JJBS

Jordan Journal of Biological Sciences

Volume 3, Number 4, December 2010
ISSN 1995-6673
Pages 141 - 146

Effect of Selenium and its Compounds on Oxygen Uptake in Freshwater Fish *Gambusia affinis* after Exposure to Lethal Doses

Rajashri. R Naik [a] and Huchhappgoudar.S.Patil [b]

[a] *Faculty of Pharmacy and Medical Sciences, Amman University, Amman, Jordan ;* [b] *Department of Zoology, Karnataka University, Dharwad , Karnataka India*

.

Abstract

The purpose of this study was to investigate the change in oxygen consumption of *Gambusia affinis* after exposure to selenium and its compounds. Male and female fish used in this study were exposed to known concentration of selenium and its compounds. All the forms of selenium showed concentration and exposure dependent inhibition. At the higher concentration of selenium and selenite male fish showed decrease in consumption, which later recovered, with increase in exposure period. Selenate showed relative decrease in uptake of oxygen. In female fish, selenium at lower concentration showed uniform decrease, at higher concentration after initial decrease recovery in uptake was observed. In selenate at lower concentration there was increase in oxygen uptake and at higher concentration there was decrease in uptake. All forms of selenium showed inhibitory action with decline at lower concentration and fluctuations at higher concentration of metals, with increase in exposure period there was slight recovery in oxygen uptake.

Keywords: Selenium, Selenite, Selenate, Oxygen uptake, *Gambusia affinis* and Fish.

1. Introduction

Freshwater are highly vulnerable to pollutants since they act as immediate sink for the consequences of human activity always associated with danger of accidental discharges or criminal negligence (Vutukuru, 2003). Heavy metals constitute a core group of aquatic pollutants and additional concentrations of these metals accumulate in the aquatic ecosystem as a result of land based activities. Fish mostly have the tendency to bioaccumulate heavy metals and human might be at great risk some time even lethal, through contamination of food chain (Ui, 1972). Selenium widely used in glass manufacturing industries chemical and pigment factories; it is also released from municipal waste, combustion of fossil fuels and industrial loses. Selenium is a naturally occurring element required in trace amounts for plants and animals. It is found in four oxidation states, selenate and selenite are highly soluble in water and are know to be toxic to biological system at relatively low concentration. Selenate and selenite predominate in aquatic environment because of their high solubility in water (Massecheleyn et al 1990). Studies have indicated that selenite was found to be more toxic then selenite (Maier *et al.,* 1988 b). Selenium is essential metal for number of domestic animals, the optimal concentration ranges for fish growth and reproduction are narrow and both excess and deficiency are harmful to the fish.

Information on lethal exposure of selenium and its compounds on the physiology of fish are limited and its effect on *Gambusia affinis* is not known. Knowledge of acute toxicity of a xenobiotic often can be very helpful in preventing and predicting acute damage to aquatic life in receiving water and as well as in regulating toxic waste discharges (APHA,1998). In view of this oxygen uptake by both male and female *Gambusia affinis* were studied after exposing it to lethal dose of selenium and its compounds. The corresponding results is being discussed in this paper and compared with other fishes exposed to various other metallic and environmental stresses.

Gambusia affinis is freshwater member of poecilidae is a diminutive fish rarely exceeding 46 mm in standard length, fish of this genus are well know for their consumption of insect larvae. This fish is used as biological control in the infestation of mosquito larva and is commonly known as mosquito fish. It is used world wide in control of mosquito larvae, native of southeastern United States and northeastern Mexico. Now it is one of the most widely distributed freshwater fish (Krumholz, 1948).

2. Materials and methods.

Fish collected from the local pond were transferred to fish tank in laboratory with continuous flow of dechlorinated tap water, fish were fed every alternate day

* Corresponding author. rajashrinaik4@yahoo.com.

with Shalimar fish feed. Male and female fish were separated based on their size (female being larger), coloration and presence of gonopodial hook in male and gravid spot in female fish. Prior to the study all the glassware were washed thoroughly and both male and female fish of approximately equal size transferred to rectangular glass for experimentation, each trial was conducted with parallel control, feed was not given during the study and 24 hr prior to the study. Water quality characteristic in each aquarium were determined at the initial stage by following APHA 1998.

2.1. Preparation of stock solution

Selenium: Metallic selenium (Se purity – 99.5%) was digested with 2.5 ml concentrated nitric acid in a beaker; the digested solution was transferred to 100 ml volumetric flask and diluted with distilled water upto the mark. Oxygen uptake was studied using this stock solution with a parallel control (with same amount of concentrated nitric acid).

