

Possible Toxicological Effect of Pesticides, Cypermethrin on Earthworm (*Eisania fetida*)

Purvi Patel^{1*}, Rakesh Prajapati²

¹Student, Department of Life Science, M. N. College, Visnagar, India

²Assistant Professor, Department of Biology, M. N. College, Visnagar, India

*Corresponding author: purvipatel291097@gmail.com

Abstract: Pollution is the preface of infect into an environment, may also defined as an unwanted modify in the physical, chemical and biological characteristics of air, water and soil which influence human life, lives of other useful alive plants and animals, industrial progress, alive conditions and florescent property Water is the most necessary & major necessities of life. One can rarely survive without water also for a few days. At that 2/3 of the world's float up region is enclosed by water but yet we are blank. An incubation study was conducted to know the toxicity level of Fipronil in soil fauna by using earthworm species *Eisania fetida* as a biomarker. The earthworm were incubated in the spiked soil, with concentration of 0.5 ppm, 0.6 ppm, 0.7 ppm, 0.8 ppm, 0.9 ppm for a period of 24 hours and 48 hours. From the study, 50% mortality was observed in 0.5 ppm. The LC50 concentration was calculated from that it was clear that Cypermethrin is toxic to earthworm.

Keywords: Bioassay test, Cypermethrin, *Eisania fetida*, Non-target animal.

1. Introduction

Soil is an environmental establishment consequential from the alteration of superficial rock by mixture of weather, plant and animal life with ageing. The configuration and belongings of the soil are observed related to ecosystem. The latter fixes the activator and progression of soil. Soil can also be defined as biological body, which is created at the borderline among lithosphere and atmosphere by, inter sequences of all elements complex in soil construction studying both active and inactive. So, soil covers not only components but living (human) and biological-element mixture. The pH of good soils should be about 7 but due to industrialized effluents the pH rises or losses because pollution in Soil.

Pollution is the preface of infect into an environment, may also defined as an unwanted modify in the physical, chemical and biological characteristics of air, water and soil which influence human life, lives of other useful alive plants and animals, industrial progress, alive conditions and florescent property. Water toxic waste become when unnecessary equipment goes in to water, modification the verity of water (Alrumman *et al.*, 2016) and damaging to atmosphere and human health (Briggs, 2003). Water is a significant natural source used for consumption and other developmental aim in our life (Bidi *et al.*, 2016). An earthworm is a tube-shaped,

segmented worm found in the phylum is Annelida. Earthworms are commonly found in living in soil, feeding on live and death organic matter. An earthworm's digestive system runs through the length of its body. It conducts respiration through its skin. I have a double transport system composed of coelomic fluid that moves within the fluid filled coelom and a simple, closed blood circulatory system. It has a central and a peripheral nervous system. They change soil structure, fertility, develop plant growth and are significant in sustaining productivity (Bucker field, 1998). The more Chemical cluster of germicides that are generally useful Organophosphate, Carbamate, Chlorinated Hydrocarbons, Pyrethroids and Nicotinoids. Dangerous spraying and shocking management of the chemical pesticides may due to very danger of the health hazard. Cypermethrin is a synthetic pyrethroid that is a widely used pesticide for insect control in livestock, household and animal husbandry mainly for cracking, crevice and spot care. (Valles & Koehler, 1997).

Pesticides are the physiological toxicants, which are necessary by the man to kill the insects, pests and also man's fight facing the circulation of illness. Now, pesticides utilization happens a necessary and integral type of world agriculture. Though, the compact use of these substance agriculture and masses performances has modification the environmental stability of several non-target animals like fishes (Todd *et al.*, 2000). There are 234 kinds of pesticides used in India in which 24 are used generally and other 28 of them have been bared in India and other region because of their compact toxicity to the non-target creatures. The environmental term like temperature, pH and dissolved oxygen play main character to improved pesticide toxicity in the relevant of leavings particles. The organic action of phenthoate, which straight inhibit enzyme, acetyl cholinesterase (Ache) activity (Rao, 1980) in fish, rise protein curve completed (Kabeer, 1979) and intensity the glycolytic activity (Rao, 1980). Fishes are specially very impressible to the water pollution. Additional completed the pesticides mostly effects on liver of fish (Gijare *et al.*, 2010) and even downfall glycogen substance in liver and the intestine of *Ophiocephalus punctatus* unguarded to sub lethal intensity of Cypermethrin, these costs of glycogen substantiate distraction in carbohydrate metabolism cause by toxic compression (Kumble & Muley, 2000). In usual, pesticides are

