may be insufficient, which leads to inaccurate water quality assessment. To overcome this problem, most researchers use biochemical analysis of benthic organisms as monitors of both levels and long term influence of pollutant within an ecosystem.

It is assumed that, even at low concentrations, toxicant causes biochemical responses within individual organisms, before these effects are observed at higher levels of organization (Sarkar *et al.*, 2006). The responses are specific to particular toxicant. Biochemical responses in aquatic organisms have been used in several monitoring programs to study the anthropogenic pollution (Cajaraville *et al.*, 2000).

In past, monitoring programs were often carried out to investigate water quality. These include chemical and biological parameters. The use of biochemical markers was less frequent. But recently, more attention has been given to propose these biomarkers of exposure and effect, in toxicity testing aiming an application in pollution monitoring (Goulet *et al.*, 2005). Changes in biological structures and functions in response to heavy metal pollution helps to assess the health of aquatic animals as early warning signals (EWS) of various environmental risks (Venier and Zampieron, 2005).

Recently, the studies on the physiological and biochemical responses of the molluscs to environmental agents have been expanded significantly. Changes in biochemical components such as proteins, ascorbic acid, DNA and RNA

of organisms are useful to know effect of different toxicants and defensive mechanism against toxic effects of heavy metals of the body. These biochemical components are indices of pollution as they are useful to determine nutritional status, health and vigor of an organism.

Protein is most abundant biological macromolecules and is extremely versatile in many physiological functions like homeostasis, detoxification of toxicants etc. The secretion or suppression of the proteins is greatly influenced by metal pollution (Kohler *et al.*, 2001). Protein is one of the main targets of free radicals attack. Radicals react with protein and amino acids to oxidize and cross-link them. This leads to impair the function of cellular and extracellular proteins like enzymes and connective tissue proteins permanently.

II. RELATED WORK

Many researchers reported that accumulated heavy metal causes biochemical alterations in organism (Rajkumar and Milton, 2011; Deshmukh, 2013; Rahane, 2014). David *et al.*, (2003) studied nickel induced changes in some aspects of protein metabolism in the tissues of *Pila globosa*. Satyaparneshwar *et al.*, (2006) observed that decrease in protein level, glutamate dehydrogenase activity in the tissues like muscle, gills and mantle exposed to chromium. 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. Hence, the estimation of protein is one of the most important parameter in toxicity testing to evaluate metal pollution in the organism.

III. METHODOLOGY

The total protein, determined from whole soft body tissues of *Bellamya bengalensis*, *Melanoides tuberculata* and *Lymnaea acuminata* collected seasonally during summer, monsoon and winter from four reservoirs of Dhule district. Whole soft body tissues were removed and dried at 70^o to 80^oC in the oven till the constant weight of dry tissues was obtained.

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 supernatant was used for ascorbic acid estimation. 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^oC. 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.

The protein contents in different tissues were calculated by referring standard graph value and it was expressed in terms of mg protein/100 mg of dry tissue. The Bovine serum albumen was used as a standard.

IV. EXPERIMENTAL RESULTS

The protein, contents were determined from whole soft body tissues of freshwater snails *Bellamya bengalensis*, *Melanoides tuberculata* and *Lymnaea acuminata* collected from Dedargaon, Latipada, Malangaon and Jamkhedi reservoirs during three seasons and obtained results were presented in table no; 1. The results of ANOVA test indicated that the difference between the mean values of protein, were varied significantly between reservoirs, seasons and snail species (P<0.05, P<0.01, P<0.001).

1. Dedargaon reservoir:

Protein contents in whole soft body tissue of *Bellamya bengalensis* were 35.65, 42.79 and 39.70(mg/100mg dry weight) in summer, monsoon and winter season respectively.

In whole soft body tissue of *Melanoides tuberculata*, protein contents were 24.70, 33.27 and 30.51 (mg/100mg dry weight) in summer, monsoon and winter season respectively.

In whole soft body tissue of *Lymnaea acuminata* protein contents were 28.92, 38.08 and 35.24 (mg/100mg dry weight) during summer, monsoon and winter season respectively.

2. Latipada reservoir: In whole soft body tissue of *Bellamya bengalensis* protein contents were 41.75, 46.14 and 43.64 (mg/100 mg dry weight) during summer, monsoon and winter season respectively.

On the other hand protein contents in whole soft body tissue of *Melanoides tuberculata* were 31.41, 40.20 and 37.85 (mg/100mg dry weight) during summer, monsoon and winter season respectively.

