Macedonian Journal of Ecology and Environment
Vol. 18, issue 1
pp. 8-13
Skopje (2016)
ISSN 1857 - 8330
Original scientific paper
Available online at www.mjee.org.mk

Alterations in the Levels of Ions in Vital Organs of Indian Major Carp, *Labeo rohita* Exposed to Profenofos

Промени во нивото на јони во витални органи на индискиот голем крап (*Labeo rohita*) експониран на профенофос

Bantu Nagaraju[1*], Erhan Ünlü [2], Zenebe Teka [1], Zenebe Hagos [1], Mulugeta Referral[1]

[1] Department of Chemistry, Aksum University, Axum-1010, Tigray, Ethiopia, Eastern Africa
[2] Biology Department, Faculty of Arts and Science, University of Dicle, Diyarbakir 21280, Turkey

Freshwater fish take up salts from their ambient medium to compensate their loss through excretion. This obviously necessitates a high metabolic demand for the regulation between the energetic and osmoregulation in aquatic animals. Sodium, potassium and calcium are not only important for the maintenance of osmoregulation of body fluids but also for the transport of nutrients from the lumen of the digestive tract into intestinal cells and uptake of neurotransmitters in the brain. In the present study, the decrease in the levels of Na^+ - K^+, Ca^{2+} ions in the gill, muscle, liver and kidney exposed to lethal and sub lethal concentrations of profenofos indicates changes in the permeable properties of the cell membrane of these organs and of deranged Na^+ - K^+ and Ca^{2+} ionic pumps due to the probable consequences of tissue damage. Disturbances in ion regulation induced by toxicants are manifested by altered ion concentrations. A number of studies have revealed that the functional properties of macromolecules are altered under pesticide stress. To gain an insight into the ion fluxes, the ions of biological significance like Na^+- K^+ and Ca^{2+} were determined in important tissues of freshwater fish, *Labeo rohita*.

Keywords: Sodium, nutrients, neurotransmitters, kidney and membrane

Слатководните риби ги усвојуваат солите од средината за да ја компензираат загубата преку екскреција. Ова очигледно преставува високо метаболитичко побарување и е рамнотежа помеѓу потребите за енергија и осморегулацијата кај акватичните животни. Натриумот, калиумот и калциумот не се важни само за осморегулацијата на телесните течности туку тие се важни за транспорт на хранливите материи од луменот на дигестивниот тракт во интестиналните клетки, како и за усвојување на невротрансмитерите во мозокот. Во оваа студија, намалувањето на нивото на Na^+ - K^+ и Ca^{2+} јоните во жабрите, мускулите, црниот дроб и бубрезите експонирани на летални и сублетални концентрации на профенофос, индицира промени во пермеабилноста на клеточните мембрани на овие органи и нарушување на Na^+-K^+ и Ca^{2+} јонските пумпи како резултат на оштетување на ткивото. Нарушувањата во јонската регулација индуцирана од токсични материи се манифестира преку променети концентрации на јоните. Бројни студии покажаел дека функционалните својства на макромолекулите се менуваат во услови на стрес предизвикан од пестициди. За да се добие увид во јонските протоци, беа анализирани јоните со биолошко значење како Na^+-K^+и Ca^{2+} во значајните ткива на слатководната риба, *Labeo rohita*.

Клучни зборови: Натриум, хранливи материи, невротрансмитери, бубрези, плазмалема.

