CHAPTER : 3

RESPIRATORY METABOLISM
3.1 INTRODUCTION

Energy is essential for survival and proper maintenance of life of organism. It is obtained through oxidative metabolic processes, wherein oxygen is one of the essential factor required. Oxygen is obtained through the process of respiration. Hence, the respiration is a vital phenomenon of life of organism, through which metabolic rate of animals can be measured. The metabolic response of an organism to environmental changes, is an overall indicator of adaptive capacity of an organism. Generally, the metabolic status of an organism is assessed by measuring rate of oxygen consumption. The rate of oxygen consumption by animal as a whole and its tissues as specific, indicates its respective metabolic rates.

Survival of living organism is depend on the capacity of its cells which contain complex molecules necessary for proper cellular functions. The energy required for these various chemical processes and proper maintenance of cellular activities, must be obtained from oxidation of high energy phosphate esters. This entire process is dependent upon the availability of molecular oxygen to the cells through the process of respiration.
The rate of oxygen consumption of an organism is known to be influenced by certain environmental factors like temperature, pH, dissolved oxygen, carbon dioxide, salinity and photoperiod (Wright, 1971). The relationship between the respiratory activity of animals and pollution has been studied considerably in aquatic animals (Roberts 1972; Davis, 1973 and Percy, 1977).

The respiratory responses of freshwater and marine animals after exposure to various concentrations of pollutants can be useful devices for quantitative assessment of sublethal effects without analyzing biochemical composition and histological structures. In addition to this response of organisms, the respiratory phenomenon plays an important role in studying the aquatic toxicology. The initial response of an organism to pollutant action is change in oxygen uptake, which reflects by changing biochemical processes and metabolism.

Suchitra and Fang (1966) suggested, the decrease in oxygen consumption is brought about by surviving links between oxidative and phosphorylative processes. The oxygen utilization by an organism was commonly used as index of metabolic activity (O’Hara, 1971). Grandel and Good night (1963) suggested that prolonged exposure of animal to long level of heavy metal pollution subjects them to stress which
causes hormonal imbalance ultimately leading to variety of internal pathological changes.

Cairns (1966) claims that testing of oxygen is one of the best method of sub-lethal bioassay. Sprague (1973) has stated that “understanding physiological action of a toxicant is the key to predict important sub-lethal effects”. Respiratory distress is one of the important manifestations of acute heavy metal toxicity and is known to produce physiological imbalance, since the respiratory potential of an animal is important physiological parameters to assess the toxic stress as it is a valuable indicator of energy expenditure and metabolism in general. Waiwood and Johnson, (1974) the parameter oxygen consumption has been used to determine the effect of toxicant on average metabolism of exposed animals.

The heavy metals released in aquatic environment, become a part of environment and they are bound to accumulate in the tissues of aquatic organism. Rice et al, (1977) have reported that part of the increased oxygen is utilized by the petroleum hydrocarbons exposed animals to support the enhanced physiological activity in metabolising and eliminating the pollutants. Oxygen consumption is a very sensitive physiological process and the change in respiratory activity has been
used as an indicator stress of toxicant exposed animals (Sharp et al., 1979).


Oxygen uptake which reflects biochemical processes is a good analyser of the physiological state of an organism. The essential elements are required in trace amounts to all forms of life, but toxication occurs when present in excess causing disturbances in normal metabolism in aquatic and terrestrial organisms. Much disturbance in metabolism results into death of an organism (Alam and Lomte, 1984).

Oxygen consumption of the snail, *Bellamya bengalensis* exposed to lead, selenium and lead selenium mixture was studied by Joshi (1993), oxygen consumption was significantly affected by lead,

Several toxicologists have studied, the effects of heavy metals on alterations in the oxygen consumption of freshwater snails, Ishak and Mohamed (1975), Mule and Lomte (1994), Sivaram-akrishna *et al.* (1991), Alam and Lomte (1984), Muley and Mane (1989), Joshi (1993) and Huil Gol and Marathe (1986) and in marine pulmonate, *Onchidium verruculatum* (Nagabhushanam and Deshpande, 1982).