used much widely in agriculture, forestry, public health and veterinary experiments and are gaining immense importance due to their ability to control weeds, pests along insects, plant sicknesses, aquatic weeds and aquatic snails (Gagnaire *et al.*, 2004; Jain *et al.*, 2005; Mustapha, 2008; Naeem *et al.*, 2010; Abu-Darwish *et al.*, 2011).

A. Cypermethrin

Cypermethrin (chemical abstract (CA) name:(*RS*)- cyano- 3-phenoxybenzyl (1*RS*,3*RS*;1*RS*,3*SR*)-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropane carboxylate and International Union of Pure and Applied Chemistry (IUPAC) name (*RS*)-alpha-cyano-3-phenoxybenzyl (1*RS*) *cis-trans*-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropane carboxylate) is one of the highest widely used synthetic pesticides for agricultural and domestic purposes, globally (Crawford *et al.*, 1981).

Many epidemiological and practical studies have been did to decide the health dangers connected with Cypermethrin exposure and measured Cypermethrin level in the blood and urine of the insecticides sprayers and exposed personal (Keenan *et al.*, 2009; Eads forth & Baldwin, 1983; Chol *et al.*, 2006; Azmi *et al.*, 2009; Lin *et al.*, 2011; Khan *et al.*, 2010; Liao *et al.*, 2011). Cypermethrin has been recognized as one of the significant component pesticides associated with human health dangers (Keenan *et al.*, 2009).

In India, Cypermethrin has a broad application in agriculture as a very impressive general pesticide. Cypermethrin has commonly appeal in actually all sectors of insect control in agriculture, in the home, and in the garden as good extra of few organophosphate, carbamate, or organochlorine insecticides (Jee *et al.*, 2005). It is used against aggregation produces pests, and control of ecto-parasites which swarm cattle, sheep, poultry and some associate animals, and aquatic animals (Treasurer and Wadsworth, 2004). Cypermethrin, like all synthetic pyrethroids, kills insects by distracting normal implementation of the nervous system. Cypermethrin is widely used for the killing of insect pests in the home. Cypermethrin and certain products containing Cypermethrin are sensitizers to the skin. (US EPA, 1989).

Cypermethrin can affect populations of birds by killing insect larvae which are usually used for food. Cypermethrin, *Eisenia fetida* (Roberts & Dorouh, 1984), is "extremely poisonous" to the earthworm. Earthworms are not Cypermethrin responsive. Fish are especially susceptible to Cypermethrin (WHO, 1989). Cypermethrin has relatively low vapor pressure and is not necessarily toxic in the atmosphere. The study showed that routine ingestion of cypermethrin up to 50 ppm in the 28-day diet had no effect on dairy cows lactating. The experimental results suggested that cypermethrin does not penetrate the bark and yet, after 60 days from the application, it is still 100 percent effective in destroying bark beetles (Jin & Webster, 1998).

2. Proposed Study

Output and efficiency have improved according to the

statistic. The high chemical usage of pesticides to bring about these spectacular increases in food production is not without its problems. A visible parallel correlation between higher productivity, high chemical input use and environment degradation effects is evident in south Gujarat were commercial agriculture is widespread. Crop like rice, maize, jowar, cotton, mangoes, cheeku, bajra, wheat, tobacco, ground nuts, tur, gram all types of vegetables are grown in north Gujarat to get more production farmers use various types of pesticides. Cypermethrin is most commonly used pesticides which are used as insecticides. Therefore, present work is aimed to evaluate the toxicity effects of widely used pesticides, fipronil an economically important animal *Eisenia fetida*. An earthworm is a tube- shaped, segmented worm found in the phylum is Annelida. Earthworms are commonly found in living in soil, feeding on live and death organic matter. An earthworm's digestive system runs through the length of its body. It conducts respiration through its skin. I have a double transport system composed of coelomic fluid that moves within the fluid filled coelom and a simple, closed blood circulatory system. It has a central and a peripheral nervous system.

