



THE EVALUATION OF NICKEL TOXICITY ON *LAMELLIDENS MARGINALIS*

A.V. Andhale*, H. M. Pawar and S. P. Zambare

Department of Zoology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad-431004 (M.S.), India

Abstract

The study deals with calculation of LC10 and LC50 values for 24 and 96 hours. In present study, the toxicity of Nickel Chloride on *Lamellidens marginalis* showed that with increasing time and concentration, mortality rate increase. From this investigation, the exact cause of their death is not known but it is proved that nickel chloride brings some changes in physiochemical properties of water and it affects the metabolic rate severally leading to the death of animals. The toxicity study provides the level of safe concentration of toxicant.

Keywords: *Lamellidens marginalis*, Nickel, Toxicity

Introduction

Comprising over 70% of the earth's surface, water is undoubtedly the most precious natural resource that exists on our planet. Without the seemingly invaluable compound compromise of hydrogen and oxygen, the life on earth would be non-existent, it essential for everything on our planet to grow proper. Although we human recognize this fact, we disregard it by polluting our rivers, lakes and oceans. Subsequently, we are slowly but surely harming our planet to the point where organisms are dying at very alarming rate. In addition to innocent organism dying off, our drinking water has become greatly affected as is our ability to use water for recreational purposes. In order to combat water pollution, we must understand the problem and become part of solution. According to the American college dictionary, pollution is defined as, to make foul unclean dirty. Water pollution occurs when a body of water is adversely affected due to addition of large amount of materials to the water. When it is unfit for its intended use, water is considered polluted. Two types of water pollutants exist, point source and non point source. 95% of all fresh water on earth is ground water. Global environmental collapse is not inevitable. But the developed work with developing world to ensure that new industrialized economics do not add to the world's environmental problems. In order to protect the biotic components, it is necessary to know the impact of toxic compounds on biotic components. Therefore the toxicity evaluation is necessary.

Due to wide spread use, the heavy metals are widely released in the environment which ultimately finds their way in the aquatic ecosystem, contaminates the drinking water and enters in the food chain. Generally, the potential impact of pollutants is greater for aquatic organisms (Murty, 1986). The pollutants are known to alter the physiochemical properties of water (Richardson, 1988). These in turn interferes with the

various physiological activities of organism including reproduction. A different type of water pollutions causes untold hazards to several non target organisms such as fishes, frogs, prawns and mussels. The promiscuous dumping of industrial, agriculture and domestic pollutants had impact on water quality in our major waterways, ponds, bays and ground water supplies. Mostly toxicant are released into water through anthropogenic activities such as industry and agriculture (Saygdeger, 2000). Undesirable effect and toxicity caused by pesticide to aquatic organisms were reviewed by some workers (Woodward and Mauck, 1980). The toxic compounds causes a kind of stress to organisms and organism responds to developing necessary potential to contract the stress.

Polluted environment is less suitable for existing life forms (Menzel, 1977). The environment is polluted by in general by animal and plant waste, and industrial waste, municipal discharge, factory effluents, automobile waste and agricultural chemicals. This has often resulted in the transformation of lakes, rivers and costal water into sewage depots. It would now be extremely difficult to find a natural ecosystem anywhere in the world that has not been exposing to traces of modern pesticide. According to industrial toxicological research center Lucknow (India), the amount of pesticide in agriculture and public health operation and their consumption is increasing everyday in country.

Heavy metal toxicants have posed a serious problem of environmental pollutions, posing a threat to human health, wild life and biotic and biotic components. Due to urbanization, modernization and industrial development lead to exploitation of natural resource at large scale. As a result guzzling, industries, electroplating, fertilizers, processed foods, dairy product, soap, detergent, plastics, thermal and nuclear

* Corresponding Author, Email: atmubiotech@gmail.com

power plant etc. have come up, most of which produces hazardous waste.