The present snail *Lymnaea*, has been worked out in detail from the point of various diversified aspects of physiology, biochemistry and histopathology. From the point of toxicological studies, in the present topic on *L. acuminata* was selected as a experimental model to check the impact of hexavalent chromium on respiratory metabolism.
3.2 MATERIAL AND METHODS.

The medium sized, experimental freshwater snails, *Lymnaea acuminata* were collected from Kham River near Aurangabad. The snails were immediately brought to the laboratory and acclimatized for 3 days to laboratory conditions (temperature 27 ± 2 °C, pH 7-8, dissolved oxygen 5.2 ± 0.5 mg/liter and total hardness 175 – 180 mg/liter). After acclimation normal sized (with an average shell length 1.8 + 2.0 cm) healthy snails were selected for experiment.

The snails *L. acuminata* were exposed for two different concentration of chromium. The acclimatized active snails were divided into three groups. One group of snails was exposed to 1/10\(^{th}\) concentration of LC\(_{50}\) value of 24 hours of hexavalent chromium , 12.690 ppm as acute treatment upto 96 hours. The second group of acclimatized active snails was exposed to 1/10\(^{th}\) concentration of LC\(_{50}\) value of 96 hours of hexavalent chromium, 4.967 ppm as acute treatment upto 96 hours and the last and third group of acclimatized active snails was kept as control group.

The amount of oxygen consumed by control as well as experimental animals was measured after 1, 2, 4, 8, 12, 24, 48,72 and 96 hours exposure to hexavalent chromium.
After respective exposure periods 10 animals were taken out and allowed for respiration for 1 hour, simultaneously a control group of animals was also maintained for respiration throughout the experimental time period. After 1 hour consumption, the water was siphoned out from control as well as experimental jars, and subjected for oxygen determination by standard Winkler’s technique (Welsh and Smith, 1961). 

At the end of experiment, the snails from both jars were removed and deshelled, blotted dry and weighed. The oxygen consumed was calculated and results are expressed in ml O$_2$/liter/hour/gm weight of snail at N.T.P.

Each experiment was repeated at least thrice. The data of oxygen consumption was statistically analysed to determine average value with standard deviation and to test the level of significance.
3.3 OBSERVATIONS AND RESULTS

The freshwater snail, *Lymnaea acuminata* showed decrease in oxygen consumption when exposed to 1/10\(^{th}\) of sublethal (LC\(_{50}\)) concentration of chromium for 24 hours and 96 hours exposure period. The amount of oxygen consumed by the control and experimental snails after exposure to two different concentrations of hexavalent chromium is summarized in Tables 3.1 and 3.2. The oxygen consumption results are expressed as ml O\(_2\)/liter/hour/gm wt. of the snail at N.T.P.

(A) Effect of hexavalent chromium on the rate of oxygen consumption of *L. acuminata after exposure to 1/10\(^{th}\) concentration of LC\(_{50}\) value of 24 hour exposure (12.690 ppm):

After exposure to 12.690 ppm chromium it was observed that there is an increase in rate of oxygen consumption initially upto 4 hours exposure period (0.1174 ± 0.0039 to 0.1191 ± 0.0054 respectively after exposure for 1 and 4 hours) when compared with control snails, ranged from 0.1034 to 0.1038 ml O\(_2\)/liter/hour/gm weight at N.T.P. and then the rate of oxygen uptake was declined continuously. However, the rate of oxygen uptake decreased significantly after further exposure for 24, 48, 72 and 96 hours (See
Table 3.1). But the decrease in amount of oxygen consumption by the exposed snails is not significant at 8 and 12 hours exposure time periods. After surveying the Table 3.1 depicts initial increase in the rate of oxygen consumption after exposure for 1, 2 and 4 hours, but there starts decrease in oxygen uptake from 8, 12, 24, 48, 72 and 96 hours of exposure period by the pond snail *Lymnaea*.