3. **Malangaon reservoir:** During seasonal study periods summer, monsoon and winter season protein contents were 43.68, 51.18 and 47.83(mg/100mg dry weight) in whole soft body tissue of *Bellamyia bengalensis* respectively.
In whole soft body tissue of *Lymnaea acuminata* protein contents were 33.76, 42.61 and 38.76 (mg/100mg dry weight) during summer, monsoon and winter season respectively.
4. **Jamkhedi reservoir:**
During seasonal study periods summer, monsoon and winter season, protein contents were 47.44, 55.10 and 51.91(mg/100mg dry weight) in whole soft body tissue of *Bellamyia bengalensis*.

Table 1:- Profile of protein contents in whole soft body tissues of freshwater snails from four reservoirs of Dhule district (Values are in mg/100mg of dry weight).

Sr. No.	Snail Species	Reservoir	Summer	Monsoon	Winter	P-Value	
01	<i>Bellamyia bengalensis</i>	Dedargaon	35.65±1.05	42.79±0.98	39.70±1.59	0.00910**	0.51287 ^{NS}
02	<i>Bellamyia bengalensis</i>	Latipada	41.75±1.60	46.14±1.31	43.64±1.14	0.11891 ^{NS}	0.84369 ^{NS}
03	<i>Bellamyia bengalensis</i>	Malangaon	43.68±1.70	51.18±1.07	47.83±1.58	0.02986*	0.82720 ^{NS}
04	<i>Bellamyia bengalensis</i>	Jamkhedi	47.44±1.67	55.10±0.38	51.91±1.27	0.00694**	0.56639 ^{NS}
05	<i>Mellanoidea tuberculata</i>	Dedargaon	24.7±0.77	33.27±1.56	30.51±1.03	0.00015***	0.01313*
06	<i>Mellanoidea tuberculata</i>	Latipada	31.41±1.35	40.20±1.01	37.85±1.30	0.00582**	0.56639 ^{NS}
07	<i>Lymnaea acuminata</i>	Dedargaon	28.92±1.39	38.08±1.16	35.24±1.89	0.00302**	0.18142 ^{NS}
08	<i>Lymnaea acuminata</i>	Malangaon	33.76±0.64	42.61±1.5	38.76±1.60	0.01202*	0.77319 ^{NS}

(±) indicates standard deviations.

*P<0.05, **P<0.01, ***P<0.001, NS- Non significant.

In the present research obtained results showed lower protein contents in whole soft body tissues of snail species *Bellamyia bengalensis* collected from Dedargaon reservoir followed by Latipada and Malangaon reservoirs and higher at Jamkhedi reservoir. For *Mellanoidea tuberculata* lower protein contents were found in Dedargaon reservoir than Latipada reservoir while for *Lymnaea acuminata* lower protein contents were found in Dedargaon than Malangaon reservoir. This indicated that snail species inhabiting at Dedargaon reservoir are more under environmental stress than other three reservoirs, while snail species inhabiting at Jamkhedi reservoir are under less environmental stress. Thus order of metal pollution at four reservoirs was Dedargaon > Latipada > Malangaon > Jamkhedi. This might be due to results revealed highest concentrations of heavy metals Zn, Cu, Cd and Pb in surface water, sediments and snail species collected from Dedargaon reservoir followed by Latipada and Malangaon reservoir and lowest concentrations of heavy metals Zn, Cu, Cd and Pb in surface water, sediments and snail species collected from Jamkhedi reservoir.

The obtained results showed lowest protein contents were observed in whole soft body tissues in three snail species collected during summer season and highest during monsoon season while results were intermediate in winter season. This might be due to snails were exposed to higher level of pollutant in summer and exposed to lower level of pollutant in monsoon while intermediate in winter season. In the present study results revealed higher level of heavy metals in surface water, soil sediments and snails species collected during summer season and lower level of heavy metals in surface water, soil sediments and snail species collected during monsoon season while results were intermediate in winter.

V. DISCUSSION

Metals are major concern, among the environmental pollutants, due to their potential toxic effects and ability of bioaccumulation in aquatic ecosystem (Censi *et al.*, 2006). This causes damage to the cell membrane and produces the natural antioxidant or oxygen free radical scavenging enzyme system (Geter *et al.*, 2008). The bio-molecules of cell like nucleic acids, lipids, proteins, polysaccharides etc are substrates of ROS (Manduzio *et al.*, 2005) that causes alterations of biochemical and physiological mechanism in the body of organism to counteract in responses of toxic effects of pollutants (Shrivastava and Singh, 2004; Satyaparneswara *et al.*, 2006). Therefore alterations in biochemical constituents like proteins, ascorbic acid, DNA and RNA considered as sensitive indicator of stress (Shinde, 2013; Deshmukh, 2013; Rahane, 2014).