Millions tons of pollutants are introduced into environment by various industries by way of gaseous, aqueous and solid waste. These waste changes the natural conditions of aquatic medium, in turn causing behavioral response in mollusks, fishes and other aquatic organisms, ultimately leading to physiological adjustment. Therefore, toxicity testing is an essential component of evaluating water pollution. It is a study of change in ecological degradation, reflecting environmental pollution. Toxicant like heavy metal ions and most pesticide are non degradable, resulting into bioaccumulations within ecosystem and its biotic components.

A] Reason for the selection of Nickel in this research work: Nickel constitutes about 0.008% of earth crust, and nickel ranks as 24th elements in order of abundance. It is also present in air (cites industrial) and in tobacco smoke. Nickel compounds are also applied in agriculture; Plospate fertilizers contain traces of nickel. Nickel is often present in agricultural soils situated near fossil fuel industries. Nickel is exposed by battery (Ni-Cd), nickel miners, ceramic markers, nickel refineries, coal gasification workers, nickel smelters, dyers oil dehydrogenators, electro formers, paint markers, electroplaters, paint markers, electroplaters, sand blasters, spark plug markers, glass workers, spray painters, ink markers, stainless steel markers, jewelers, textile dyers, magnet makers, varnish makers, metal workers etc. Other exposures are diet especially in vegetables: peas, beans, lentils, lettuce, spinach, cabbage and mushrooms, margarine, seafood, chocolate, nuts, baking powder and certain species beverages: wine beer, milk-nickel leaching to food and beverages coetaneous exposure: nickel dermatitis iatrogenic exposure: nickel implantations for joint prostheses, plates and screws for fractured bones, surgical clips and steel sutures or dental implants.

B] Reason for the selection of *Lamellidens marginalis* as an Experimental model:- 1)The body wall, gills, digestive glands and mantles of this animal are soft through which substances passes easily as compare to thicker skin.2)These parts being soft are affected as well as cured early as compared to thick skin. 3) These organs always bath in toxicant contaminated water and hence effect will be prominent at low concentration does. 4) The doses of salts of heavy metals (Nickel salt) maintained in water at particular strenght so that there will be no problem of feeding or injection of doses 5) The animal is filter feeder, because of all above mentioned organs always bath in toxicant contaminated water, large surface of the body is exposed to water at particular streanght, so that there is no problem of feeding or injection.6) These

animals are easily available in large number and are easily to rear in laboratory.

In present investigation Nickel chloride is used as salt of heavy metal pollutant and *Lamellidens marginalis* as an experimental model to evaluate toxicity of this pollutant. Because *Lamellidens marginalis* (Mollusca-Bivalvia- *Eulamellibranchiata*) is an important member of the freshwater ecosystem of India, which is sustained is filtering phytoplankton, bacteria and other particulate organic matter from the available water. Hemocytes, the circulating blood cells of bivalves, function as immunological effector cells under exposure to toxins and /or parasites. The hemocytes of *Lamellidens marginalis* have been identified morphologically and enumerated as being qualified for use as a biomarker to analyze the threat of freshwater contamination by sub lethal concentrations of nickel chloride, a natural pollutant of freshwater ecosystem. Under normal conditions nickel does not react with water; so solubility of nickel and nickel Compounds required. Elementary nickel is water insoluble at T=200C pressure = 1 bar. However, nickel compounds may be water soluble. Nickel chloride is most water soluble; 553g/L at 200 C, to 880g/L at 99.90C. Salt of nickel will be tested separately for safe concentration. LC-10 and LC-50 dose by using standard Probit Analysis method as given by Finney D. J. (1971). The toxicity test is necessary because chemical and physical test are not sufficeint to assess the potential effect of toxicant on aquatic biota. The susceptibility of different tolxicant is variable and therefore responses are dose dependent. The toxicity test is useful for various purposes as: i) to determine favorable and unfavorable concentration of chemical in environment to the organism. ii) To determine safe and lethal concentration to the organism. The toxicity of nickel salt to the bivalve *Lamellidens marginalis* determined and LC-10 and LC-50 value for 24 and 96 hours were calculated.