(B) **Effect of hexavalent chromium on the rate of oxygen consumption of *L. acuminata* after exposure to 1/10th concentration of LC$_{50}$ value of 96 hours exposure period (4.967 ppm):**

The data of oxygen consumption by snail *L. acuminata* after exposure to 4.967 ppm are summarized in Table 3.2. The pond snail, *L. acuminata* when exposed to 1/10th concentration of LC$_{50}$ value of 96 hours (i.e. 4.967 ppm) shows increase in rate of oxygen consumption upto 4 hours (0.1184 ± 0.0036 ml O$_2$/liter/hour/gm wt. of snail at NTP) when compared with control snails (0.1040 ± 0.0048 ml O$_2$/liter/hour/gm Wt. of snail at NTP). This increase in oxygen uptake by snails is not significant. The amount of oxygen consumed by simultaneous control animals ranged from 0.1034 ± 0.0041 to 0.1038 ± 0.0045 ml O$_2$/liter/hr/gm/wt of snail at NTP.

After 8 hours exposure onwards upto 96 hours of treatment with hexavalent chromium, has inhibitory effects on the rate of respiration.
by snail *Lymnaea*, thereby showing continuous decrease in oxygen uptake by the snails up to 96 hours exposure period (See Table 3.2) After 3rd and 4th day of exposure the rate of oxygen consumption significantly decreased over the control animals.
3.4 DISCUSSION

The respiratory physiological response of both freshwater and marine water organisms to various types of pollutants, plays an important role in studies on aquatic toxicology. Heavy metal toxicity to freshwater ecosystem and organisms studied by various workers (USEPA, ERS, 1975; Forster and Prosi, 1979 and Balavenkatasubbaiah et al, 1983). The impact of pollutants on oxygen uptake, reflects many physiological and biochemical process of aquatic organisms. The oxygen consumption process is influenced by a number of factors such as temperature, hydrogen ion concentration, body size, salinity, oxygen tension and chemical pollutants of the medium. Amongst these the chemical pollutants are most hazardous and are represented in the form of pesticides, herbicides and molluscicides, etc.

Chromium potentially possesses toxicological, carcinogenic and mutagenic properties and is extremely hazardous to aquatic biota. Hence, the removal of chromium by Albizia lebbeck pods as suggested by Verma and Rehal (1996) or by other treatment procedures from the industrial effluents is very essential before it is released in any form into the aquatic environment.
The freshwater pulmonate snail *Lymnaea acuminata* showed variations in respiratory response to hexavalent chromium treatment. After surveying through the results depicted in the form of observation Tables 3.1 and 3.2, it is evident that, the chromium has induced to increase in oxygen consumption at least during initial phases of experimentation. The heavy metal pollutants may constitute a physiological stress to the freshwater organisms. However, the interpretation of pollutant induced changes in respiratory metabolism is very difficult, because such type of changes vary from pollutant to pollutant, from species to species and from one environmental condition to another. Thurnberg *et al*, (1973) are of the opinion about the interpretation of metal induced changes in respiratory physiology becomes complicated by the fact that alterations differ not only from metal to metal and species to species, but from one experimental condition to another. Alterations in the metabolic processes following exposure to heavy metals have always been used as an indicator of stress.

Sprague (1971) has stated that “under standing physiological action of a toxicant is the key to predict important sub-lethal effects”. Respiratory distress is one of the important manifestations of acute heavy metal toxicity and is known to produce physiological imbalance.
The rate of oxygen consumption is often considered as a reflection of total metabolism and hence the metabolic state of the organisms.

The initial increase in oxygen consumption by snail, *L. acuminata* after exposure to sub-lethal concentration of metal chromium may exhibit a new steady state of metabolism to compensate physiologically with the stress caused by toxic metal pollutant (Nagabhushanam *et al*, 1985; Jayasuriya *et al*, 1991 and Rajamannar and Manohar, 1998). According to Ghosh (1987); Singh (1994) and Kumta *et al*, (1998), a part of an increased oxygen consumption is required to support enhanced physiological activities in metabolizing and eliminating the pollutants by exposed animals.

