International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 12 (2014) pp. 268-275**
http://www.ijcmas.com

Original Research Article

Toxic effects of AlCl3 on Biochemical profile and fecundity of brine shrimp (*Artemia parthenogenetica*)

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Keywords

Artemia, Aluminium chloride, Sub lethal, Fecundity, LC_{50}

A B S T R A C T

The present study was performed to determine the toxic effects of an aluminium chloride on changes in the biochemical profile and fecundity was evaluated in *Artemia parthenogenetica.* It exposed to lethal (LC_{50} value 14.453µg $AlCl_3$/l for 96hrs) and sub lethal concentrations of aluminium chloride for a month. Alteration in the amounts of these biomolecules in the whole body extracted of the $AlCl_3$ exposed *Artemia* were recorded after 96 hrs and day 1 to 30 days compared with control. Fecundity rate was reduced in the higher concentrations and this result showed statistically significant at $P<0.05$ levels. Maximum toxic level was obtained when the concentration was increased and in biochemical levels were reduced. Hence, it is evident that in direct menace metal toxicity effects though all levels.

Introduction

Aluminium is among the most abundant element in the environment. It is present in the environment due to anthropogenic activities, such as industrialization, desalination plants and sewage was to enter into various water bodies. These discharges have been found to cause deleterious effects to the biota of the marine environment (Bu-olayan and Thomas, 2004). Campbell *et al.* (2000) reported that result shows significant accumulation of Al by the freshwater snail *L. Stagnalis* at neutral pH and changes in behaviors (Truscott *et al.,* 1995). Kadar *et al.* (2001) studied the effect of aluminium on the filtering behavior of freshwater bivalve *Anodonata cygnea*; aluminium content was higher in lower dose exposures than high dose exposure since the latter inhibited filtering activity due to value closure which may have prevented the uptake of aluminum. The toxicity of the effluent from an aluminium plant on *Crassostrea gigas* oyster embryogenesis (lethal effects) and larval growth (sub lethal effects) is tested by His *et al.* (1996).

Schofield (1976) investigated that a decrease in reproductive capacity is normally considered to be the overall limiting factor which controls the population of salmon stock in acidified environments. Nelson (1982) observed that low pH reduced growth, increased mortality and delayed the process of hatching. Haven (1993) investigated the effects of the pH and aluminium on the survival of littoral macro-invertebrates by acute bioassay. Although they have not been always well understood, the effects of aluminium on invertebrates have been reviewed by Sparling and Lowe (1996).

Scancar and Milacic (2006) reported that the human are frequently exposed to aluminums from various food additives, therapeutic treatments and also accumulates in all tissues of mammals such as the kidneys, liver, heart, blood, bones and brain (Sanchez-Iglesias *et al.*, 2007; Gonzalez *et al.*, 2009).

Many aquatic invertebrates, including the brine shrimp, can accumulate and tolerate a very high level of metals in the tissues. Although the concentration of metals in most saline water systems is rather low point source discharges such as municipal and industrial wastewater falls out which can lead to high concentrations (Moore and Ramamoorthy, 1984; Salomons and Forstner, 1984) of effects the aquatic environment. The endeavors of present studies propose to observe the effects of $AlCl_3$ on survival, reproduction (fecundity) and biochemical changes of *Artemia* were recorded.

Materials and Methods

Artemia parthenogenetica (Bowen and Sterling, 1978) cysts were collected from Tamaraikulam saltpan (08^0 04' N to 77^0 68' E) Kanyakumari district, Tamilnadu, India. They were stored in a dark place at 5°C until used for testing. Cysts were hatched out in seawater (salinity $28g.L^{-1}$) at approximately 27° ± 2C under vigorous aeration. Nauplii < 48 hrs old were used to initiate the study. Water quality parameters (pH, dissolved oxygen, and salinity) were measured in each test bowls. 1mg of aluminium chloride (reagent-grade) was dissolved in 1L of deionized water and mixing for 2 h to achieve a $1mgL^{-1}$ stock solution.