The earthworms are a group of invertebrates belonging to the phylum Annelida and Class Oligochaeta and represented by more than 1000 species. Earthworm is a face organism and it is present in moist and dark places in mud. Earthworms are of great economic value to mankind because they improve the soil quality by their action. Earthworms ingest organic material and facilitate the redistribution of crop residues and organic matter throughout the soil profile. In the Indian subcontinent earthworms are represented by 509 species in 67 genera under 10 families. They are segmented worms, bilaterally symmetrical, with an external gland (clitellum) for producing the egg case (cocoon), a sensory lobe in front of the mouth (prostomium), and an anus at the end of the animal body, with a small number of bristles (setae) on each segment.

Earthworms are a group of soil macro fauna well known for their remarkable contribution in organic matter recycling. The worms have long been related with creative soils. They change soil structure, fertility, develop plant growth and are significant in sustaining productivity (Bucker field, 1998). Earthworm is one which has always interested to mankind; it is nature's own farmer, aerator, crusher, composter, and humidity builder of the top soil and above all is soil's intimate friend and benefactor (Watanab, 1975 and Lal, 1988). Earthworms play a vital role in soil fertility through the influence on water regime, aggregation, litter involment, soil organic dynamics and microbial activity (Briones *et al.*, 1998). Assessment of fipronil toxicity was designed to carry out with TLm (Median Tolerance Limit) test for 96 hrs. The median tolerance limit (TLm) is the attention which is used to articulate the result of toxicity test (bioassay) to point to the stamina of 50% survival of the test animals throughout certain experimental epoch. At the same time, behavioral modify of the earthworm with the fipronil were undertaken.

3. Method and Material

A. Experimental Animal

Healthy and active Earthworm *Eisenia fetida* having size of 6.5 ± 1.5 cm were procured from the Ganpat University, Kherva village of Mahesana district, India. Before investigation Earthworm maintained at least for 10 days in laboratory floor containing dough of fertilizer. All dough of fertilizer were kept in the laboratory in cool place and covered with bag of jute and also use iron net to prevent the escape of animals and the foreign particles falling in. Dead specimens were removed immediately. Dough of fertilizer renewed every week. Chose different concentration like 17ml, 18ml, 19ml, and 20ml this concentration of solutions were prepared from the stock solution, and these are used for the bioassay test. The soil was mixed carefully with a glass rod.



Fig. 1. *Eisenia fetida*



Fig. 2. *Eisenia fetida* measuring

B. Pesticides

The technical formulation of Cypermethrin (EC 25%) a type of organophosphorus pesticide was selected for the study. The pesticide was purchased from the local pesticide supplier, Visnagar, Gujarat, India and used to check its impact on Earthworm.

C. Bioassay Test

The median tolerance limit (TLM-96 hrs) of Fipronil for the was *Eisenia fetida* determined in the laboratory through static renewal bioassay test using standard method as described in APHA, (1995). For the purpose of bioassay test, earthworms

from the vermicompost (100gm) were used. For determining the LC_{50} selected 20 worms having similar size were inserted into each earthen pot containing pesticide in different concentrations (ppm). First, the animals were exposed to different concentrations for range finding test where the lowest concentration at which 0% and the highest concentration for 50% mortality occurred.

No feed was given before 24 hrs and during the bioassay test. Test medium was renewed for every 24 hrs with their respective test concentration of the toxicant without aeration. Dead specimens were removed immediately as and when found mortality rate was recorded at the end of 24, 48, 72 and 96hrs. The median tolerance limit (TLM 96 hrs) was obtained by plotting graph with concentration series against the mortality rate for 96hrs. All the exposures were repeated at least three times to maintain the consistency in the results.

4. Result and Discussion

A. Bio-assay Test

Depicts the mortality and survival of *Eisenia fetida* exposed to different concentration of Cypermethrin. Concentration at which minimum mortality (50%) *Eisenia fetida* was recorded at 0.5 ppm and maximum concentration where 100% mortality was noted at 0.9 ppm. The exposure of *Eisenia fetida* Cypermethrin treatment was most critical at 24 hrs.

