International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 7; Issue 6(K); June 2018; Page No. 13855-13858
DOI: http://dx.doi.org/10.24327/ijcar.2018.13858.2489

Research Article

HEAVY METAL EXPOSURE TO FRESHWATER SNAILS BELLAMYABENGALENSIS, MELLANOIDESTUBERCULATA AND LYMNAEAACUMINATA FROM DEDARGAON RESERVOIR OF DHULE DISTRICT (M. S.) INDIA, IN RELATION TO ASCORBIC ACID CHANGES

Petare R. K[1] and Waykar B. B[2]

[1]Department of Zoology, K. A. M. P. & K. N. K. P. College, Pimpalner, Dhule, (M. S. India)
[2]Department of Zoology, Dr. B. A. M. University, Aurangabad (M. S. India)

A R T I C L E I N F O

Article History:

Received 12th March, 2018
Received in revised form 24th April, 2018 Accepted 5th May, 2018
Published online 28th June, 2018

Key words:

Ascorbic acid content, Dedargaon, Snails, Heavy metals, *Bellamyabengalensis, Mellanoidestuberculata, Lymneaacuminata*

A B S T R A C T

Alterations in ascorbic acid contents after chronic exposure to zinc, copper, cadmium and lead for 10 and 20 days, were observed in whole soft body tissue of three experimental snail species, *Bellamyabengalensis, Mellanoidestuberculata* and *Lymnaeaacuminate* collected from Dedargaon reservoir of Dhule district of Maharashtra, India. The results indicates that ascorbic acid contents of three experimental snail species were decreased as compared to those of control snails. Among all treated heavy metals, Cd caused more decrease as compared to Zn, Cu and Pb in three snail species. The ascorbic acid decreased was more after 20 days as compared to 10 days of exposure. A maximum ascorbic acid contents were observed in whole soft tissue of *Lymnaeaacuminata* in response to accumulated levels of Zn, Cu and Pb while *Mellanoidestuberculata* for Cdas compare to studied snail species. Therefore it is concluded that, snail species *Lymnaeaacuminata* is the sentinel organism for biochemical study of heavy metals Zn, Cu and Pb, and *Mellanoidestuberculata* for Cd in freshwater ecosystem.

INTRODUCTION

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), which attack unsaturated fatty acids of the cell membrane which leads to formation of lipid peroxidation (Viarengo, 1989). Heavy metals are responsible for biochemical and physiological changes in the organisms. Changes in biochemical composition serve as the initial sensitive indicators of toxic effect on tissues (Thaker and Haritos, 1989).

Biochemical analysis of benthic organisms as monitors is the best option for conventional metal pollution monitoring system which may be insufficient, that leads to inaccurate water quality assessment. It is evident that, even at low concentrations, toxicant causes biochemical changes within individual organisms, before these effects are observed at higher levels of organization (Sarkar *et al.,* 2006). Biochemical responses in aquatic organisms have been used in several monitoring programs to study the anthropogenic pollution (Cajaraville *et al.,* 2000).

Corresponding author: **Petare R. K**
Department of Zoology, K. A. M. P. & K. N. K. P. College, Pimpalner, Dhule, (M. S. India)

Recently, more attention has been given to propose biomarkers of exposure and effect, in toxicity testing aiming an application in pollution monitoring (Goulet *et al.,* 2005). The studies on the physiological and biochemical responses of the molluscs to environmental toxicants 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.

Ascorbic acid is important biomolecule. It is responsible for several metabolic reactions (Kaya, 2003). It is one of the most important antioxidant that prevents oxidative damage in tissues of organisms. The ascorbic acid has antioxidant property which helps to prevent free radical formation from toxic water soluble molecules that may cause cellular injuries. It also acts as a hydrogen carrier and plays an important role in carbohydrate, protein or both metabolisms.

Lackner (1998) studied that ascorbic acid level was decreased during chronic exposure to different stressors. Waykaret al., (2001) studied the effect of cypermethrin on ascorbic acid content in the mantle, foot, gill, digestive gland and whole body tissues of freshwater bivalve, *Parreysiacylindrica.* The study of change in ascorbic acid content in molluscs exposed to heavy metals can be useful as an indicator of toxicant stress condition. Nandi *et al.,* (2005) reported that, ascorbic acid prevents oxidative damage to the membranes against peroxydation by increasing the activity of tochoperol. It also inhibits the in vitro lipid peroxydation (Verma *et al.,* 2007).