Selenite: Sodium selenite ($NaSeo_3$ $5H_2O$- purity 99.0%) was weighed and transferred to volumetric flask, dissolved with distilled water and volume made upto 100ml, the concentration of selenite in salt was 0.478 g and Na and H_2O and impurities is 0.527 g per 100ml of solution. Therefore, each ml of stock solution contains 4.78 mg selenite. Oxygen uptake was calculated on the basis of amount of selenite in solution using distilled water as control.

Selenate: Sodium selenate 1.0 g ($NaSeo_4 10H_2O$ purity 97.0%) was weighed and transferred to 100 ml volumetric flask, selenate was dissolved in distilled water and the volume was made upto 100 ml. Selenate is 0.375 g and sodium and other impurities are 0.625 g per 100ml of solution. Therefore each ml of stock solution contains 75 mg of selenate. Oxygen consumption was calculated on the basis of selenate in the solution and using distilled water as control.

2.2. Oxygen uptake

For oxygen uptake Winkler's Azide modification method (APHA, 1998) was employed. In this method one molecule of O_2 = 2 molecules of iodine produced at the end of reaction in bottle.

Therefore 1 ml of standard sodiumthiosulphate (0.025 N) = 0.2 mg of dissolved oxygen as mg/l = 0.2×5 = 1 mg/l. Hence, burette reading directly gives amount in weight of oxygen dissolved. Choubey and Pandey (1993) method was adapted for measurement of oxygen consumption with surfacing prevented and surfacing allowed to the experimental fish, fishes were confined to fixed volume of water in respiratory jar for particular length of time, volume of water was in proportion to size and weight of fish, for each set of experiment a parallel control with fish and without toxicant and a blank was set. Blank was used to detect oxygen consumption of microorganism and other oxidizing materials. After a know interval of time dissolved oxygen of water in jar was estimated, from dissolved oxygen of blank dissolve oxygen of water in which control fish were put and dissolved oxygen of water in which experimental fish, the amount of oxygen consumed by test fishes was calculated, weight of control and exposed fish was noted and oxygen consumed was expressed as mg of O_2 $h^{-1}g$ body weight, the consumption rate of control was taken as 100% (Normal rate) around 10 samples were taken.

3. Results

Male and female fish were exposed to three different concentrations of selenium, selenite and selenate for 24, 48, 72, and 96 hrs and oxygen uptake of the stressed fish was measured at intervals of 24, 48, 72, 96 hr of exposure

At lower concentration of 5 mg selenium, male fish showed an initial increase (24 hr) of 36.70% in percent utilization of oxygen which dropped to 15.745 at 48 hr, there after a rapid increase in uptake was observed from 50.21 to 59.39% at 72 and 96 hr exposure. At 6 mg/l, small amount of 5.06% of oxygen was consumed followed by marked depletion in uptake at 48 hr (-62.96%) which later increased to 21.81%. Higher concentration of 10 mg /l showed an initial enhancement of 20.88% followed by sudden drop in uptake of -76.85% at 48 hr, which recovered from 72 hr with 3.43% and 12.04% at 96 hr.

On exposure to different concentration of selenite, it was found to be time and concentration dependent. Lower concentration of selenite (8 mg /l) showed steady increase of 28% which raised to 59.76% at 96 hr. Percent utilization of oxygen ranged from 23.40 at 24 hr to 55.85 at 96 hr on exposure to 9.5 mg/l. Oxygen consumption was reduced at 13 mg/l to 7.09% which thereafter increased gradually to 53.12%. It is evident from the data that percent uptake of oxygen declined with increasing concentration in the test medium.

Selenate a less toxic compound when compared to other two forms also induced fish to consume more oxygen available in test medium. At 65 mg/l selenate, a constant uptake of 32.74% in consumption of oxygen was observed upto 48 hr of exposure, thereafter a sudden increase in uptake of 53.19% was observed at 72 hr which further rose to 61.81% at 96 hr. At 75 mg/l, percent oxygen uptake ranged from 15.20 at 24 hr to 31.03 at 96 hr, high concentration of 85 mg/l selenate lowered rate of uptake was recorded, with the values that varied between 2.92 at 24 hr and 29.88% at 96 hr.