The experiment consists of seven concentrations and control. The nominal concentrations of 0.001, 0.002, 0.004, 0.008, 0.016, 0.032 and 0.064mg $AlCl_3$/l and were used in the definitive study. These concentrations were based on a range-finding (Probit analysis for determination LC_{50} value calculated by SPSS-version 11) study was done with the results in 4-days for brine shrimp survival. The test solutions were not aerated during the exposure period. On the other hand, chronic assays (0.001, 0.002, 0.004 and 0.008mg $AlCl_3$/l) were fed with rice bran was added every second day. Unwanted debris and unfed in culture has been regularly removed. Once in every 5^{th} day seawater was changed and freshly prepared stock solutions were added in the bowls. The solutions were aerated during the exposure period. The tests were carried out by triplicates with control. Biochemical and reproductive characteristics of experimental animals were recorded. The fecundity rates were determined by counting the number of nauplii released by individual *Artemia* respectively. As we were interested in the synergistic or antagonistic effects of the selected metal substances, we compared the results obtained in the experimental group and evaluated them statistically.

Biochemical components such as total protein, total free sugar and total lipid were

estimated using standard procedures. About 10mg of the whole body (*Artemia*) was homogenized with 10% TCA and centrifuged for 5min at 4000rpm.The supernatant was used for sugar content estimation (Roe, 1955) and the precipitated was dissolved in 0.5ml of 1N NaOH. These samples were done in protein assay by Lowry *et al.,* 1951. The 10mg of wet samples were extracted (2:1) for evaluation total lipid content by using standard methods of Folch *et al.* (1957) and Barnes and Black Stock (1973). The biochemical contents in the samples were calculated by the following formula and results were expressed in µg/mg. Biochemical component = O.D. of the sample / O.D. of the estimated × Concentration of the standard. The data obtained by exposing the *Artemia parthenogenetica* to different concentrations metals were subjected to statistical analyses such as standard deviation, Probit analysis, one-way analysis of variance and their significance expressed as $P < 0.05$ levels.