Ascorbic acid is well known to play protective and therapeutic role against pollutant or metal toxicity (Rao *et al.,* 2001). Changes in ascorbic acid content due to exposure to heavy metals were reported by Deshmukh (2013) and Rahane (2014).

MATERIALS AND METHODS

Three species of freshwater snail, *Bellamyabengalensis*, *Melanoidestuberculata* and *Lymnaeaacuminata* were collected from four water reservoirs of Dhule district, Maharashtra state, India, and were brought to laboratory and acclimatized for 10 days. After acclimatization, the active, medium, uniform sized and healthy snails of each species were selected by measuring their shell length and divided into six groups as below.

1st group was maintained as control.
2nd group was exposed to chronic concentration 0.1990 ppm ($LC_{50/10}$) of Zn up to 20 days.
3rd group was exposed to chronic concentration 0.5451 ppm ($LC_{50/10}$) of Cu up to 20 days.
4th group was exposed to chronic concentration 1.7345 ppm ($LC_{50/10}$) of Cd up to 20 days.
5th 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). Medium sized 10 animals from *Bellamyabengalensis,* 30 animals from *Mellanoidestuberculata* and 20 animals from *Lymnaeaacuminata* 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. Digestion of each sample was carried out by above mentioned methods, respective metal was analyzed by using Atomic Absorption Spectrophotometer (AAS). Dry weight of each animal was used to calculate the metal concentration per unit body weight (µg/g) and metal body burden (µg/individual).

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 analyzed by Student's'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 ascorbic acid were studied comparatively in three experimental freshwater snails species, *Bellamyabengalensis, Mellanoidestuberculata and Lymnaeaacuminata* after exposure to chronic concentrations of Zn, Cu, Cd and Pb for 10 and 20 days along with control animals in dry tissues. The results are summarized in table no.1. Significant variations (P<0.05,P<0.01,P<0.001) were observed in the mean values of ascorbic acid for three snail species.

After chronic exposure the results indicates that the ascorbic acid 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 ascorbic acid as compared to Zn, Cu while Pb in three snail species. The ascorbic acid decreased was more after 20 days as compared to 10 days of exposure. A maximum depletion in ascorbic acid contents were observed in whole soft tissue of *Lymnaeaacuminata*in response to accumulated levels of Zn, Cu and Pb while *Mellanoidestuberculata* for Cdas compare to studied snail species.

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

Sr. No.	Snail Species	Treatment	10 Days	20 Days
1	*Bellamyabengalensis*	Control	0.81±0.021	0.79±0.021
		Zn	0.76±0.017 (-6.23)	0.58±0.017 (-26.28)
		Cu	0.71±0.025 (-11.62)	0.55±0.025 (-30.09)
		Pb	0.70±0.021 (-13.70)	0.54±0.021 (-31.78)
		Cd	0.65±0.013 (-19.51)	0.53±0.017 (-32.63)
	P-Value	Metals	0.00013676**	0.0000**
		Replicate	0.1361520NS	0.8899 NS
2	*MellanoidesTuberculata*	Control	0.71±0.013	0.70±0.017
		Zn	0.67±0.021 (-5.66)	0.53±0.013 (-25.24)
		Cu	0.65±0.017 (-8.02)	0.52±0.021 (-26.67)
		Pb	0.63±0.021 (-11.80)	0.50±0.017 (-29.05)
		Cd	0.52±0.017 (-27.36)	0.35±0.013 (-50.96)
	P-Value	Metals	0.00003243**	0.0000**
		Replicate	0.29059730NS	0.0000**
3	*LymnaeaAcuminata*	Control	0.76±0.025	0.74±0.021
		Zn	0.69±0.017 (-9.21)	0.48±0.013 (-35.74)
		Cu	0.64±0.021 (-16.22)	0.44±0.017 (-40.28)
		Pb	0.61±0.017 (-20.62)	0.42±0.025 (-42.99)
		Cd	0.57±0.013 (-25.88)	0.40±0.013 (-46.61)
	P-Value	Metals	0.00**	0.0000**
		Replicate	0.0001975**	0.0604 NS

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

DISCUSSION

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). Most of the toxicants interact with enzymes, metabolites or other cellular components of the organisms and affect the integrated functions like survival, growth, reproduction and behavior of the organism (Abou*et al.,* 2005). Many researchers reported that heavy metals stress leads to alterations in protein, ascorbic acid, DNA and RNA in molluscs (Deshmukh, 2013).