Introduction

Pesticides are regularly utilized in agriculture to destroy or repel pests. The presence of pesticides as well as the presence of some of their active substances in the environment is of great concern. Pesticides from agriculture runoff and other sources into aquatic systems have been increasing their contamination by these products in several areas around the world with potential adverse effects (Lavanya et al. 2011). Unfortunately, after use pesticides do not stay in their place of application but move to the other parts of the environment and ultimately to the aquatic environment through surface runoff. Since pesticides are poisons and are meant to kill, repel or destroy during their sojourn through the different compartments of the globe, they kill the host of other non-target organisms (Toni et al. 2011; Zubair 2012). The inorganic ions play an important role in osmotic phenomena and in the regulation of cellular metabolism, significant mechanisms of neurotoxicity of pesticides; interaction with Na^+ channels on nerve cell membranes; disruption of K^+ membrane permeability in nerve cells; inhibition of Na^+, K^+-ATPase, Mg^{2+}- ATPase, Ca^{2+}, Mg^+-ATPase, and/or Ca^{2+}-ATPase; and inhibition of ionic channels. These are necessary by all animals to provide appropriate medium for protoplasmic activity. The principle cation of extra cellular fluids of most animals is Sodium (Na+). It maintains internal sodium concentrations and electro-neutrality (Maetz & Romu 1964). It also

Submitted: 18.03.2016
Accepted: 10.06.2016

* Author for correspondence: nagaraju.bantu301@gmail.com

plays an important function in the osmotic regulation of body fluids and serves as a vital activating ion for specific enzyme system. The increased ion of sodium content may cause a shift in ionic symmetry with a consequent change in functional efficiency and membrane permeability of sodium- potassium (Na^+-K^+) pumps.

Potassium (K^+) ion is an important co-factor in the regulation of osmotic pressure and acid-base balance (Ezhilmathy et al. 2014; Saxena 1957). It plays a role in protein biosynthesis by ribosome and is critical for the maintenance of normal membrane excitability. Many enzymes require potassium for their maximum activity (Nelson et al. 2008). Potassium ions activate certain enzymes and are essential for the maintenance of normal membrane excitability. It plays an important role as an osmotic inorganic effecter in animals. Calcium (Ca^{2+}) ion is one more significant osmotic effectors and is involved in conferring stability to the cell membrane. It is also a co-factor for several proteases, oxidoreductases, and ATPases. Calcium couples the oxidation with contraction in muscle, for the maintenance of structural integrity of mitochondria and rate of enzyme catalysis. Calcium content of tissues is an important factor (Murray et al. 2012). High calcium level generally decreases permeability and low calcium increases it. Hence, calcium level can be taken as index of mitochondrial integrity and cellular metabolism. Any change in calcium level can alter the protein synthesis, mitochondrial function, and enzymatic reactions (Reddy et al. 1979). All these ions exist in bound as well as in free forms. Bound ionic forms involve in metabolic functions and free ions involve in osmoregularity in order contributing to homeostasis of the cell system. Any inequity in the levels of these ions in animals will lead to impairment in various physiological actions (Leone and Ochs, 1987; Baskin, et. al. 1981). The literature available put forth by many researchers gives an understanding on the effects of many pesticides on ionic composition, associated enzyme activities of freshwater fish. There is a necessity to understand and establish relationship between the concentration of profenofos and its responses on ions. In view of this, an attempt has been made to study levels of sodium, potassium and calcium ions in different tissues such as gill, muscle, liver and kidney of the freshwater fish, *Labeo rohita* at lethal and sublethal exposure of profenofos.

Material and Methods

The freshwater fish *Labeo rohita* (size 6-7 cm and 6.5 -7.5 g weight) were acclimatized to laboratory conditions for 15 days. During the acclimation period fish were fed twice a day with commercial fish pellets *ad libitum* and rice bran. At the same time water was renewed with freshwater every two days. The water used in the experiments and water had following physic-chemical characteristics (Tab. 1, APHA 2005). The same water supply was used during acclimation period and subsequent lethal and sublethal toxicity tests.

Commercial grade formulation of organophosphorus pesticide profenofos, available in 50% EC (emulsifiable concentrate active ingredient), were used as the test pesticide. Based on the preliminary lethal toxicity tests (96 h LC_{50} 100 μg L^{-1}; 1/10th 96 hr LC_{50} i.e.10 μg L^{-1}) were selected as lethal and sublethal concentrations. After the acclimation, fish were exposed to sublethal concentrations in groups of 10 fish in 15 L of the test water in glass chambers for 1, 8 and 16 days. The experiments were conducted in triplicates for each concentration of pesticide. Control group were maintained, concentrations of the pesticide were re established to maintain the original levels while the test solution was renewed each day to improve the water quality (APHA 2005; OECD 1992) and remove the faecal matter. The levels of sodium, potassium and calcium ions were estimated in gill, liver, kidney and muscle of fish.

