Summary and conclusions

Earthworm are economically important in fields, hence in the present study the effect of temperature, salinity and pesticides on the survival of earthworm *Eudrilus eugeniae* and on its biological aspects are tested. Earthworms are the Golden Bough of our agricultural history and are of enormous ecological importance to mankind, particularly in agricultural efforts, as witnessed by the effect of the common earthworm on soil fertility. They act as aerators, crushers, mixers, grinders, chemical degraders and biological stimulators. Climate change and in particular an increase in temperature have a direct impact on the moisture of the soil and indirectly on the earthworm. Temperature has a profound effect on the metabolism of organism and in the modern world, farmers use excessive heavy irrigation and chemical fertilizers. Plant protection products can affect soil organisms and thus might have negative impacts on soil functions. Due to heavy irrigation and use of chemicals the soil become saline and toxic which badly affect the earthworm activity. The work is presented through following 3 chapters.

**Chapter 1:** In this chapter various aspects of soil ecology including types of soils, importance of soils, soil impacts on earthworms, bionomics of earthworms and survey of earthworms are included. 8 species of earthworms are found in this region. Which are given with their systematic position and characters. Earthworm species include *Perionyx excavatus, Polypheretima elongata, Lampito mauritii, Perionyx sansibaricus, Pheretima elongata, Pheretima posthuma, Metaphire houlleti,* (all 7 belong to the family Megascolecidae) and *Eudrilus eugeniae* (belongs to the family Eudrilidae). The *Eudrilus eugeniae* has been selected for research work.

**Chapter 2:** In this chapter analysis of physico-chemical characters like pH, salinity, organic matter, nitrogen, phosphorus, etc. of selected site has been done. Changes are tracked every month for the period of two years. The values of pH salinity, organic matter, nitrogen, phosphorus, during the period October 2010 to September 2012 were averaged. Variations in physico-chemical parameters were correlated with each other. Results of physico-chemical parameters are as follow.
pH

In the present study, average seasonal record of pH of soil showed, maximum (8.32) in summer season and minimum (7.56) in monsoon season. A statistical correlation of pH with other parameters showed that pH has significantly positive correlation (P<0.01) with salinity and organic matter and negatively correlated (P < 0.05) with nitrogen and phosphorus. The pH values of soil under study show the alkaline nature. Generally alkaline (7 to 9) soil condition may be due to accumulation of CaCO$_3$ and other salts in the predominately evaporating moisture regimes.

Salinity

Average seasonal record of salinity showed maximum (495ppm) in summer and minimum (156.75ppm) in monsoon season. The statistical correlation of salinity with other parameters showed that it has significantly positive correlation (P< 0.01) with pH and organic matter and negatively correlated (P ˃ 0.05) with nitrogen and phosphorus. Salinity in the top layers of soil tended to increase due to high rate of evaporation during summer month, whereas the leaching taken place during monsoon season.

Organic matter

Average seasonal record of organic matter showed maximum in summer (2.69%) and minimum in monsoon (1.62%) season. A statistical correlation with other parameters showed that organic matter has significantly positive correlation with (P< 0.01) pH and salinity, and negatively correlated (P>0.05) with phosphorus and nitrogen. Decreased values in monsoon season may be due to uptake of both the ions by soil organisms and plant.

Nitrogen

Average seasonal record of Nitrogen showed maximum (268.7kg/h) in winter and minimum in summer (172.6kg/h) season. Nitrogen has significantly positive correlation (P< 0.01) with phosphorus and negative correlation (P > 0.05) with pH, salinity and organic matter. Maximum nitrogen content in winter may be due to increase in soil water content due to rain fall and also the favorable temperature and humidity together which
favors the luxuriant growth of nitrogen fixing bacteria, blue green algae which are ultimately responsible for nitrogen content in the soil.

**Phosphorous**

Average seasonal record of phosphorus showed high values during winter (67.15 kg/h) and low values during summer (39.51 kg/h) season. Phosphorous has shown significantly positive correlation with nitrogen and significantly negative correlation (P < 0.05) with organic matter, pH and salinity. The high value may be due to dead organic matter from the top layer and low values may be related to removal of top layer of soil.