In the present investigation the ascorbic acidcontents were determined from whole soft body tissue of three experimental snail species, *Bellamyabengalensis, Mellanoidestuberculata and Lymnaeaacuminata* after chronic exposure to heavy metals Zn, Cu, Cd and Pb. The results showed significant depletion in ascorbic acid is summarized in table no. 1. The results of ANOVA test indicated that the variations between the mean values of ascorbic acidwere statistically significant ($P<0.05$, $P<0.01$, $P<0.001$) in three experimental snails species.

Changes in ascorbic acid contents

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 ascorbic acid content was observed in whole soft body tissue of three experimental freshwater snail species *Bellamyabengalensis, Mellanoidestuberculata and Lymnaeaacuminata* as compared to snails maintained as control. Among the tested heavy metals, Cd caused more depletion in ascorbic acid content in three experimental freshwater snail species as compared to Zn, Cu and Pb. Highest depletion of ascorbic acid content was observed in whole soft tissues of *Lymnaeaacuminata* in response to accumulated levels of Zn, Cu and Pb and in *Mellanoidestuberculata* for Cd.

Ascorbic acid readily forms salts of several metals and reduces their activity. According to Chattergee *et al.,* (1995), ascorbic acid protects mammalian tissue against oxidative stress causing damage.

The ascorbic acid levels were depleted on acute and chronic exposure to different stressors such as metals (Lackner, 1998). Mahajan (2013) observed depletion in the ascorbic acid contents in *Bellamyiabengalensis* on chronic exposure to lead and copper as compare to control. Nawale, (2008) reported that depletion in the ascorbic acid contents in bivalve, *Lamellidenscorrianus* after chronic exposure to lead nitrate and sodium arsenate. The ascorbic acid level decreased during acute response to different stressors such as heavy metal, heat shock etc (Lackner, 1998). Deshmukh (2013) observed decrease in ascorbic acid level in different tissues of experimental bivalves after chronic exposure to heavy metals for 10 and 20 days as compare to control bivalves. The decreased level of ascorbic acid might be due to its contribution in detoxification or impairments in its synthesis, repairing of injuries in tissues and to face the toxic stress caused by heavy metals (Waykar *et al*., 2001).

Alterations in biochemical components likeproteins, ascorbic acid,DNA and RNA, are useful to study different toxicant defense mechanisms of the body in response to toxic effects of heavy metals. Most biochemical changes in the laboratory studies are evaluated after exposure to toxicants like metals, pesticides, etc. These changes provide sensitive and specific response to specific toxicant. The use of biomarkers to study cellular, molecular and biochemical effects due to pollutants in snails have been studied to investigate the status of the aquatic environment. The biochemical components are indicators of pollution, which are useful to determine health and nutritional status of an organism.

The overall results showed after chronic exposure to the heavy metals Zn, Cu, Cd and Pb for 10 and 20 days a significant decrease in ascorbic acid contents was observed in whole soft body tissue of experimental freshwater snail species *Bellamyabengalensis, Mellanoidestuberculata and Lymnaeaacuminata* as compared to snails maintained as control.Among the tested heavy metals, Cd caused more depletion in ascorbic acid contents in three experimental freshwater snail species than Zn, Cu and Pb. Highest depletion of ascorbic acid, contents was observed in whole soft tissues of *Lymnaeaacuminata*in response to accumulated levels of Zn, Cu and Pb and in *Mellanoidestuberculata*for Cd. Therefore, results clearly indicates that *Lymnaeaacuminata*is sentinel organism for biochemical study of heavy metals Zn, Cu and Pb and *Mellanoidestuberculata* for Cd in freshwater ecosystem. Thus, the level biochemicals such as proteins, ascorbic acid, DNA and RNA in three snail's species after exposure to heavy metals can be considered as indices for stress.