Table 1. Physico-chemical characteristics of water. Values in (mg L^{-1})

Variable	Results
Turbidity	8 silica units
Electrical conductivity at 28°C	816 Micro ohms/cm
pH at 28°C	8.1
i) Phenolpthalene	0
ii) Methyl orange as $CaCO_3$	472
Total Hardness	320
Calcium Hardness	80
Magnesium Hardness	40
Nitrite nitrogen (as N)	0
Sulphate (as SO_4)	Trace
Chloride (as Cl)	40
Fluoride (as F)	1.8
Iron as (Fe)	0
Dissolved oxygen	8-10 ppm
Temperature	28 ± 2°C

Estimation of sodium (Na^+), potassium (K^+) and calcium ions (Ca^{2+})

The weighed tissues were wet ashed in 50:50 (V/V) concentrated perchloric acid and nitric acid (Dall 1967). After keeping the wet ash solutions for half an hour, until the tissues were completely dissolved, they were evaporated at 100°C to 200°C temperature. The residues were dissolved in glass with distilled water and made up to 10 ml. It was filtered through Whatman No.1 filter paper. Further, appropriate dilutions were made prior to estimations and the sodium, potassium and calcium ions were estimated with the help of Spectrophotometer. Standard solutions of sodium, potassium and calcium were prepared by using AR (analytical grade) chemicals. The values are expressed as µg/g wet weight of the tissue.

Statistical analysis

The statistical analysis of data was done using analysis of variance (ANOVA). The significance of results was ascertained at $p < 0.05$. All the data are represented as means± standard deviation of the means.

Results

Changes in the levels of sodium, potassium and calcium ions after lethal and sublethal exposure regimes in gill, muscle and liver of fish, *Labeo rohita* were shown (Table 2-4).

Effect on Na^+ levels*:* Decreases in the levels were observed in lethal and sublethal concentrations in all the tissues (Table. 2). The maximum decrease was observed in kidney (19.54%) followed by muscle (14.23%), gill (9.37%) and liver (7.58%) at 24 h lethal. At 24 h sublethal, maximum decrease (27.65%) and minimum (21.48%) was noticed in kidney followed by liver (26.19%) and muscle (25.10%), whereas variations in decrease were observed at sublethal concentrations. The maximum of 47.82% decrease was recorded in kidney on 8 days sublethal exposure followed kidney (64.49 %) and gill (37.55%) on 16 days. Changes in the levels of sodium, potassium and calcium ions after lethal and sublethal exposure regimes in gill, muscle and liver of fish, *Labeo rohita* were shown (Table 2-4). Effect on Na^+ levels*:* Decreases in the levels were observed in lethal and sublethal concentrations in all the tissues (Table. 2). The maximum of (47.82%) decrease was recorded in kidney on 8 days sublethal exposure followed kidney (64.49 %) and gill (37.55%) on 16 days. Effect on Ca^{2+} levels: Calcium ion levels also exhibited comparable propensity of gradual decrement at lethal level and sublethal level in gill, liver, kidney and muscle. The maximum decrease in kidney (27.58%), followed by liver (15.47%) at 24 h lethal concentration. At 24 h sublethal concentrations, maximum decrease in kidney (37.93%), minimum in liver (21.42%). Under sublethal 8 days exposure, maximum (43.67%) in the kidney tissue followed by gill (42.64%), (40.19%) liver and (39.48%) at 16 days sublethal exposure minimum (47.62%), maximum (57.89%) was recorded in muscle.