**Chapter 3:** This chapter has been presented in two parts. Part one deals with tolerance of *Eudrilus eugeniae* to temperature (Upper and lower limits), salinity and toxicity (LD$_{50}$) of pesticides like endosulfan and monocrotophos. Upper tolerance limit of temperature was found to be 33$^\circ$C and lower to be 11$^\circ$C. Tolerance limit of salinity was found to be 4.23 LC$_{50}$ values of endosulfan was found to be 0.047 ppm and monocrotophos 0.1 ppm after 24 hr of exposure.

Impact assessment of temperature, salinity and pesticides on nervous system, digestive enzymes, excretory products, reproductive organs and whole body biochemical contents has been done. It was observed that exposure of these worms to various experimental regimes of tolerance limit showed drastic changes in histological structures of vital organs.

**Neurosecretory changes in *Eudrilus eugeniae* in response to temperatures.**

The T. S. of brain of worms maintained in the laboratory temperature are considered as control (26$^\circ$C) with Mallory’s Triple stain showed two distinct lobes with healthy appearing neurosecretory cells A and B. *Eudrilus eugeniae* when exposure to various temperature for 24 hr produce drastic changes in the brain.

**At lower temperature (11$^\circ$C)**

When worms were exposed to lower temperature (11$^\circ$C). A cell neurosecretory material (NSM) intensity of brain was increased by 50 %, whereas cell area and nuclear diameter increased by 21.6 % and 12.7 % respectively as compared to control worms.
cell neurosecretory material (NSM) intensity was increased by 50 %, whereas cell area and nuclear diameter increased by 16.8 % and 21.8 % respectively as compared to control.

At higher temperature (330°C)

When worms were exposed to higher temperature (330°C) A cell neurosecretory material (NSM) intensity of brain was decreased by 50 %, whereas cell area decreased by 10.28 % and nuclear diameter increased 55.55 % as compared to control. B cell neurosecretory material (NSM) intensity of brain was also decreased by 50 %, whereas cell area decreased by 11.45 % and nuclear diameter increased by 34.88 % as compared to control.

Neurosecretory changes in *Eudrilus eugeniae* after treatments with lethal dose of sodium chloride

The results indicate that exposure of *Eudrilus eugeniae* to lethal concentration of sodium chloride (4.23%) for 12 hr produced remarkable changes in the neurosecretory activity of brain.

In the treated worms A cell neurosecretory material (NSM) intensity of brain was decreased by 66.66 %, whereas cell area and nuclear diameter increased by 8.6 % and 29.2 % respectively as compared to control worms B cell neurosecretory material (NSM) intensity of brain was also decreased by 66.66 %, whereas cell area and nuclear diameter increased by 20 % and 36.8 % respectively as compared to control.

Neurosecretory changes in *Eudrilus eugeniae* after treatments with lethal dose of endosulfan (0.047ppm) and monocrotophos (0.1ppm)

**Endosulfan**

The results indicate that exposure of *Eudrilus eugeniae* to lethal concentration (24h LC50) of endosulfan and monocrotophos for 12 hr produced drastic changes in the neurosecretory activity of brain.
Summary and Conclusions

In worms exposed to 0.047 ppm of endosulfan. A cell neurosecretory material (NSM) intensity of brain was decreased by 66.66 %, whereas cell area and nuclear diameter increased by 8.2 % and 84 % respectively as compared to control. B cell neurosecretory material (NSM) intensity of brain was also decreased by 66.66 %, whereas cell area decreased by 3.43 % and nuclear diameter increased by 80.8 % as compared to control.

**Monocrotophos**

In worms treated with 0.1 ppm of monocrotophos A cell neurosecretory material (NSM) intensity of brain was decreased by 66.66%, whereas cell area and nuclear diameter increased by 5.5 % and 62% respectively as compared to control. B cell neurosecretory material (NSM) intensity of brain was also decreased by 66.66 % whereas cell area increased by 4.5% and nuclear diameter increased by 59.6% as compared to control.

The influence of salinity stress on the neurosecretory cells of brain. The earthworm *Eudrilus eugeniae* after exposure to sodium chloride produced a considerable reduction in the quantity of NSM. Depletion of NSM suggests that probably NSM plays a significant role in maintaining the hydrostatic and salt balance in this earthworm. It may be possible that rate of transport and release of neurosecretory material in ‘A’ and ‘B’ cells was increased cell area and nuclear diameter. Under the stress of pesticides there was an increase in the rate of synthesis of neurosecretory material as indicated by increased nuclear diameters. The rate of transports or release of neurosecretory material seem to be faster than the synthesis indicated by the severe decrease in neurosecretory material.