Consumption of oxygen in male fish was found to be concentration and time dependent, the rate of oxygen uptake reduced with increase in concentration of selenate, however with increase in exposure period there was increase in uptake of oxygen. The results were statistically analyzed with two way ANOVA and was found to be significant at $P<0.001$. Results are presented in Table 1 and Fig 1.

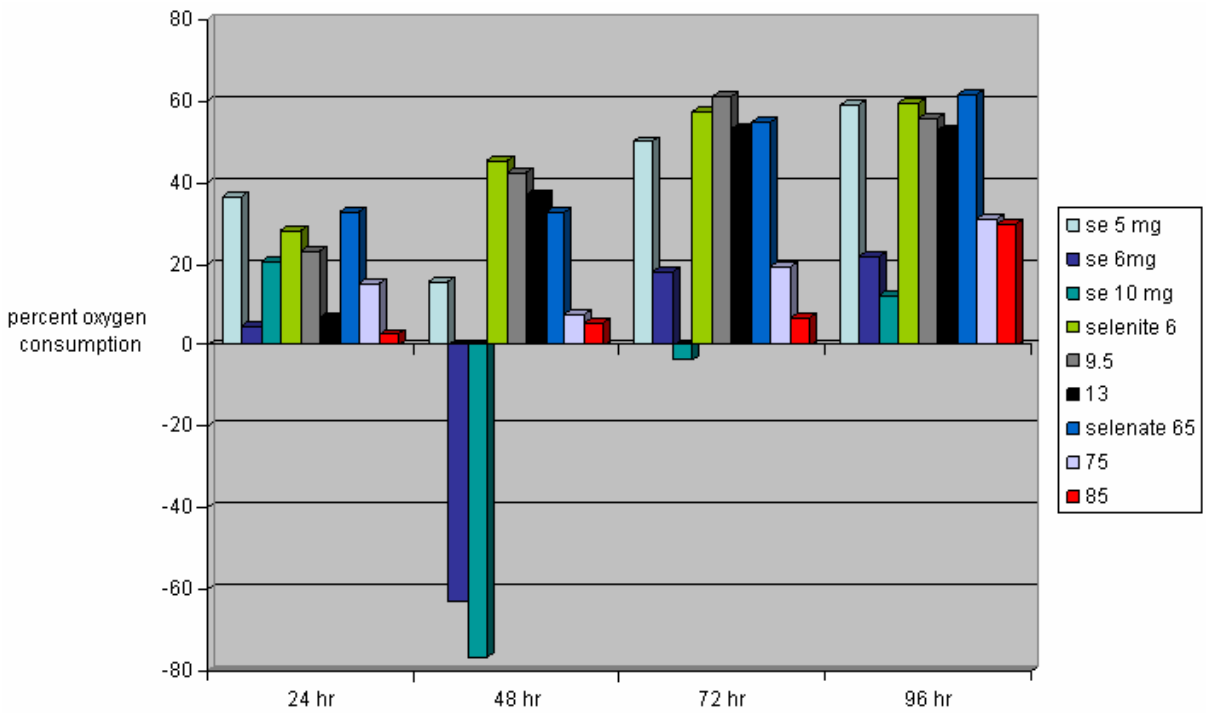

Figure 1. Oxygen consumption in male fish.