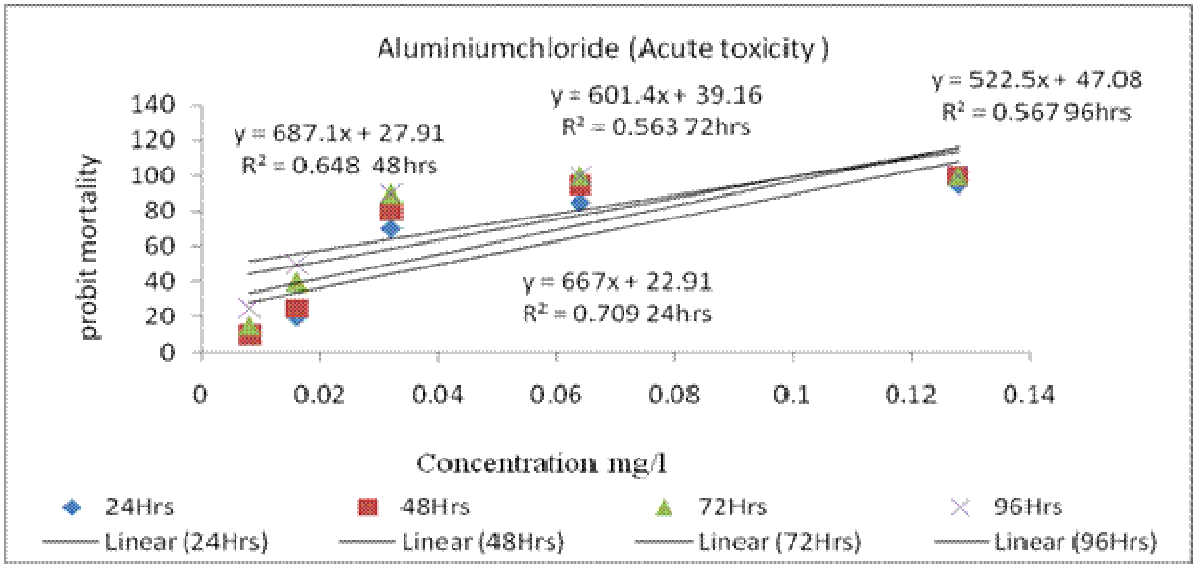
Result and Discussion

The results of acute (96 h) toxicity of aluminum chloride on the mortality rate of *Artemia parthenogenetica* (48 h old) were plotted (Figure 1). The $AlCl_3$ was found to be acutely toxic to nauplii of the test *Artemia* at was high concentrations ranging from 0.016 to 0.128 mg $AlCl_3$/l. The calculated LC_{50} value 0.014 mg $AlCl_3$/l for 96h. These samples were estimated in various biochemical analyses. Total free sugar content decreased from 0.001to 0.016 mg $AlCl_3$/l concentrations and the sugar content of 0.001 mgL^{-1} was 0.111µgmg^{-1}. When compared to control with 0.129 µgmg^{-1}. The total protein content of the control 0.368µgmg^{-1} and it decreased from 0.001 (0.330 µg mg^{-1}) to 0.016 mg (0.270 µgmg^{-1}) $AlCl_3$/l concentrations. The total lipid contents were of 0.001mg $AlCl_3$/l (0.225µgmg^{-1}) with reference to the control (0.241µgmg^{-1}). These results have shown all biochemical levels were decreased significantly ($P<0.05$) in a Figure 2.

The chronic toxicity studies and no significant mortality were observed in different concentration level and no significant growth effected for 30th day. The biochemical components were significant ($P<0.05$) decreased from while comparing with control. The results were observed as follows (Figure 3). The total protein content of control in *Artemia* exposed to $AlCl_3$ was 0.331µgmg^{-1} and was found to decrease from 0.001 (0.316 µgmg^{-1}) to 0.008mg (0.265µgmg^{-1}) $AlCl_3$/l and with control. The maximum levels of total free sugar were recorded in lower concentration of 0.001 mg $AlCl_3$/l (0.132 µgmg^{-1}) with reference to the control (0.141 µgmg^{-1}). The sugar content of 0.002 to 0.008 mg $AlCl_3$/l were (0.132, 0.120 and 0.105 µgmg^{-1}) compared with that of control. The total lipid contents of the control were 0.261 µgmg^{-1} and it decreases from 0.002 to 0.008mg $AlCl_3$/l concentrations (0.230, 0.220, 0.210 and 0.180µgmg^{-1}) when compared to that control.