Materials and Methods

The bivalves *Lamellidens marginal* is were collected from Dhondwadi Dam of Borna River, (Nearly 25 Kms away from Tq. Parli Vaijanath) Dist. Beed, (M.S.), India and brought to the laboratory. Bivalves of similar size (40-50mms long) and average weight (60-75 gms with shell) were cleaned by removing fouling biomass and algal biomass and allowed to acclimatize in laboratory conditions 50 L capacity glass aquarium without food for 3 to 4 days, prior to experimentation. During this period no mortality was observed.

Ten bivalves were exposed to 10 to 15 different concentrations of nickel chloride in troughs containing five liters of water. The water of appropriate concentrations pesticides from through was changed after every 24 hours. The dead animals were counted

in each concentration and the resulting mortality was recorded for the duration of 24, 48, 72 and 96 hours. The criterion for death was inability of bivalve to close the shell valves and failure to respond to probing of foot with needle. In order to obtain constant results, each experiment was repeated for three times. Simultaneously control was maintained along with each set. The statistical analysis of obtained data was done by means of the probit tables. The toxicity curve (% mortality vs oog of concentration) was transformed in to regression lines (Mortality in probit vs. log of concentration) (Finney, 1971). By using this data LC₁₀ and LC₅₀ values were calculated for 24 and 96 hours exposure to nickel chloride.

Calculation of percent mortality:-The percent mortality was calculated by using Abbot's formula. Mortality of bivalves from the control group was subtracted from that of the treated group.

Calculation of regression lines:-The method described by Finney (1971) and simplified by Busvine (1971) was used to obtain a well studied straight line between log of concentration and probit kill. The bivalve were exposed to different concentrations of nickel chloride up to 96 hours and the regression equation and regression line was calculated.

Calculation of LC₁₀ and LC₅₀ values:-The LC₁₀ and LC₅₀ values of nickel chloride in ppm for 24 and 96 hours were calculated from regression equation using Y=3.7184 and Y=5.00 respectively.

Table 1 Calculation of regression equation for LC₁₀ and LC₅₀ values of *Lamellidens marginalis* exposed to Nickel chloride for 24 hrs

Sr. No.	Conc. of NiCl (PPM)	Log of conc. to base 10 'x'	No. of bivalves exposed 'n'	Mortality for 24 hrs. 'y'	% mortality P= $\frac{100y}{n}$	Empirical probit 'X'	Expected probit 'Y'	Weighting coefficient 'w'	Weight W=wn	Working probit 'y'	Wx	Wy	Wx ²	Wy ²	Wxy	Improved expected probit 'y'
1	2	0.3010	10	1	10	5.7184	5.425	0.25735	2.573	5.806	0.7149	9.0405	0.2151	34.4075	2.7211	5.5579
2	3	0.4771	10	2	20	4.1584	4.200	0.50260	5.026	4.159	2.3979	20.9051	1.1440	36.9559	9.9725	4.2607
3	4	0.6020	10	4	40	4.7467	4.750	0.61609	6.160	4.747	3.7058	29.2457	2.2326	158.829	17.6058	4.7592
4	5	0.6989	10	5	50	5.0000	5.175	0.63451	6.345	5.000	4.4551	31.7155	3.0582	158.577	22.1659	5.1459
5	6	0.7781	10	7	70	5.5244	5.524	0.58099	5.809	5.524	4.5206	32.0958	3.5174	177.256	24.9721	5.4620
6	7	0.8450	10	8	80	5.8416	5.825	0.50260	5.026	5.841	4.2469	25.5668	3.5896	171.475	24.5064	5.7280
									$\Sigma W=30.7412$		$\Sigma Wx=20.0222$	$\Sigma Wy=73.9591$	$\Sigma Wx^2=162.3582$	$\Sigma Wy^2=67.5091$	$\Sigma Wxy=102.2442$	