Discussion

Exposure of freshwater fish *Labeo rohita* to pesticide profenofos at lethal and sublethal concentrations, pesticides are increasingly becoming the predominant environmental contaminants due to their extensive use particularly in the developing countries. The pesticide profenofos have been selected for the experiments are being used indiscriminately. The potential of the pesticide in causing adverse effects both in the environment and its constituents is well documented. Sodium, potassium and calcium are not only important for the maintenance of osmoregulation of body fluids but also for the transport of nutrients from the lumen of the digestive tract into intestinal cells and uptake of neurotransmitters in the brain. In the present study, the decrease in the levels of Na^+ - K^+, Ca^{2+} ions in the gill, muscle, kidney and liver exposed to lethal and sub lethal concentrations of profenofos indicates changes in the permeable properties of the cell membrane of these organs and of deranged Na^+ - K^+ and Ca^{2+} ionic pumps due to the probable consequences of tissue damage.

The results in the present study suggest that the sodium content decreased as a function of time of exposure to profenofos. Sodium is the major component of the cations of the extracellular fluid. It is largely associated with chloride and bicarbonate in maintenance of acid base balance. It maintains the osmotic pressure of body fluid and thus protects the body against excessive fluid loss. It is known that sodium content in tissues mainly depends on the permeability functional efficiency of biomembrane and efficient functional role of Na+ pump, which regulates ionic content of tissues. The level of Na^+ signifies its importance in the mobilization of water transport, since sodium content in the membrane facilitates the water movement among the tissues (Wilbur 1972; Dietz 1979). From the result, it is evident that the Na^+ loss is higher in the case of gill indicating the derangement in Na^+ transport. Also, the decreased sodium content in the tissues of exposed fish indicates changes in

Table 2. Sodium ion levels (µM / g wet weight) in the organs of fish, *Labeo rohita* on exposure to the lethal and sublethal concentrations of profenofos.

Organs	Control	Lethal 1 day	Exposure periods (Sublethal days)		
			1	8	16
Gill	64.59±0.01	58.47±0.12[a]	46.73±0.22[d]	39.39±0.21[c]	62.88±0.30[b]
Muscle	49.71±0.35	42.76±0.28[c]	37.28±0.39[a]	33.51±0.48[a]	38.63±0.36[d]
Liver	53.26±0.30	49.99±0.33[d]	42.59±0.03[c]	29.43±0.04[b]	51.79±0.28[a]
Kidney	46.55±0.23	37.32±0.24[c]	36.54±0.27[b]	24.84±0.27[c]	43.44±0.31[b]

Data represents means ± SD of six individual values; different letters indicate significant differences between the values of control and pesticide profenofos exposed groups are based on 1, 8 and 16 days exposure. a) $p \leq 0.05$ denotes significant when compared with control values, b) $p \leq 0.02$ denotes significant when compared with control values, c) $p \leq 0.01$ denotes significant when compared with control values, d) $p \leq 0.005$ denotes significant when compared with control values.

Table 3. Potassium ion levels (µM / g wet weight) in the tissues of fish, *Labeo rohit* on exposure to the lethal and sublethal concentrations of profenofos.

Organs	Control	Lethal 1 day	Exposure periods (Sublethal days)		
			1	8	16
Gill	72.27 ±0.12	59.85±0.45[c]	49.96±0.22[a]	56.35±0.48[c]	59.34±0.30[a]
Muscle	65.84± 0.25	54. 17±0.22[a]	46.35±0.14[d]	51.19±0.49[a]	49.56±0.29[c]
Liver	69.50± 0.27	58.72±0.84[d]	53.45±0.05[a]	52.97±0.72[d]	51.29± 0.33[b]
Kidney	57.76±0.25	47.32±0.49[c]	36.52±0.29[c]	41.28±0.84[c]	44.97±0.45[d]

Data represents means ± SD of six individual values; different letters indicate significant differences between the values of control and pesticide profenofos exposed groups are based on 1, 8 and 16 days exposure. a) $p \leq 0.05$ denotes significant when compared with control values, b) $p \leq 0.02$ denotes significant when compared with control values, c) $p \leq 0.01$ denotes significant when compared with control values, d) $p \leq 0.005$ denotes significant when compared with control values.