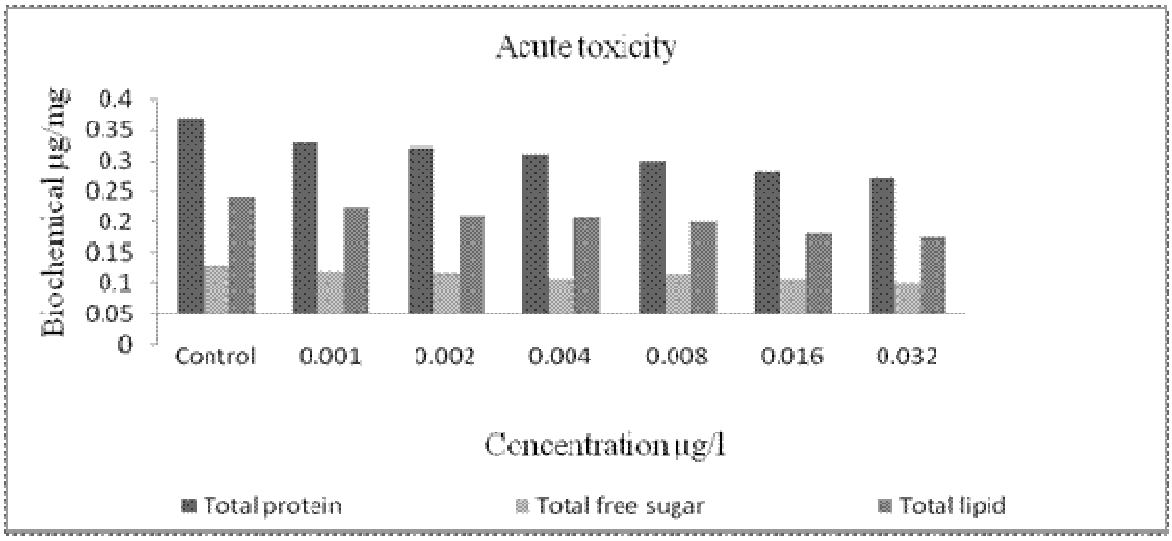
The nauplii were exposed to $AlCl_3$ during the chronic and each concentration of individual's mature *Artemia* were transformed to separate bowls and Figure 4 shows the daily released nauplii were recorded. It appears that nauplius occurs at variation peak levels during releasing time correspondingly. The fecundity rate was decreased from the highest concentration 0.008mg $AlCl_3$/l (240) to lower concentrations 0.001mg $AlCl_3$/l (270) was increased with respectively. The control shows the number of nauplii (302) were that resulted in the Figure 4 as a statistician ($P<0.05$).

Figure.1 LC_{50} Determinations for aluminum chloride toxicity in *A.parthenogenetica* (concentrations / Probite regression analysis)



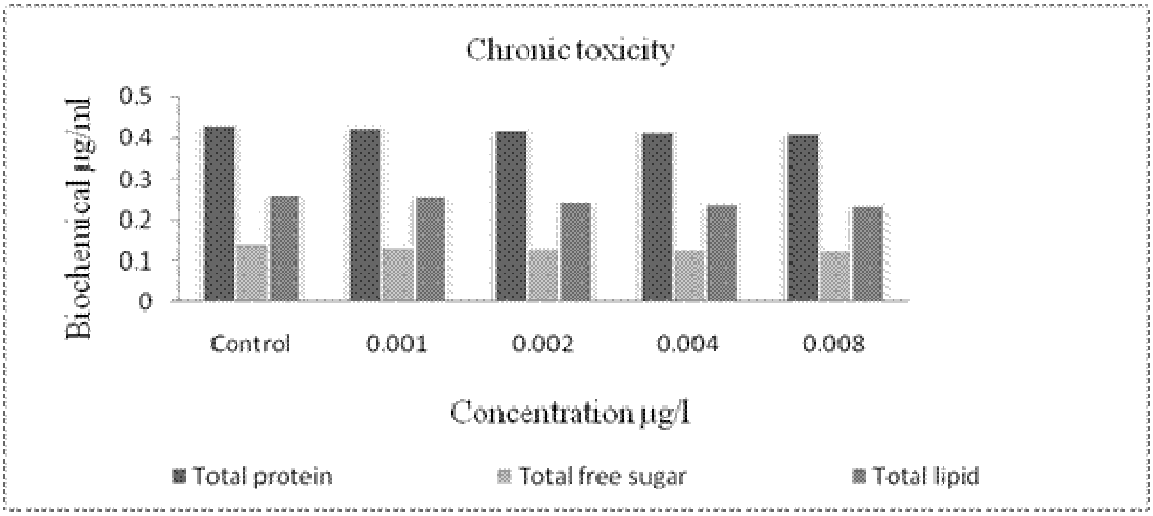
Significant value P<0.05% level

Figure.2 Total biochemical fluctuations of aluminium chloride exposed to *Artemia* at acute toxicity studies (96 hrs)