1) $\bar{y} = \frac{\Sigma Wy}{\Sigma W} = \frac{73.9591}{30.7412} = 2.4057$

2) $\bar{x} = \frac{\Sigma Wx}{\Sigma W} = \frac{20.0222}{30.7412} = 0.6513$

3) $b = \frac{\Sigma Wxy - \bar{x}\Sigma Wy}{\Sigma Wx^2 - \bar{x}^2 \Sigma W} = \frac{102.2442 - 0.6513 \times 73.9591}{162.3582 - 0.6513^2 \times 30.7412} = \frac{30.153}{13.7959} = 2.1899$

$LC_{10} = \frac{3.7184 - \bar{y}}{b}$

$LC_{50} = \frac{5.00 - \bar{y}}{b}$

$LC_{10} = \text{Antilog } 0.6623$

$LC_{50} = 4.5954$

$LC_{10} = \frac{3.7184 - \bar{y}}{b}$

$LC_{50} = \frac{5.00 - \bar{y}}{b}$

$LC_{10} = \text{Antilog } 0.3411$

$LC_{50} = 2.1936$

Regression equation
 $Y = \bar{y} + b(X - \bar{x}) = 2.4057 + 2.1899(X - 0.6513)$
 $= 3.9902X - 2.5994 + 4.9560 = 3.9902X + 2.3566$

Table 2 Calculation of regression equation for LC₁₀ and LC₅₀ values of *Lamellidens marginalis* exposed to Nickel chloride for 96 hrs

Sr. No.	Conc. of NiCl (PPM)	Log of conc. to base 10 'x'	No. of bivalves exposed 'n'	Mortality for 96 hrs. 'y'	% mortality P= $\frac{100y}{n}$	Empirical probit 'X'	Expected probit 'Y'	Weighting coefficient 'w'	Weight W=wn	Working probit 'y'	Wx	Wy	Wx ²	Wy ²	Wxy	Improved expected probit 'y'
1	1.5	0.1760	10	2	20	4.1584	4.15	0.47144	4.714	4.160	0.8297	19.6119	0.1460	81.5855	5.4515	4.08
2	2.0	0.3010	10	4	40	4.7467	4.74	0.61609	6.160	4.747	1.5544	29.2457	0.5581	158.829	5.8028	4.71
3	2.5	0.3989	10	5	50	5.0000	5.12	0.63451	6.345	5.000	2.5259	31.7155	1.0042	158.577	12.6195	5.20
4	3.0	0.4771	10	7	70	5.5244	5.52	0.58099	5.809	5.524	2.7719	32.0958	1.3224	177.256	15.5119	5.60
5	3.5	0.5440	10	9	90	5.8416	5.94	0.47144	4.714	6.216	2.5446	29.5047	1.5842	152.158	15.5172	5.94
									$\Sigma W=27.74$		$\Sigma Wx=10.5245$	$\Sigma Wy=41.149$	$\Sigma Wx^2=141.9716$	$\Sigma Wy^2=58.4564$	$\Sigma Wxy=56.0029$	

1. $\bar{y} = \frac{\Sigma Wy}{\Sigma W} = \frac{41.149}{27.7427} = 1.4832$

2. $\bar{x} = \frac{\Sigma Wx}{\Sigma W} = \frac{10.5245}{27.7427} = 0.3793$

3. $b = \frac{\Sigma Wxy - \bar{x}\Sigma Wy}{\Sigma Wx^2 - \bar{x}^2 \Sigma W} = \frac{56.0029 - 0.3793 \times 41.149}{141.9716 - 0.3793^2 \times 27.7427} = \frac{14.1531}{4.4250} = 3.1982$