Our work provides information of heavy metal pollution in the surface water and soil sediment, bioaccumulation of heavy metals in whole body tissues of snails inhabiting in Dedargaon reservoirs of Dhule district of North Maharashtra. Seasonal variation of heavy metals in surface water, soil sediment and, bioaccumulation in whole body tissue and their effect on biochemical constituents are focused in this study. This study helps to develop database about bioaccumulation response under laboratory condition, which might be used for future biomonitoring of heavy metal pollution. This study also helps to determine the most sentinel snail species for monitoring of heavy metal pollution in the freshwater ecosystem.

CONCLUSION

1. Highest depletion in ascorbic acid contents was observed in whole soft body tissues of *Lymnaeaacuminata* after exposure and bioaccumulation of heavy metals Zn, Cu and Pb, while in *Mellanoidestuberculata* for heavy metal Cd as compare to other snails species.
2. It is concluded that, snail species *Lymnaeaacuminata* is the sentinel organism for biochemical study of heavy metals Zn, Cu and Pb, and *Mellanoidestuberculata* for Cd in freshwater ecosystem.

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.

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.

Chatteijee C., Mukhopadhyay K. and Ghosh K. (1995): Vitamin 'C a potential sever against free radical induced oxidative damage. *Current Science*, 69(9): 10.

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

Fernandes, C., Fontainhas-Fernandes, A., Cabral, D., Salgado, M. A. (2008)*:*Heavy metals in water, sediment and tissues of Liza Saliens from Esmorizparamos lagoon, Portugal. Environ. *Monit. Assess.,* 136: 267 – 275.

Finney, D. J. (1971): Statistical methods for biological analysis. 3rd Edition, London.

Goulet, R. R., Lalonde, J. D., Munger, C., Dupuis S, Dumont-Frenette G, Campbell P. G. C (*2005):*Phytoremediation of effluents fromaluminum smelters: a study of Al retention in mesocosms containing aquatic plants. *Water Res*, *39 (11): 2291–2300.*

Kaya, B. (2003)*:* Anti-genotoxic effect of ascorbic acid on mutagenic dose of three alkylating agents. *Turk. J. Biol*., 27: 241 - 246.

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.

Lackner, R., (1998): Oxidative stress in fish by environmental pollutants. In: Fish Exotoxicology (Eds.: T. Braunbeck, D.E. Hinton and B. Streit). BrikhauserVerlag Basel, Switzerland, pp. 203-224.

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

Nandi, D., Patra, R. C. andSwarup, D. *(*2005*):* Effect of cysteine, methionine, ascorbic acid and thiamine on arsenic-induced oxidative stress and biochemical alterations in rats. *Toxicology*. 211:226–235.

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. BabasahebAmbedkarMarathwada University, Aurangabad (M.S.) India.

Rahane, B. B. (2014):Biomonitoring of heavy metal pollution in some water reservoirs of Nasik district using bivalves as bioindicators. Ph. D. thesis submitted to Dr. B. A. M. University, Aurangabad.

Rao, M.V., Chinoy, N. J., Suthar, M. B. and Rajvanshi, M. I.,(2001): Role of ascorbic acid on mercuric chloride-induced genotoxicity in human blood cultures. *ToxicolIn Vitro*, 15:649-654.

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.

Thaker, A. A., Haritos, A. A. *(*1989*):*Comp Biochem Physiol. 94C: 63-70.

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., Lomte, V. S. and Zambare, S. P. (2001): Effect of cypermethrin on ascorbic acid content in the mantle, foot, gill, digestive gland and whole body tissues of freshwater bivalve, Parreysiacylindrica. *J. Aqua. Biol*., (16): 57-61.

How to cite this article:

Petare R. K and Waykar B. B (2018) 'Heavy metal exposure to freshwater snails bellamyabengalensis, mellanoidestuberculata and lymnaeaacuminata from dedargaon reservoir of dhule district (m. S.) India, in relation to ascorbic acid changes', *International Journal of Current Advanced Research,* 07(6), pp. 13855-13858.
DOI: http://dx.doi.org/10.24327/ijcar.2018.13858.2489

****