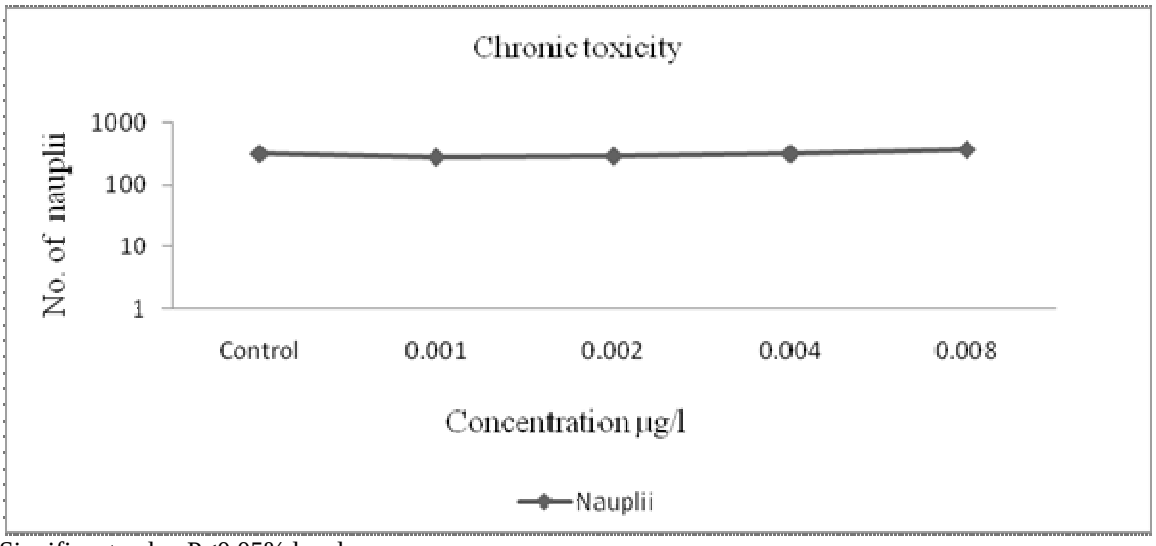
Significant value P<0.05% level

Figure.3 Total biochemical fluctuations of aluminium chloride exposed to *Artemia* at chronic toxicity (30th day)



Significant value P<0.05% level

Figure.4 Frequency of spawning in *A. parthenogenetica* during chronic toxicity (1–30 days) after exposure to aluminium chloride



Significant value P<0.05% level

The present data clearly indicated that the increased concentration levels of aluminium chloride have reduced the biochemical levels in test organisms. It is unlikely that the poor growth is the result of energetic deficiency brought on by the need to mobilize and eliminate toxicant (Moles *et al.,* 1987). The findings were also closely consistent with the above statement and changes in growth rates in adults also associated with the relationship between aluminium chloride accumulations and decreased of growth rate also noticed. Wickins (1972) investigation suggested that a nutritional deficiency was involved. This result suggests in direct menace that total biochemical levels to alter in chronic test,

but no mortality occurs at the end of the day.

Artemia is a major food source in marine and freshwater aquaculture **(**Bengtson *et al.,* 1991; Brix *et al.,* 2006), and exposure of *Artemia* populations to environmental contamination may result in population decline and the production of contaminated cysts which are potentially harmful if used in aquaculture.

Several scientific reports have indicated that the acute toxicity tests with early stage supply information on mortality and numerous other significant endpoints such as malformations, growth inhibition, and developmental process delays (Herkovits *et al.,* 1997; Nebeker *et al.,* 1985; Mehrle and Mayer, 1985). Thonga-ar *et al.* (2003) reported that the nauplii can tolerate very high concentration of a heavy metal and also absorb the metal from test solution. The same tendency was observed by correlating with the acute effects of biochemical levels as well as survivals were recorded.