$LC_{10} = \frac{3.7184 - \bar{y}}{b}$

$LC_{50} = \frac{5.00 - \bar{y}}{b}$

Antilog of 0.1044 = 1.272

Antilog of 0.3562 = 2.27

$LC_{10} = 1.272$

$LC_{50} = 2.27$

Regression equation:
 $4. Y = \bar{y} + b(X - \bar{x}) = 1.4832 + 3.1982(X - 0.3793)$
 $= 5.0900X - 5.0900 \times 0.3793$
 $= 5.0900X - 1.9306 + 5.1174 = 5.0900X + 3.1868$

Results and Discussion

When bivalves of control group were introduced to a tap water they imprisoned themselves inside the shell valves. After some period they had extended their foot out side the shell valves. There was mucus secretion. In nickel chloride exposed bivalves shells were closed for longer time to avoid the direct contact of toxicant. Their movements were restricted and excreta were eliminated in large amount. Dead bivalves were with open shell valves and shrunken foot. The results of toxicity evaluation are summarized in table 1 and 2. The LC₁₀ values for 24 and 96 hours exposure to nickel chloride were 2.1936 and 1.272 ppm of nickel chloride while LC₅₀ values of the respective periods of exposure were 4.5954 and 2.27 ppm.

The toxicity of heavy metals to the invertebrate has been studied by many workers such as Marigomez *et. al.*, (1986), Ajmalkhan *et. al.* (1986); Nagabhushanam *et. al.*, (1986) and Khan *et. al.* (1987). Survival and Behavior of the freshwater gastropod *Viviparus bengalensis* after exposure to mercuric salt in different season was observed Mule and Mane (1988). The contamination of Soil by heavy metal may be from irrigation water source such as deep wells, rivers, lakes or irrigation canals (Ross, 1994) have reported that new swage water content toxic elements and heavy metals such as Fe, Cu, Zn, Mn, Pb, Cd, Co, Cr etc. Arsenic, cadmium and lead are highly toxic heavy metals and they do not have a role in biological process in living organisms. Thus, even low concentration, both cadmium and lead could be harmful to living organisms (Burden *et. al.* 1998).

Heavy metal induces change in physiology and survival of aquatic organisms under metabolic stress accomplished because such change differ from metal to metal, species to species and from one experimental conditions to another (Shrinivas *et. al.*, 2000). The mortality of aquatic organism due to pollutant increases with increasing in time of exposure period and lethal dose decreases with increasing exposure time (Nimmo and Behner, 1971). The physiological factors also influence the toxicity of the aquatic pollutants. Eisler (1977) found that in static bioassay, temperature influenced the toxicity of pesticide. Moreover, he had also found that different salinity's have little effect on the toxicity of agrochemicals, temperature and pH had greatest effect on toxicity of pollutants.

In present research work on toxicity of Nickel chloride on *Lamellidens marginalis* showed that with increasing time and concentration, mortality rate increases. From this investigation, the exact cause of their death is not known but it is proved that Nickel Chloride brings some changes in physiochemical properties of water and it acts as pollutants against life. The mortality rate is directly proportional to

concentration and period of exposure and main cause is its effect on physiological activities.

Clearly, the problems associated with water pollution have the capabilities to disrupt the life on our planet to great extent. Congress has passed laws to try to combat water pollution thus acknowledging the fact that water pollution is indeed serious problem. But the government alone cannot be solving the entire problem. It is ultimately up to us, to be informed, responsible and involved when it comes to the problem we face with our water. We must become familiar with our local water resources and learn about ways for disposing harmful household waste so they don't end up in sewage treatment plants that can't handle them or landfills not designed to receive hazardous materials. In our yards we must determine whether additional nutrients are needed before fertilizer are applied and look for alternatives where fertilizers might run off into surface water. We have to preserve existing trees and new plants / trees and shrubs to help to prevent soil erosion and promote infiltrations of water into the soil. Around our house, we must keep litter, pet waste, leaves and grass clippings out of gutter and storm drains. These are just a few of the many ways in which we as human have the ability to combat water pollution. The awareness and education will most confidently to be two the most important ways to prevent water pollution. If these measures were not being taken in consideration and water pollution continues and life on earth will suffer severally.