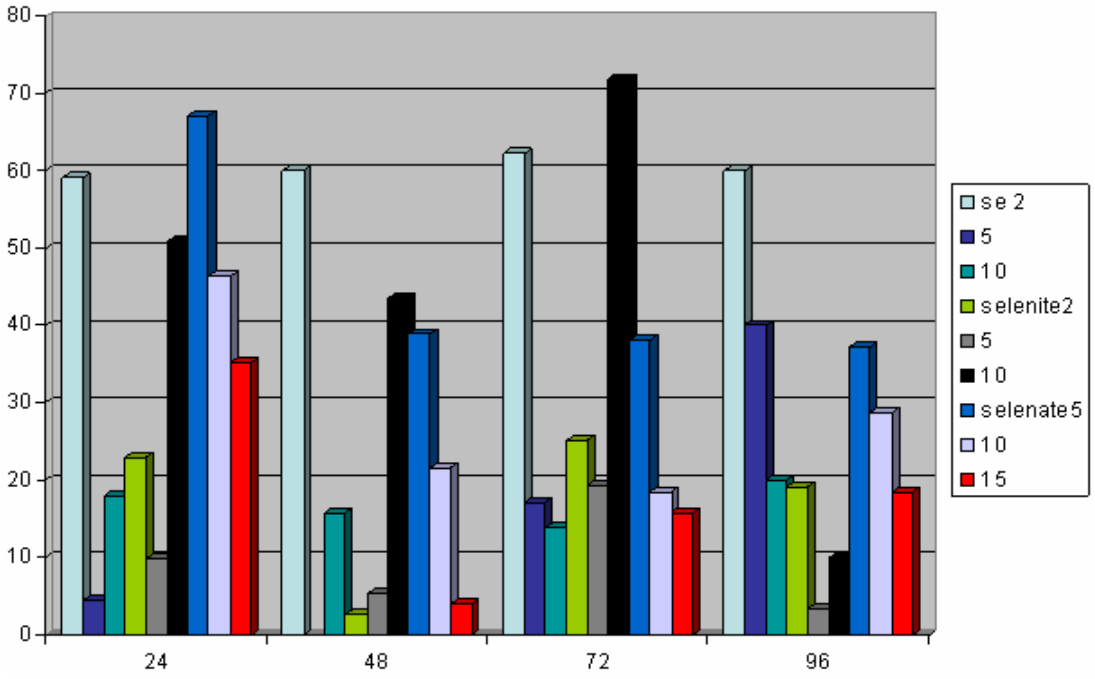

Figure 2. Oxygen consumption in female fish.

Female fish was exposed to three lethal concentrations of selenium and its compounds. The three lethal concentration of selenium chosen were 2, 5 and 10 mg/l and the response to oxygen uptake was studied. There was a uniform decrease in oxygen utilization at the lowest dose of 2 mg/l, percent utilization fluctuated between 59.01% and 62.24%. At 5 mg/l, the rate of uptake was drastically affected and a slow recovery in uptake with increase in exposure period was observed. Fish after exposure to 10 mg/l showed initial increase in uptake, which decreased gradually from 18.03% to 13.69% (at 72 hr) and later recovery in uptake of 40% was observed at 96 hr of exposure. Data revealed that first two days of exposure, percent uptake was not found to be concentration – dependent and decrease in last two days of uptake was concentration – dependent.

In response to selenite exposure (2, 5 and 10 mg/l), fish did not show uniform consumption of oxygen however the uptake rate fluctuated with time of exposure and concentration selenite concentration. Lower concentration of selenite showed initial increase in uptake fallowed by sharp decline to 2.61% and in subsequent observation an enhancement fallowed by decline in uptake was noticed. Similar trend in uptake was observed at 5 and 10 mg/l of selenite. The results were statistically analyzed with Two way ANOVA and the results were statistically significant at $P<0.001$ and $p>0.005$ at exposure period.

The pattern of variation in oxygen uptake in fish on exposure to three different concentrations of 5, 10 and 15 mg/l selenate showed a different trend. Fish dosed with 5 mg/l showed rapid increase in uptake at 24 hr. Thereafter there was a steep decline and later uniform pattern in uptake was observed through out the experimental period. Oxygen uptake reduced from 46.67% to 18.42% at 72 hr, followed by recovery in uptake of 28.81% at 96 hr. At higher concentration of 15 mg/l fish consumed 35.29% oxygen at 24 hr, which dropped to 3.88%, thereafter steady increase in uptake was observed. Results were statistically analyzed by two way ANOVA and were found to be significant at $P<0.001$, except at exposure period $P>0.0054$. Results are presented in table 2 and Fig 2.