Proteins are the most abundant biological macromolecules and forms the building blocks of organism and also act as a source of energy. It is one of the most suitable substances that show the effects of heavy metals by changing their levels in the body. Waykar and Lomte, (2001), reported that, environmental stress reduces the rate of protein synthesis or increase the proteolysis to cope with the high energy demands under toxicants stress. Hence, proteins can be used as an indicative tool to determine the physiological responses.

In the present research obtained results showed lowest protein contents in whole soft body tissues of snail species collected from Dedargaon reservoir followed by Latipada and Malangaon reservoirs and higher at Jamkhedi reservoir. This might be due to snails inhabiting at Dedargaon reservoir were exposed to higher load of pollutants than other three studied reservoirs. In the present study results revealed highest concentrations of heavy metals Zn, Cu, Cd and Pb in surface water, sediments and snail species collected from Dedargaon reservoir than other three studied reservoirs.

The oxidation of protein leads to conformational changes related with the decreased catalytic activity of enzymes and resulting in a breakdown of proteins by proteases due to increased susceptibility (Almroth *et al.*, 2005; Jagtap *et al.*, 2011). The reactive oxygen species (ROS), can leads to damage the proteins due to oxidative attack and results in modifications of amino acids, fragmentation of the peptide chain, aggregation of cross-linked reaction products, altered electrical charge and increased susceptibility to breakdown of proteins (Grune, 2000; Requena *et al.*, 2003). At high pollution stress, protein synthesis can be suppressed representing disturbance of regular metabolic processes (Pottinger *et al.*, 2002).

Many researchers reported that pollution stress caused decreases in protein content in variety of animals. Bislimi *et al.*, (2015) studied comparative analysis of some biochemical parameters in hemolymph of garden snail *helix pomatia* of the kastriot and ferizaj regions, Kosovo and found a decrease in total protein exposed to heavy metal concentration medium. Mahajan and Zambare, (2012) observed decrease in protein content of hepatopacreas and gonad of *Bellamya bengalensis* due to heavy metal toxicity and concluded that decrease in protein contents was due to severe disturbances of the metabolism in the animal. El-Shenawy *et al.*, (2012), recorded decreased protein content in terrestrial snail, *Eobania vermiculata* collected from higher polluted Qurwa farm as compare to less polluted El-Shreef farm and Wadi Waj garden of in Taif city, Saudi Arabia. Deshmukh (2013) and Rahane (2014) reported that bivalve species inhabiting at higher polluted reservoir showed lower protein content, while bivalve species inhabiting at lower polluted reservoir showed higher level of protein content.

Seasonal variation in biochemical composition of aquatic organisms depends on the variation in temperature, salinity and many industrial sewage contaminants added in the water. The results of seasonal study indicated that, the protein contents in whole soft body tissue of *Bellamya bengalensis* from all the four reservoirs were found lowest during summer, highest in monsoon and intermediate in winter season. In whole soft body tissue of *Mellanoides tuberculata*, collected from Dedargaon and Latipada reservoir, the seasonal changes in biochemical contents showed lowest protein contents during summer, highest in monsoon and intermediate in winter season. Similarly, in whole soft body tissue of *Lymnaea acuminata*, collected from Dedargaon and Malangaon reservoir, the seasonal changes in biochemical contents showed lowest protein contents during summer, highest in monsoon and intermediate in winter season. This might be due to snails were exposed to highest level of pollutant in summer and exposed to lowest level of pollutant in monsoon. In the present study results were correlated with higher level of heavy metals in surface water, soil sediments and snails species collected during summer season and lower during monsoon season.

VI. CONCLUSION

Seasonal variations in protein content were studied by many researchers. Salaskar and Nayak, (2011) reported seasonal changes in protein content of *crassostrea madrasensis* and *Perna viridis* in the Kali estuary, Karnataka, India. Kamalkanth *et al.*, (2014) observed higher protein content in gastropod *Thais mutabilis* in summer as compare to winter and monsoon in foot and gonad.

Thus results clearly indicated that order of protein content in snails from four studied reservoirs was Dedargaon > Latipada > Malangaon > Jamkhedi. The results also indicated that protein contents were lowest in summer and highest in monsoon and intermediate in winter season in three snail species *Bellamya bengalensis*, *Mellanoides tuberculata* and *Lymnaea acuminata* at four studied reservoirs.

This work provides information regarding biochemical contents like protein in three snails species *Bellamya bengalensis*, *Mellanoides tuberculata* and *Lymnaea acuminata* in relation to heavy metal accumulation at four reservoirs of Dhule district of North Maharashtra. Seasonal variation of biochemical components are also aimed in this study.

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