Table 4. Calcium ion levels (µM / g wet weight) in the tissues of fish, *Labeo rohita* on exposure to the lethal and sublethal concentrations of profenofos

Organs	Control	Lethal 1 day	Exposure periods (Sublethal days)		
			1	8	16
Gill	68.88±0.25	61.13±0.36 [a]	54.99±0.29[c]	39.27±0.42[d]	32.27±0.30[d]
Muscle	76.32±0.32	63.38±0.32 [c]	49.43±0.22[a]	46.64±0.46[a]	32.86±0.29[b]
Liver	84.59±0.47	71.44±1.41 [d]	66.94±0.27[b]	51.58±0.29[c]	44.94±0.80[a]
Kidney	87.51±0.27	63.87±0.39 [c]	54.32±0.24[b]	49.03±0.89[b]	36.65±0.42[d]

Data represents means ± SD of six individual values; different letters indicate significant differences between the values of control and pesticide profenofos exposed groups are based on 1, 8 and 16 days exposure. a) $p \leq 0.05$ denotes significant when compared with control values, b) $p \leq 0.02$ denotes significant when compared with control values, c) $p \leq 0.01$ denotes significant when compared with control values, d) $p \leq 0.005$ denotes significant when compared with control values.

permeable properties of different bio-membrane systems to different extent by altering the Na^+ pump (Rafat 1986) and rupture in the respiratory epithelium of gill tissue (Anand Kumar 1994). The findings of present investigation are in strong agreement with the previous studies under pesticide stress in fishes (Walser 1960; Moorthy, et. al. 1984); Edwards 1973; Siddiqui et. al. 1993; David 1995; Narendra et. al. 1993; Dava Prakasa Raju 2000; Durairaj 2001).

Sodium, potassium and calcium are not only important for the maintenance of osmoregulation of body fluids Baskin et al, (1981) but also for the transport of nutrients from the lumen of the digestive tract into intestinal cells Crane (1977) and uptake of neurotransmitters in the brain Iverson & Kelly (1975). Freshwater fishes are hyper osmotic to their medium. They gain water osmotically and tend to loose solutes by diffusion. In the regulation of osmolarity of system sodium, potassium and calcium ions play main role to stay the hyperosmotic properties of the animals according to Narasimhan et al. (1983). The increased sodium ion content may cause a shift in ionic balance with a consequent change in membrane permeability and functional efficiency of Na+-K+ pumps. Potassium ion an important role in protein biosynthesis by ribosome and is critical for the maintenance of normal membrane excitability. A group of enzymes require potassium for their maximum activity by Nelson et al. (2008). Calcium content of tissues is a significant factor (Murray et al. 2012). Therefore calcium level can be in use as key mitochondrial integrity and cellular metabolism. Any change in calcium level can change the protein synthesis, mitochondrial function and steady state of enzymatic reactions reported by Reddy et al. (1979). Disturbances in ion regulation induced by toxicants are manifested by altered ion concentrations. A number of biochemical studies have revealed that the functional properties of macromolecules are altered under pesticide stress. The ions of biological importance like Na^+- K^+, Ca^{2+} and Mg^{2+} were determined in important tissues of fish.

A continuous decrease of K^+ content in the tissue was observed in the present study. Furthermore, the ions are actively taken up from water via the chloride cells in the gill epithelium. For the ionic movement, the membrane system in the chloride cells is important as this is the structure with which Na^+ and K^+ ATPase is associated (Epstein et al. 1980). It is known that any remarkable decrease in K^+ level might be accompanied by serious disturbances in muscular irritability, myocardial function and respiration (Coles, 1967). The decrease in K^+ content in the tissues of *Labeo rohita* exposed to profenofos might be attributed to the alterations observed in respiration at whole animal as observed in the present investigation.