Fecundity is one of the potential reproductive capacities of an organism or population, measured by the number of gametes. *Artemia* fecundity was defined as the total sum of all recoverable gametes produced by a single female throughout her reproductive history (Squire, 1979)**.** Aluminium chloride significantly were reduced the fecundity rate of the f1 generation. Fecundity and the growth showed characteristic response to environmental stress. By, the interfering with this response on aluminium chloride concentrations in the effects of increasing the vulnerability of brine shrimp to their environment and have been shown to be toxic (Tisler and Zagorc-Koncan, 2002; Donaldson, 1990).

It is concluded from the present study on aluminium chloride toxicity reveals the *Artemia parthenogenetica* is very sensitive to the toxic in adult than nauplii. The biochemical changes were shown in all the experimental levels of brine shrimp and fecundity was not produced in higher concentrations of *Artemia*. These species are linked directly or indirectly to the human food chain. Because of this, it is important that we should be observing of their exposure to environmental mutagens and carcinogens. Likewise, the organisms have the capacity to transform these agents to biologically active metabolites and accumulate toxicants in their cells and tissues at concentrations several orders of magnitude above that found in the environment**.**

Acknowledgement

The authors are grateful to Centre for Marine Science and Technology, M.S.University, Rajakkamangalam and official of Department of Zoology, Thiagarajar College, Madurai-625009, Tamilnadu, India

References

Barnes, H., Black Stock, 1973. Estimation of lipids in marine animals and tissue: Detail investigation of the sulphophosphovanillin method for total lipids. *J. Exp. Mar. Biol. Ecol.*, 12: 103–118.

Bengtson, D.A., Léger, P., Sorgeloos, P. 1991. Use of *Artemia* as a food source for aquiculture. In: R.A. Browne, P. Sorgeloos and C.N.A. Trotman (Eds.), *Artemia* Biology, CRC Press, Boca Raton, Florida. Pp. 255–285.

Bowen, Sterling, 1978. *Artemia partheniogenetica* Taxonomic Serial No: 624245.

Brix, K., Gerdes, R., Adams, W., Grosell, M. 2006. Effects of copper, cadmium, and zinc on the hatching success of brine shrimp (*Artemia franciscana*). *Arch. Environ. Contam. Toxicol.*, 51(4): 58.

Bu-Olayan, A.H., Thomas, B.V. 2004. Effects of trace metals, harmful algal blooms, nutrients and hydrological variables to mullet *Liza klunzingeri* in Kuwait Bay. *Biosci. Biotech. Res. Asia.*, 2(1): 1–8.

Campbell, M.M., White, K.N., Singh, R.J., Powell, J.J., McCrohan, C.R. 2000. Effect of aluminium and silica acid on the behavior of the freshwater snail *Lymnaea stagnalis*. *Can. J. Fish. Aquati. Sci.*, 57: 1151–1159.

Donaldson, E.M. 1990. Reproductive indices as measures of the effects of environmental stressors in fish. *Am. Fish. Soci. Sym.*, 8: 109–122.

Folch, J., Lees, M., Slonae – Stanlley, G.H. 1957. A simple method for isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, 226: 497–507.

Gonzalez, M.A., Bernal, C.A., Mahieu, S., Carrillo, M.C. 2009. The interactions between the chronic exposure to aluminium and liver regeneration on bile flow and organic anion transport in rats. *Biol. Trace Elem. Res.*, 127: 164–176.

Haven, K.E. 1993. Acid and aluminium effects on the survival of littoral Macro -Invertebrates during acute bioassays. *Environ. Pollut.*, 80: 95–100.

Herkovits, J., Cardellini, P., Pavanati, C. Perez-Coll, C.S. 1997. Susceptibility of early life stages of Xenopus *laevis* to cadmium. *Environ. Toxicol. Chem.*, 16(2): 312–316.

His, E., Beiras, R., Seama, M.N., Pagona, G., Trieff, N.M. 1996. Sublethal and lethal toxicity of aluminium industry effluents of early developmental stages of the *Crassostrea gigas* oyster. *Arch. Environ. Contam. Toxicol.*, 30: 335–399.