B. Behavioral Responses

After application of pesticides in soil, instantly animals were trying to jump out side of the culture tray. When the earthworm was exposed to various concentration of Cypermethrin, they move immediately to the surface of the culture tray. The movement of earthworm was observed to be disrupted from 24 hrs of exposure. Irregular movements followed by imbalanced slide activity were observed. The sliding behaviour was sudden, rapid non-directed spurt of surface movement. The earthworm progressively showed signs of higher concentration (0.5, 0.6, 0.7, 0.8, 0.9 ppm) showed that animal lost their equilibrium and response to external stimuli such as touch by drowning to the soil trap and correspondingly died. *Eisenia fetida* body colour changes dark red to light red.



Fig. 3. Changing of colour



Fig. 4. Death part of *E. fetida*

Table 1
Mortality of *E. Fetida* exposed to Cypermethrin

S. No.	Concentration of Cypermethrin in (ppm)	No. of Earthworm Exposed	Mortality in Numbers		Mortality in %
			24 hrs	48 hrs	
1.	0.5	20	10	00	50
2.	0.6	20	13	00	65
3.	0.7	20	16	00	80
4.	0.8	20	18	00	90
5.	0.9	20	19	01	100

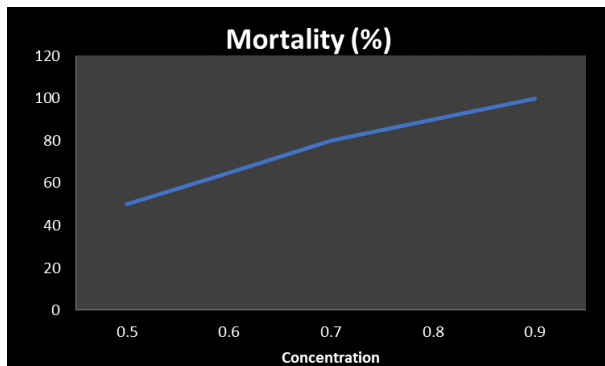


Fig. 5. Sigmoid curve between percentage mortality of *Eisenia Fetida* and concentration of Cypermethrin

Assessed as the category of urea toxicity, it was graded as "very toxic" to *E. Fetida: Fetida*. An average estimate showed various toxic effects in filter paper substratum 28 µg/cm² with clear area of contact exposure of urea to the earthworm in different concentrations. The comparable medium on the plate bed of Petri is thus. A series of test solution geometric concentrations (5, 10, 20, 40, and 80 mg/5 ml) were prepared and tested in which earthworm mortality was observed at 20, 40, and 80 mg concentrations after 18, 12, and 6 h respectively. Even the concentration of 5 mg/5 ml was for 48 h not harmful to earthworm. The concentration of 48 h lethal to *E. Fetida* had a concentration of 10 mg/5 ml. The earthworm was not able to move around after 40 hr, neural retention and defoliation starts, and the body parts separate leading to the death. The lethal effect of the regularly used chemical fertilizer urea is killing the friend of farmer in a drastic way. In this preliminary screening