The decline of Ca^{2+} ion levels in the tissues on exposure to profenofos indicating increased decalcification. It is known that Ca^{2+} plays an important role in the regulation of cellular metabolism. It is required for regulation of muscle contraction, transmission of impulses neuromuscular excitability and regulation of protein binding capacity (Walser 1960). Mitochondria and endoplasmic reticulum are the two important subcellular organelles involved in the maintenance of the calcium homeostasis. Potassium ions activate certain enzymes and are necessary for the maintenance of normal membrane excitability. The consistency of intracellular potassium, even with varying total osmotic concentration of habit, may represent a very old cellular chamber (Prosser 1973). It plays a significant role as an osmotic inorganic effecter in animals.

Moreover decreased calcium content during pesticide stress corresponds to structural changes in mitochondrial integrity (Miroslaw 1973). It appears that the decreased Ca^{2+} in the present study might attribute to the disturbances in mitochondrial integrity and subsequent respiratory distress. Hoar (1976) suggested that the levels of amino acids and metabolites, pyruvate and lactate will be increased under stress conditions to compensate the loss of inorganic ions. Amino acids and lactate were found increased in the tissue of *Labeo rohila* exposed to sub lethal concentration of cypermethrin (Malla Reddy et al. 1991).

The decrease in sodium, potassium and calcium ion levels in the organs of fish, exposed to profenofos could be attributed to the decreased activities of Na^+ - K^+ and Ca^{2+}.The decrease in these ions can be attributed to inhibition of their carriers like ATPases which are found to be inhibited as reported by different authors in fishes exposed to pesticides (Richards and Fromm 1970; Thebault and Decaris 1983; Dikshith et. al. 1978) suggesting that the pesticide affects the active transport processes in the membrane. The reduction in ATPase activities also suggests a drastic decrease in the prolactin release, which might be particularly responsible for the hypocalcemia as reported by Nelson (1975), David (1995), Dava Prakasa Raju (2000) and Durairaj (2001). It is evident that the fish, *Labeo rohita* under profenofos stress affects functional regulation of the ionic transport and water permeability. The imbalance in bio-chemically changed components like amino acids could be attributed to imbalance of ionic composition.

In the present investigation, we found that during sublethal exposure of profenofos to fish, the Na^+ - K^+ and Ca^{2+} levels significantly decreased ions in all the tissues. This disruption may be due to the effect of profenofos on passive movement of ions i.e., the permeability characteristics. The decrease in activities may also be due to interaction of pesticide with Mg^{2+} and Na^+ - K^+ ATPases by this means inducting inhibition. May be the inhibition of ATPase activity is dependent on the functional groups of the enzyme and the amount of profenofos available for the competitive replacement of the substrate. Further recruitment of chloride cells proposed as a fundamental and physiologically significant response of freshwater fish to increase the capability to take up Na^+- K^+ and Ca^{2+} from water.

Conclusion

It is concluded that measurement of the activities of Na^+ - K^+ and Ca^{2+} may provide useful indicator of exposure to stress inducing chemical contaminants in fish. The laboratory data as reported in this study warrants its application in field investigations and it is proposed that sodium, potassium and calcium measurement may be included in the battery of biomarkers that are employed in the biomonitoring programmes for exposure monitoring and risk assessment. The decrease in these activities indicates the destruction of cellular ionic regulations in the vital organs of the fish.

References

Anand Kumar, A. (1994). Endosulfan induced biochemical and pathophysiological changes in freshwater fish, *Clarias batrahus*. PhD thesis, *Osmania University*, *Hyderabad, India*.

APHA, AWWA, WPCF. (2005). Standard methods for the examination of water and wastewater, 21st Edn. Washington, DC: *American Publication of Health Association.*

Baskin, S.I., Kuhar, K.P., Uricchio, F.J and Harper, G.R. (1981). The effect of age on five ions of the kidney in the Fischer 344 rat. *Reprod. Nutr. Dev,* **21**: 689-694.

Toni, C., Loro, V.L., Santi, A., De Menezes, C.C., Cattaneo, R., Clasen, B.E., and Zanella, R. (2011). Exposure to tebuconazol in rice field and laboratory conditions induces oxidative stress in carp (*Cyprinus carpio*). *Comparative Biochemistry and physiology, Part C*, **153**:128-132.