The heavy metals released in aquatic environment, become a part of environment and they are bound to accumulate in the tissues of aquatic organisms. The freshwater snail, *L. acuminata* can not be an exception from the point of bioaccumulation of metal chromium in its body tissues. It is also a well known fact that aquatic invertebrates have the capacity to concentrate toxic (Cd, Ag, Hg, Pb and Cr) and nutritionally important (Cu, Zn, Mn and Co) trace metal accumulate, may there must be metabolic strategies to utilize or sequester these metals depending upon their nutritional value or toxic potential. May be due to this fact, there is an increase in respiration by the snail upto 4
hours of exposure. However, this increased state of respiration was not continued during further exposure period. Similar type of changes in oxygen consumption was recorded during acute phosphamidate exposure by freshwater snail, *Bellamya bengalensis* (Rohankar and Kulkarni, 2005). According to their observations there was significant rise in oxygen consumption from first hour to 12 hours exposure and gradual decline was noticed from 24 hours up to end of experiment.

Copper and mercury is reported to be inhibitory to many enzymes and they affects respiratory rate (Jakim, 1974). The decrease in oxygen consumption due to heavy metal mercury is reported by Luckey and Venugopal (1977). They have concluded that primary effect of mercury on cells appears to be binding with sulfadryl groups on surface membrane protein. Manley (1983) studied the effects of copper on the behaviour, respiration and ventilation activity of *Mytilus edulis*. Mathew and Menon (1983) studied effect of heavy metals on oxygen consumption of tropical bivalves *Perna viridis* and *Meretrix casta*. Variations in oxygen consumption on exposure to metals are supposed to be due to variation in shell closure, gill irrigations, filtration rate or ciliary activity of bivalves.

The decrease in respiration might also be due to penetration of the pollutant molecule as their action alters metabolic cycle at sub-

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The freshwater basommatophoran snail, *L. acuminata* showed gradual decrease in rate of respiration after exposure to both concentrations i.e.1/10\textsuperscript{th} of LC\textsubscript{50} value for 24 and 96 hours exposure to chromium. The consistency in decreased respiratory is observed in both experimental group. Muley and Lomte (1994) while working on effect of heavy metals, copper, and mercury on the oxygen consumption of the freshwater snail, *Thiara tuberculata* shown that after acute and chronic treatment, the rate of oxygen consumption was decreased, concluding mercury was more toxic as compared to copper. Reduced oxygen consumption at higher concentrations of heavy metals could also arise as a result of respiratory inhibiting factors that come into play. In the present study, copious mucous secretion and bulging of mantle folds was observed externally. The drop in the metabolic
rate of *L. acuminata*, when exposed to chromium may be due to clogging of pulmonary cavity by mucous. The decrease in the metabolic rate can also be attributed to the reduction in the mantle cavity surface area due to chromium induced damage in terms of atrophy, bulging of mantle epithelium, hypertrophy and hyperplasia of mantle layer, necrosis and separation of epithelial cells from inner muscular layers. Hingoroni, *et al*, (1979) while studying the effect of industrial effluents on the oxygen consumption of fish *Labeo rohita* also observed a decline in the oxygen uptake and attributed this to coagulation of gill mucus causing asphyxiation and inhibition of enzyme systems at mitochondrial level.

In the present snail, *Lymnaea*, when treated with two sub-lethal concentrations of chromium upto 96 hours showed initial increase in respiration upto 4 hours, later slight decrease in respiration from 8 hours to 24 hours exposure when compared with control snails. But after 24 hours onwards there was significant decrease in respiration upto 96 hours exposure. This decrease in rate of respiration was more significant in animals after exposure to 1/10\(^{th}\) concentration of LC\(_{50}\) for 24 hours.