References

- Ajmalkhans, Rajendra K. and Natarajan R. (1986): Effect of Cu. On the larval development of estuaries hermit crab *Clibanarius olivaceus* Henderson. Indian J. of Mar. Sci; 15: 260-264.
- Burden VM, Sandheinrich MB, Caldwell CA (1998): Effects of lead on the growth and δ-aminolevulinic acid dehydratase activity of juvenile rainbow trout, *Oncorhynchus mykiss*. Environ. Pollut. 101:285-289.
- Busvine, J. R. (1971): Critical review of the techniques for testing insecticides. Commonwealth Agricultural Bureau, England PP 267-282.
- Eisler, R. and Henneykey, R.J. (1977): Acute toxicities of Cd²⁺, Cr⁶⁺, Hg²⁺, Ni²⁺ and Zn²⁺ to estuarine macrofauna. *Arch. Environ. Contam. Toxicol.*, 6:315-319.
- Finny, D. J. (1971): Probit analysis, Cambridge, university Press, London, pp1-138.
- Khan A. K. Raos. Alam., Patil M. V., Nagabhushanam R and Sarojini R. (1987): Heavy metal toxicity to marine juvenile prawns, *Peanus monodon*, and *Peanus; incus*. J. Ind, Pollut, Cont, (1): 7-10.
- Marigomez, J. A; Anguloe and Moya J, (1986): Copper treatment of the digestive gland of the slug, *Arion*

- arel, Bioassay conduction and histochemical analysis. (Detoxification, calcium metal pollution). Bull. Environ. Contam. Toxicol: 36(4): 600-607.
- Menzel (1977): Controlled environmental experiments in pollution studies, ocean management, issue 24 december 1978 page 319-344 oceanology international 78.
- Muley D. V. and Mane U. H. (1988): Survival and behavior of the freshwater gastropod *Viviparus bengalensis* (Lam.) after exposure to mercurial salts in different seasons; *Trop. Ecol*, (29): 71-78.
- Murthy, A. S. (1986): Toxicity of pesticides to fish, CRS Press, Inc., Boca Raton, Florida, U.S.A.
- Nagabhushanam R., K. Sambasivarao and Sarojini R. (1986): Acute toxicity of three heavy metals to marine edible crab, *Scylla serrata* J, Adv. Zoo. 7(2): 97-99.
- Nimmo D.R. Lightner D.V. and Bahner L.H. (1971): Effect of cadmium on the shrimp *Panaeus duorarum*, *Palaemonetes pugio* and *Palaemonetes vulgaris*. In : physiological response of marine biota to pollution; *Academic press, New York*.
- Richardson, (1988): Constructed wetland in water pollution program press. New York, NY. 605 p reports no 212.
- Ross SM (1994): Sources and forms of potentially toxic systems. In: Toxic metals in soil-plant systems (Ed: S. M. Ross). Wiley and Sons New York pp3-25.
- Saygdeger (2000): Effect of lead and pH on lead uptake chlorophyll and nitrogen, water air, soil, pollution, 101:323 sorption of cadmium and their effects of growth, portion contents and photosynthetic. [www.fspublisher.org/ijb/postissue / ijabovl.6 no 39 pdf](http://www.fspublisher.org/ijb/postissue/ijabovl.6.no.39.pdf).
- Srinivas S Vutukuru, Balaparameswara Rao M (2000): Impact of hexavalent chromium on survival of the freshwater fish, *Sarotherodon mossambicus*. J. Aqua. Biol. Vol. 15(1&2):71-73.
- Woodard and Muck (1980): Relative toxicity of technical grade and formulated carbaryl and 1-nepthol, and carbaryl induced biochemical changes in the fish *Cirrhinus mrigata*, Environmental pollution series A, Ecological and Biological Volume 34 issue 1984 page 47.54.