Coles, E.H. (1967). Blood chemistry. In: *Veterinary Clinical Pathology*, W.B. Sounders Company, Philadelphia. London, 116-165.

Crane, R.E. (1987). The gradient hypothesis and other models of carrier-madiated active transport*. Rev. Physiol. Biochem. Parmacol,* **78**: 99-159.

Dall, W. (1967). Hypo-osmoregulation in crustacea. *Comp. Biochem. Phyisiol,* **21**: 653-678.

David, M. (1995). Effect of fenvalerate on Behavioural, Physiological & Biochemical aspects of freshwater fish, *Labeo rohita* PhD thesis, S.K. University, Anantapur, Andhra Pradesh, India.

Desai, I., Dobronyl, I., and Verga, L. (1986). Immuno, neuro and general toxicological animal studies on a synthetic pyrethroid, cypermethrin. *Ecotoxicol. Environ. Safety,* **12**:220-232.

Dietz, T.H. (1979). Uptake of sodium chloride by freshwater mussels. *Can. J. Zool,* **57**: 156-160.

Dikshith, T.S.S., Tandon, S.K., Datta, S.K., Gupta, P.K. and Jay Raj, B. (1978). Comparative response of male rats to parathion and linden: Histopathological and biochemical studies. *Environ. Res,* **17**: 1-19.

Durairaj, D.S. (2001). Quinalphos, phenthoate and their combined effect on brain ATPases of fish, *Oreochromis mossambicus*. *Poll. Res,* **20(1)**: 139-142.

Edwards, C.A., (1973). In: Environmental pollution by pesticides (C.A. Edwards ed.) Plenum press, London; 409-439.

Ezhilmathy, R., Rajalakshmi, K., and Chezhian, A. (2014). Ameliorative effect of external calcium on the combined toxicity of pesticide and metal in relation to Ionoregulation of fish, *Lates calcarifer* (Bloch, 1790). *International Journal of Research in Biological Sciences,* **4(3)**: 82-88.

Epstein, F.H., Silva, P. and Kormanik, G. (1980). Role of Na+-K+ ATPase in chloride cell function. *Am. J. Physiol.* 238: 246-250.

Hoar, W.S. (1976). General and Comparative Physiology. 2nd edition, Prentice-Hall of India Pvt., New Delhi.

Iverson, L.L., and Kelly, J.S. (1975). Uptake and metabolism of L-aminobutyric acid by neurons and neuroglical cells. *Biochem. Pharmacol,* **24**: 933-938.

Lavanya, S., Ramesh, M., Kavitha, C., and Malarvizhi, A. (2011). Hematological, biochemical and ionoregulatory responces of Indian major carp *Catla catla* during chronic sublethal exposure to inorganic arsenic. *Chemosphere* **82**:977-985.

Leone J. and Ochs S. (1987). Anoxic block ad recovery of axoplasmic transport and electrical excitability of nerve. *J. Neurobiol*. **9 (3)**: 229-245.

Maetz, J. and Gracea Romu, F. (1964). The mechanisms of sodium and chloride uptake by the gills of freshwater fish, *Carassius auratus*, II Evidence for NH4/ Na+ and HCO3-/Cl- exchanges. *J. Gen. Physiol,* **47**: 1209-1227.

Malla Reddy, P. (1987). Effects of fenvalerate and cyermethrine on the oxygen consumption of a fish, *Cyprinus carpio; Mendel,* **4:**209-211.

Malla Reddy, P. and Harold Philip, G. (1991). Changes in the levels of respiration and ions in the tissues of freshwater fish, *Labeo rohita* under cypermethrin toxicity. *Chemo,* **25(6)**: 843-852.

Miroslaw, S.R. (1973). Ion association: The effect of multivalent ions on the protein bound and the complexed calcium in serum*. J. Cell. Comp. Physiol.* **55**: 245-250.