experiment, the chemicals are shocked or absorbed into the earthworm body mainly through the outer dermal layer of skin when it is moving around the filter paper (Smetak *et al.*, 2007, Estevez *et al.*, 1996, Potter *et al.*, 1985). The study found the organophosphorus pesticide chlorpyrifos is toxic to be earthworm species *Eudrilus eugeniae* at a concentration of 100 ppm, which showed 100 per cent mortality within 24 hours. Earthworm is one of the macro fauna indicator species. From the prohibit analysis high level of LC value can be seen from prohibit analysis so the study gives the information that the chlorpyrifos is toxic to earthworms at high concentrations levels from 100 ppm. *L. Rohita* was silvery white in body in the control group throughout the experiment. The body colour changed from original silvery white to dark colour in pesticide treated fish. The fish maintained in freshwater behaved normal as body colour changed from original silvery white usual. But when the fish was exposed to pesticides monocrotophos and lambda cyhalothrin, erratic swimming, abnormal posture, disbalance, sluggishness, imbalance in posture, increase in surface activity, opercula movement, and gradual loss of equilibrium and spreading of excess of mucus all over the surface of the body were observed. A survey of LC50 values of different pesticides to the fish for different periods of exposure reveals the occurrence of a wide differences between duration of exposure and types of fishes (Macek and McAllister, 1970; Holden, 1972; Carter and Graves, 1973; Bakthavathasalam, 1980; Koundinya and Ramamurthi, 1980; Padmini, 1980; Rani *et al.*, 1990; Dhanalakshmi, 1991; Sadhu, 1993; Pickering and Henderson, 1966; Santhakumar and Balaji, 2000; Mathivanan, 2004 and Ramasamy *et al.*, 2007). Changes in body colour have been reported in *Anabas testudineus* after exposure to monocrotophos (Santhakumar and Balaji, 2000), *C. punctatusto* organophosphorus (Sandhu, 1993) and *Cyprinus carpio* ammonia stress (Israeli-weinstein and Kimmel, 1998). The lethal (LC₁₀₀), median (LC₅₀) and healthy sub-lethal (LC₀) toxicant values were then tested during the experimental exposure duration of 24-96 hours. Developmental use to pesticides during amphibian metamorphosis results in a decrease in growth and low reproductive activity. Youth are more susceptible than adults, and can die from chronic pesticide exposure. Skin is the main source of exposure to the pesticides. Exposure to pesticides is more likely in amphibians than in other vertebrate species. Various concentrations of Cypermethrin have significantly impacted amphibian hatching success (Greulich *et al.*, 2003). The relative insensitivity of turtle embryos hatching success to environmental pollutants has also been found in other species. Cypermethrin is a widely used pesticide for destroying pests in homes, farm crops and for a variety of other uses. Cypermethrin has caused many health hazards resulting in physiological effects, neurotoxicity, reproductive toxicity, molecular toxicity and so on due to the significant increase in the use of Cypermethrin in everyday life and its exposure to humans to such a degree. Cypermethrin's physiologic impact on male reproductive health is also seen.

The findings clearly show the awareness that the respondent farmers had about the effects of pesticide use. We realized that pesticides cause waste, can affect soil fertility and can have toxic effects on soil Razali, (1997).

5. Conclusion

After this experiment it was found that when pesticides mixing in soil, within 24 hours, the effects are most pronounced on the earthworm. After 72 hours, the earthworms become stable. The pesticides concentration in the soil decreases so it does not have an effect on the earthworm after 24 hours – 96 hours.

Finding of the carried out during the experiment *Eisenia fetida* different concentration of Cypermethrin which is used in agriculture to control the mite and indicated that the pesticide was hyper toxic. Due to high humidity and low temperature, the effect of pesticides on earthworms are less effect and low humidity and high temperature, the effect of pesticides on earthworms are more effect. From the study, 50% mortality was observed in 0.9 ppm. The LC50 concentration were calculated from that it was clear that Cypermethrin is high toxic to earthworm.

Acknowledgement

I am very thankful to my guide Dr. Rakesh Prajapati, Assistant Professor, Department of Biology, M. N. College, Visnagar for given me an excellent and perfect guidance at any movement during this work. I also indebted for his suggestion and constructive comments which enable me to complete this work in time. I own my success to him.

References

[1] Abu-Darwish, M.S., Al-Fraihat, A.H., Al-Dalain, S.Y.A., Afifi, F.U. and Al-Tabbal, J.A., Determination of essential oils and heavy metals accumulation in *Salvia officinalis* cultivated in three intra- raw spacing in ash-shoubak, Jordan. *Int. J. Agric. Biol.* 13, 2011.

[2] Alrumman, S. A., Elkott, A. F. and Kehsk, M. A., Water pollution: Source and treatment. *American journal of Environmental Engineering*, 6(3):88-98, 2016.

[3] Azmi, M.A., Naqvi, S.N., Akhtar, K., Moinuddin, Parveen, S., Parveen, R., and Aslam, M., Effect of pesticide residues on health and blood parameters of farm workers from rural Gadap, Karachi, Paki- stan. *J. Environ. Biol.*, 30, 747-756, 2009.

[4] Briggs, D., Environmental pollution and the global burden of disease. *British medical bulletin*.68:1-24, 2003.

[5] Briones, M.J.I., Bol, R. and Sleep, B. Isotopic ecology of earthworms under grassland and arable cropping system. 6th International Symp. Earthworm Ecol, Vigo, Spain, pp. 90. 1998.

[6] Buckerfield, J. C., Earthworms are indicators of sustainable production. 6th international symp. Earthworm Ecol. Vigo, Spain, 95, 1998.