4. Discussion

The results of the present study show that fish under toxic stress to different forms of selenium altered oxygen uptake. In general, initial decrease of oxygen uptake followed by an increase in its consumption was observed. All forms of Se have shown inhibitory action on rate of oxygen utilization and at time irregular uptake was noticed. However, as exposure period advanced a slight recovery was found in both male and female fish (Koti, 1996) has shown that Cu, Zn and Ni during lethal and sub lethal exposure (Individual and mixture of metals) altered oxygen consumption in both sexes of *G.affinis*. It was observed that fish in response to metal exposure secreted mucus in the test medium and red patches appeared at gill region and basal part of pectoral fins. Environmental factors such as temperature, pH, and hardness of the test medium, have shown cumulative effect on oxygen uptake of *G.affinis* in the presence of Cu, Zn and Ni (Kallangoudar and Patil, 1997). Lemly (1993) demonstrated metabolic stress in bluegills *Lepomis macrochirus* due to elevated concentration of selenium. He also showed reduced respiratory activity with increase in respiratory demand and oxygen consumption due to gill damage. According to (Tovell *et al.*, 1975) metals mainly enter fish through respiratory system. The mechanism for metal uptake through gill probably occur through pores by simple diffusion (Bryan, 1979) these metals are then absorbed through cell membranes (Opperhuizen *et al.*,1985) Metals then coagulate in protoplasm after absorption into the bodies of aquatic animals (Skidmore, 1964). The decrease in oxygen uptake observed following exposure to selenium, selenite and selenate was possibly due to mucus precipitation on gills, during the present study mucus was present on the gills which appeared as reddish patches and was possibly secreted in response to the irritation caused by metalloids (Carpenter, 1930) investigated lethal action of dissolved metals salts on fish leading to death. According to him death resulted from interaction between metallic ions and mucus secreted by the gills and not from internal poisoning. A layer of coagulated mucus is formed on the gill surface which impairs respiratory efficiency to such an extent that fish becomes asphyxiated (Rani and Ramamurthi, 1987; Gosh and Chakraborti, 1990). Increased utilization of oxygen during later part of lethal exposure to selenium and its forms indicate that under stress fish might have used more oxygen to meet metabolic demand. Similar effect of metal stress on respiratory activity in other fish has been reported, (Davis, 1975; Van Resburg1989; Shivraj, 1990; Koti, 1996; Vijayamohanan *et al.*, 2000). Other possible alternate reason of inhibitory action of pollutants include gill damage (Natarajan, 1981; Koti, 1996) and internal action of pollutants (Natarajan, 1981; Tuurala and Soivio, 1982)

Selenium and its compounds has shown to affect oxygen uptake in both male and female fish after exposure to lethal dose, which is evident from the gradual decline in uptake at lower concentration and fluctuations at higher concentration. Which may be due to over secretion of mucus resulting in blocking of gills or due to onset of severe hyoxia, which alter the metabolic pathways or due to damage caused my metals to gill. Increase in uptake may be due to increase in demand under toxicant stress.

Table 1. Percent oxygen uptake in male fish *G.affinis* after exposure to lethal concentration of selenium and its salts.

Toxicant	Dose(mg/l)	Exposure period (hours)				F-Ratio	P-value
		24	48	72	96		
Control	-	39.57±0.86 (100%)	35.25±0.96 (100%)	22.62±1.16 (100%)	18.69±1.28 (100%)	-	-
Se	5	36.70±0.48	15.74±0.44	50.21±0.57	59.39±0.53	0.530	0.001
	6	5.06±0.73	-62 .96±1.53	18.02±0.93	21.80±1.02	6.630	0.001
	10	20.88±0.61	-76.85±0.93	-3.43±1.18	12.40±1.14	5.570	0.001
selenite	8	28.36±0.51	45.59±0.53	57.44±0.50	59.76±0.52	70.260	0.001
	9.5	23.40±0.56	42.40±0.57	61.27±0.45	55.85±0.59	7.610	0.001
	13	7.09±0.65	37.17±0.60	53.19±0.57	53.12±0.61	52.220	0.001
selenate	65	32.74±0.60	32.96±0.65	55.08±0.55	61.68±0.50	186.854	0.001
	75	15.20±0.73	7.85±0.87	19.49±0.94	31.03±0.89	25.504	0.001
	85	2.92±0.81	5.75±0.90	6.77±1.08	29.88±0.90	12.914	0.001

Results are Significant at $P<0.001$

Table 2**.** Percent oxygen uptake of female fish *G.affinis* after exposure to lethal concentration of selenium and its salts.

Toxicant	Dose (mg/l)	Exposure period (hours)				F-Ratio	P- value
		24	48	72	96		
Control	-	39.92±0.83 (100%)	38.98±0.96 (100%)	33.77±1.13 (100%)	27.41±1.16 (100%)	-	-
Se	2	59.01±0.36	60.09±0.40	62.24±0.44	60.00±0.48	31.250	0.001
	5	4.37±0.85	3 .84±0.97	17.01±0.97	40.00±0.73	155.850	0.001
	10	18.03±0.73	15.86±1.50	13.69±1.02	20.00±0.97	3.460	0.001
selenite	2	22.85±0.61	2.61±0.92	25.10±0.92	19.12±1.01	73.260	0.001
	5	9.71±0.79	5.23±0.89	19.27±0.93	3.18±1.20	2.650	0.054*
	10	50.85±0.43	43.45±0.56	71.86±0.37	9.96±1.12	43.760	0.001
selenate	5	67.05±0.13	38.88±0.57	38.15±0.70	37.28±0.74	43.760	0.001
	10	46.47±0.45	21.66±0.70	18.42±0.92	28.81±0.82	176.260	0.001
	15	35.29±0.81	3.88±0.85	15.78±1.89	18.45±0.95	4.258	0.001

Result are significant at $P<0.001$, except the exposure period $P>0.054$

References

APHA; AWWA; WPCP 1998. **Standard Methods for the Examination of Water and Wastewater.** 20th ed. American Public Health Association, Washington, DC.