Moorthy, K.S., Reddy, B.K., Swami, K.S. and Chetty, C.S. (1984). Changes in respiration and ionic content in tissues of freshwater mussel exposed to methyl parathion toxicity. *Toxicol. Lett,* **21**: 287-291.

Murray, K., Rodwell, V., Bender, D., Botham, K.M., Weil, P.A., and Kennelly, P.J. (2009). Harper's Illustrated Biochemistry. 29th Edition, McGraw-Hill Medical Publishing.

Nagender Reddy, A. (1991). Endosulfan induced metabolic alterations in the fresh water filed crab *Berytelphusa quarini*. PhD thesis. Osmania University, Hydrabad, India.

Reddy, M.N., Indira, K., and Swami, K.S. (1979). Changes in electromigratory pattern of Ca^{2+} atrophy of amphibian *Gastrocnemus* muscle. *Indian J. Exp. Ecol*, **17**: 1392-1398.

Narasimhan P.V.S.L., Swami K.S. and Chetty C.S. (1983). Polarisation of selected oxdoreductase in the axoplasm of sheep medulla oblongata during electro induced intracellular transport. *Indian J. Comp. Anim. Physiol*, **1(2)**: 14-17.

Nelson, D.L., Lehninger, A.L., and Cox, M.M. (2008). Lehninger principles of biochemistry, 5th edition, W. H. Freeman and Company, New York.

OECD (Organisation for Economic Co-operation and Development) (1992). Guideline for testing of chemicals, Fish, *Acute Toxicity Test*, 9 pp.

Potts, W.T.W and Parry, G. (1964). Osmotic and Ionic regulation in animals Oxford: Pergamon Press.

Prosser, C.L. (1973). Comparative animal physiology 3rd edition, W.B. *Saunder company*, Philadelphia, pp 165-167.

Rafat, Y. (1986). Physiological responses of freshwater fish*, Anabas scandens* to the toxicity of endosulfan. Ph.D. Thesis, Osmania University, Hyderabad, India.

Reddy, A.T.V and Yellamma, K. (1991). The possible metabolic diversions adopted by the cockroach, *Periplaneta americana* to counteract the toxicity of fenvalerate. *Biochem. International,* **23(2)**:359-365.

Richards, B.D. and Fromm, P.D. (1970). Sodium uptake by isolated perfused gills of rainbow trout, *Salmo gairdneri. Comp. Biochem. Physiol,* **33**: 303-310.

Saxena, B.B. (1957). Inorganic ions in the blood of *Pila globusa*. *Physiol. Zool*. **30**:161-164.

Schemidt Nielson, B. (1975). Osmoregulation: Effect of salinity and heavy metals. *Fed. Pro.* **33**: 2137-2146.

Siddiqui, A.A. (1984). Studies on the environmental toxicity of certain organochlorine, organophosphate and carbamate compounds with special reference to freshwater fish, *Channa punctatus*. PhD thesis. Meerut University, Meerut, India.

Toni, C., Loro, V.L., Santi, A., De Menezes, C.C., Cattaneo, R., Clasen, B. E., and Zanella, R. (2011) Exposure to tebuconazol in rice field and laboratory conditions induces oxidative stress in carp (*Cyprinus carpio*). *Comparative Biochemistry and physiology, Part C*, **153**:128 -132.

Thebault, M.J. and Decaris, F.X. (1983). Ca^{2+} ATPase inhibitions in trout fill microsomes following *in vitro* and *in vivo.*

Walser, M. (1960). Ion association: The effect of multivalent ions on the protein bound and the complexed calcium in serum. *J. Cell. Comp. Physiol,* **55**: 245-250.

Wilbur, K.M. (1972). Shell formation in mollusks. In: Chemical Zoology (Eds) Florkin and B.J. Scheer Vol. 3, Academic press, New York, 103-145.

Zubair A. (2012). Toxicity bioassay and effects of sublethal exposure of malathion on biochemical composition and haematological parameters of *Clarias gariepinus. African Journal of Biotechnology*, **11(34)**:8578-8585.