[7] Carter, F. L. and Graves, J. B. Measuring effects of insecticides on aquatic animals. *Louisiana Artic*. 16-14, 1973.

[8] Crawford, M.J., Croucher, A. and Hutson, D.H., Metabolism of cis- and trans-cypermethrin in rats: Balance and tissue retention study. *J. Agric. Food Chem.*, 29, 130-135, 1981.

[9] Dhanalakshmi, S., Synergistic toxicity and effects of dimecroncuman on oxygen consumption and haematological parameters of freshwater teleost, *Sarotherodonmossambicus* (Peters). *M.Phil. Thesis*, Bharathiar University, Coimbatore, Tamil Nadu, India, 1991.

[10] Eadsforth, C.V. and Baldwin, M.K., Human dose-excretion studies with the pyrethroid insecticide, cypermethrin. *Xenobiotica*, 13, 67-72, 1983.

[11] Estevez B., NEstevez B. A., Coderre D., The effect on earthworm abundance and selected soil properties after 14 he es of solid cattle manure and NPK Mg fertilizer application. *Can J Soil Sci*; 76:351-5, 1996.

[12] Gagnaire, B., Thomas-Guyon, H. and Renault, T., *In vitro* effects of cadmium and chromium on pacific oyster, *Crassostreagigas*(Thunberg) haemocytes. *Fish Shellfish Immunol.* 10, 2004.

[13] Gijare, S., I. A. Raja, V. T. Tantarapale and K. M. Kulkarni. Influence of Cypermethrin on Liver of the Freshwater Fish *Ophiocephalus Punctatus*, *Poll. Res.*, 31(1), 37-40, 2010.

[14] Holden, A. V., The effects of pesticides on life in freshwater. *Proc. Royal Soc., Lond. B.*, 180: 383-394, 1972.

[15] Jain, P., Sharma, J. D., Sohu, D. and Sharma, P., Chemical analysis of drinking water of villages of Sanganer Tehsil, Jaipur district. *Int. J. Environ. Sci. Technol.* 2, 2005.

[16] Jee, J.H., Masroor, F. and Kang, J.C., Responses of cypermethrin induced stress in haematological parameters of Korean rockfish, *Sebastes schlegelii* (Hilgendorf). *Aqua Res.* 36(9): 898- 905, 2005.

[17] Jin, H. and Webster, G.R.B., Persistence, penetration, and surface availability of cypermethrin and its major degradation products in elm bark. *J. Agric. Food Chem.* 46:2851-2857, 1998.

[18] Kabeer, A.S.I., Studies on some Aspects of Protein Metabolism and Associated Enzyme System in the Freshwater Teleost, *Tilapia Mossambica* Subjected to Malathion Exposure, Ph.D. Thesis, S. V. University, Tirupati, India, 1979.

[19] Keenan, J.J., Vega, H. and Krieger, R.I., Potential exposure of children and adults to cypermethrin following use of indoor insecticide foggers. *J. Environ. Sci. Health B.*, 44, 538-545, 2009.

[20] Khan, M.A., and Ghouri, A.M., Environmental Pollution: Its effects on life and its remedies. *Journal of arts, science and commerce*.2 (2):276-85, 2011

[21] Koundinya, P. R. and Ramamurthi, R., Toxicity of sumithion and sevin to the freshwater fish, *Sarotherodonmossambicus* (Peters). *Curr. Sci.*, 49(22): 875-87, 1980.

[22] Kumble G.B. and D. V. Muley, Effect of Acute Exposure of Endosulfan and Chloropyrifos on the Biochemical Composition of Freshwater Fish *Sarotherodon Mossambicus*, *Indian J. Environ. Sci.*, 4, 97-102, 2000.

[23] Lal, R., Effect of macrofauna on soil properties in tropical ecosystems. *Agricul. Ecosys. Environ* 24:101-116, 1988.

[24] Liao, H.T., Hsieh, C.J., Chiang, S.Y., Lin, M.H., Chen, P.C. and Wu, K.Y., Simultaneous analysis of chlorpyrifos and cypermethrin in cord blood plasma by online solid-phase extraction coupled with liquid chromatography-heated electrospray ionization tandem mass spectrometry, 2011.