Bryan GN. 1979. Bioaccumulation of marine pollutants. Philos Trans Res Soc, London B **286**: 483.

Carpenter KE. 1930. Further researches on the action of metallic salts of fishes. J Exp Zool **56**: 407 – 422.

Choubey B.G. and Pandey P.K.1993.Seasonal changes in oxygen consumption in relation to environmetal factors and ECH of thyroid gland in the fish *Clarius batrachus (L)* female. Environment and Ecology **11** (1): 243 – 245.

Davis JC. 1975. Minimal dissolved oxygen requirement of aquatic life with emphasis on Canadian species: A review. J. Fish Res, Bd Cannada **32**:2295 – 2332.

Gosh AR Chakraborti P. 1990.Toxicity of arsenic and cadmium to a freshwater fish *Notopterus notopterus*. Environ Ecol. **8(2)**: 576 – 579.

Kallangoudar YP Patil HS. 1997. Influence of water hardness on copper, zinc and nickel toxicity to *Gambusia affinis (B &G).* J Environ Biol. **18(4)**: 409 – 413.

Koti DL. 1996. Studies on the individual and combined effect of some toxic metals to a freshwater fish *Gambusia affinis* (PhD thesis) Dharwad: Karnataka University, India..

Krumholz LA. 1948. Reproduction in western mosquito fish Gambusia affinis (Baird and Girard) and its use in mosquito control. Ecol.Monog. **18**: 1-43.

Lemly AD. 1993. Metabolic stress during winter increased the toxicity of selenium to fish. Aquat. Toxicol. **27**: 133 – 158.

Natarajan GM. 1981. Effect of lethal (LC_{50}/48 hr) concentration of metasytox on sublethal oxidative enzymes, tissue respiration and histology of freshwater air breathing fish *Channa striatus(blecker)*. Curr Sci. **50**: 985 – 989.

Opperhuizen A Van der Velde EW Gobas FAPC et al. 1985. Relationship between bioconcnetration in fish and steric factors of hydrophobic chemicals. Chemosphere **14(11)**: 1871 – 1896.

Rani AV Ramamurthi R. 1987. Cadmium induced behavioural abnormalities of the fish *Tilapia mosambica.* Environ Ecol. **5**: 168 – 169.

Shivraj KM. 1990. Studies on the toxicity of some heavy metals on freshwater fishes (PhD thesis) Dharwad : Karnataka University, India.

Skidmore JF.1964. Respiration and osmoregulation in rainbow trout with gills damaged by zinc sulphate. J Exp Biol. **52**: 481 – 484.

Tovell PWA Howes D Newsome CS. 1975. Absorption metabolism and excretion by goldfish of the anionic detergent, sodium lauryl sulphate. Toxicology **4**: 17 – 29.

Tuurala H Soivio A. 1982. Structural and circulatory changes in the secondary lamellae of *Salmon gairdneri* gills after sublethal exposure to dehydroabietic acid and zinc. Aquat.Tox. **2**: 21 – 29.

Ui J . 1972. **The Changing Chemistry of the Oceans**. Almquist and Wiksells, Stockholm

Van Resburg EL. 1989. The bioconenrtation of atrazine, zinc and iron in *Tilapia sparrmanii (Chiclidae)* PhD thesis, Rand: African Univ South Africa p – 137.

Vijayamohanan Nair A Suryanarayanan H. 2000. Lethal limits and respiration in the chiclid fishes *Etroplus Maculatus (Bloch)* and *Etroplus suratensis (Bloch)* exposed to effluents from Titanium di oxide factory. J Environ Biol. **21 (3)**: 169 – 172.

Vutukuru SS. 2003. Chromium induced alterations in some biochemical profiles of the Indian major carp, Labeo rohita (Hamilton). Bull. Environ Contanm Toxicol. **70**: 118 – 123.