Kadar, E., Salanki, J., Jugdaohsingh, R., Powell, J.T., McCrobon, C.R., White, K.N. 2001. Avoidance responses to aluminium in the freshwater bivalve *Anodonta cygnea*. *Aquatic. Toxicol.*, 55: 137–148.

Lowry, O.H. Roosenborough, N.J., Farr, A.C., Randall, R.J. 1951. Protein measurement with folin phenol reagent. *J. Bio. Chem.*, 193: 265–275.

Mehrle, P.M., Mayer, F.L. 1985. Biochemistry/ Physiology. In: G.M. Rand and S.R. Petrocelli (Eds.), Fundamentals of aquatic toxicology: methods and applications. Hemisphere Washington, DC. Pp. 264–282

Moles, A., Babcock, M.M., Rice, S.D. 1987. Effects of oil exposure on pink Salmon, *Oncorhynchus gorbuscha*, alevins in a simulated intertidal environmet. *Mar. Environ. Res.*, 21: 49–58.

Moore, J.W., Ramamoorthy, S. 1984. Heavy metals in natural waters. Springer Verlag, New York, USA. 268 Pp.

Nebeker, A.V., Savonen, C., Stevens, D.G. 1985. Sensitivity of rainbow trout early life stages to nickel chloride. *Environ. Toxicol. Chem.*, 4: 233–239.

Nelson, J.A. 1982. Physiological observations on developing rainbow trout *Salmo gairdneri*, Richard son, exposed to low pH and varied calcium ion concentrations. *J. Fish. Biol.*, 20: 359–372.

Roe, J.H. 1955. The determination of sugar in blood and spinal fluid with anthrone reagent. *J. Biol. Chem.*, 212: 335–343.

Salomons,W., Forstner, V. 1984. Metals in the hydrocycle. Springer Verlag, Berlin, Germany. 349 Pp.

Sanchez-Iglesias, S., Soto-Otero, R., Iglesias-Gonzalez, J., Barciela-Alonso, M.C., Bermejo-Barrera, P., Mendez-Alvare, E. 2007. Analysis of brain regional distribution of Aluminium in rates via oral and interperitoneal administration. *J. Trace Elem. Med. Biol.,* 21: 31–34.

Scancar, J., Milacic, R. 2006. Aluminium speciation in environmental samples: A review. *Anal. Bioanal. Chem.,* 386: 999–1012.

Schofield, C.L. 1976. Acid precipitation: effects on fish. *Ambio,* 5: 228–230.

Sparling, D.G., Lowe, T.P. 1996. Environmental hazard of aluminium to plants, invertebrates, fish and wildlife. *Rev. Environ. Contam. Toxicol.,* 145: 1–127.

Squire, R.D. 1979. The effects of acute gamma irradiation on the brine shrimp, *Artemia*. III. Male F1 reproductive performance following paternal irradiation of mature sperm. *Biol. Bull.,* 144(1): 192–9.

Thonga-ar, W., Parkpian, P., Tang, A. 2003. Toxicity of mercury to growth and survival of seabass larvae, *Lates calcarifer* and the modifying effectes of salinity. *Sci. Asia.,* 29: 209–219.

Tisler, T., Zagorc-Koncan, J. 2002. Acute and chronic toxicity of arsenic to some aquatic organisms. *Environ. Contam.Toxicol.,* 69: 421–429.

Truscott, R., McCrohan, C.R., Bailey, S.E.R., White, K.N. 1995. Effect of aluminium and lead on activity in the freshwater snail *Lymnaeastagnalis*. *Can. J. Fish. Aquat. Sci.,* 52: 1623–1629.

Wickins, J.F. 1972. The food value of brine shrimp, *Artemia* salinaL., to larvae of the prawn, *Palaemon serratus* Pennant. *J. Exp. Mar. Biol. Eol.,* 10: 151–170.