[25] Macek, K. J. and McAllister, W. A., Insecticide susceptibility of some common fish family representatives. *Trans. Amer. Fish. Soc.*, 99(1): 20-27, 1970.

[26] Mathivanan, R., Effects of sublethal concentration of quinophos on selected respiratory and biochemical parameters in the fresh water fish *Oreochromismossambicus*. *J. Ecotoxicol. Environ. Monit.*, 14 (1): 57-64, 2004.

[27] Mustapha, M.K., Assessment of the water quality of Oyun Reservoir, Offa, Nigeria, using selected physico-chemical parameters. *Turk. J. Fish. Aquat. Sci.* 8, 2008.

[28] Naeem, M., Salam, A., Tahir, S.S. and Rauf, N., Assessment of the essential element and toxic heavy metals in hatchery reared *Oncorhynchus mykiss*. *Int. J. Agric. Biol.* 12, 2010.

[29] Padmini, N., Toxicity and effects of pesticide sevin on blood free amino acids level of *Tilapia mossambica* (Peters). M.Sc. Dissertation, University of Madras, Tamil Nadu, India, 1980.

[30] Pickering, Q. H. and Henderson, C., The acute toxicity of some pesticides to fish. *Ohio. J. Sci.*, 66(5): 508 -513, 1966.

[31] Potter D. A., Bridges B. L., Gordon F. C., Effect of N fertilization on earthworm and micro arthropod populations in Kentucky bluegrass. *Turf Agron J*; 77:361-12, 1985.

[32] Ramasamy, P. K., Jeyaraj, R., Rajkumar David, A. J. and Ramaswamy, M., Toxicity of an organophosphorus pesticide, quinalphos to the catfish, *Mystus vittatus*. *J. Ecotoxicol. Environ. Monit.*, 17 (4): 391-396, 2007.

- [33] Rani, S., Shaik Dawood, A. and Ramasamy, M., Toxicity of a carbamate fungicide, cuman to an edible, freshwater fish, *Sarotherodonmossambicus* (Peters). *J. Aquor.*, 3 (1): 29- 36, 1990.
- [34] Rao, K.S.P., Studies on some Aspects of Metabolic Changes with Emphasis on Carbohydrate Utilization in Cell-free System of Tilapia Mossambica (Peters) Subjected to Methyl Parathion Exposure, Ph. D. Thesis, S. V. University, Tirupati, India, 1980.
- [35] Roberts, B.L. and Dorough, H.W., Relative toxicities of chemicals to the earthworm *Eisenia foetida*. *Environ. Toxicology. Chem.* 3:67-78, 1984.
- [36] Sadhu, D. N., Toxicity of an organophosphorous insecticide monocil to the air breathing fish, *Channapunctuatus*. *J. Ecotoxicol. Environ. Monit.*,3: 133-136, 1993.
- [37] Santhakumar, M. and Balaji, M., Acute toxicity of an organophosphorus insecticide monocrotophos and its effects on behaviour of an air-breathing fish, *Anabas testudineus*(Bloch). *J. Environ. Biol.*, 21(2): 121-123, 2000.
- [38] Smetak KM, Johnson-Maynard JL, Lloyd JE. Earthworm population density and diversity in different-aged urban systems. *Appl. Soil Ecol.*; 37:161-8, 2007.
- [39] Todd, N.E. and M. V. Leeuwen, Effects of Sevin (Carbaryl Insecticide) on Early Life Stage of Zebra Fish *Deirio Sesio*, *J. Ecotoxicology Environ. Safety*, 53, 267-272, 2000.
- [40] Treasurer, J.W. and Wardsworth, S.L., Interspecific comparison of experimental and natural routes of *Lepeotheriussalmonis* and *Caligus elongates* challenge and consequences for distribution of chalinus on salmonids and therapeutant screening. *Aqua. Res.* 35: 773-783, 2004.
- [41] U.S. EPA, Office of Pesticides and Toxic Sub- stances. Office of Pesticide Programs. Pesticide fact sheet. Cypermethrin. No. 199, 1989.
- [42] Valles, S.M. and Koehler, P.G., Insecticides used in the urban Enviroment: mode of action, 1997.
- [43] Watanab, M., On amounts of cast production by the megascolecial earthworm *Pheretimalupeinsis*. *Pedobiol* 15:20-28, 1975.