In the present study activity of protease, amylase and lipase have been recorded in the gut of *Eudrilus eugeniae*. Maximum increase in enzyme activity was recorded in earthworm *Eudrilus eugeniae* after exposures to temperature, salinity, pesticides. Exposure of worm with higher temperature (33° C), salinity (4.23%), and endosulfan (0.047 ppm) and monocrotophos (0.1 ppm) after 12h produced a significant increase in
Summary and Conclusions

enzyme activity. The increase in the enzyme activity indicates that to maintain their vital activities more energy is required.

In the control worm the level of urea and uric acid was very low. In the present study no significantly notable changes were observed in urea and uric acid contents in worms exposed to lower temperature (11°C) as compared to control. Treatment of worm with higher temperature (33°C), salinity and pesticides produced a significant increase in the urea and uric acid levels in whole body and in nephridia. It is observed that concentration of urea and uric acid is more in nephridia as compare to whole body. Increase in urea and uric acid contents of earthworm *Eudrilus eugeniae* might be due to stress.

Whole body tissue and nephridia when exposed to higher temperature, salinity and pesticides there is significant decrease in protein, lipid and glycogen level. Due to stress they required more energy which is fulfilled by degradation of protein, lipid and glycogen.

The histomorphological picture of intestine of base control shows that the longitudinal and circular muscle layers are healthy with internally folded villi, microvilli and intestinal lumen But high temperature and salinity damaged folds and villi, distorted lumen observed, in monocrotophos and endosulfan treatment severely damaged folds and villi, intrusted spaces and distorted lumen.

The histomorphological picture of ovary of base control showed healthy ovarian lobes with oocytes in different stages of development, while damaged ovarian tissue after exposing to the lethal doses of temperature. The developmental and maturational stages of ovary of *Eudrilus eugeniae* decreased when exposed to lethal doses of salinity, damage in ovarian tissue observed when exposed to pesticides as compare to control. The exposure caused ooplasmic vacuolization and atresia of mature oocytes and showed severe nacrotic changes in *Eudrilus eugeniae*. The histomorphological picture of testies of control *Eudrilus eugeniae* showed healthy testicular lobes with developing spermatogonia, appearance of follicle, spermatogonia, spermatophores and stomal tissue. Testis of *Eudrilus eugeniae* exposed to stress of salinity and 33°C, distorted spermatogonia and spermatophores, loosing of stomal tissue, appearance of open spaces
in stoma noted, while in pesticides treated worm damaged follicle, distorted spermatogonia, decrease in spermatogonia, damaged spermatophore mass and pathetic appearance of stomal tissue observed. These results clearly indicate that sodium chloride, endosulfan and monocrotophos are toxic to the intestine, ovary and testis of *Eudrilus eugeniae*. 
1. 16<sup>th</sup> ALL INDIA C CONGRESS OF ZOOLOGY AND NATIONAL SYMPOSIUM ON RECENT ADVANCES IN ANIMAL research held on 21<sup>st</sup> to 23<sup>rd</sup> Sept October 2005, organized by Department of Zoology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad-431004.

2. State level seminars on strategic Approaches to Environmental problems organized by Faculty of Science, Bhosala Military College, Nasik, 6<sup>th</sup>-7<sup>th</sup> Jan. 2006.

3. Workshop 27<sup>th</sup> and 28<sup>th</sup> April 2006 on “new syllabus in Biology “iorganised by Garware College.Pune

4. One day Personality Development Workshop for Girl held on 27<sup>th</sup> Dec. 2006 organized by Pad. Dr. D.Y. Patil of Arts, Commerce and Science College, Akurdi, Pune-44.

5. One day Seminar on “Techniques in Biology” organized by Department of Biotechnology, Dr. D. Y. Patil. A. C. S. College Akurdi, held on 10<sup>th</sup> Oct. 2007.

6. One day Seminar on Impact of Urbanization on Biodiversity held on 22<sup>nd</sup> Jan. 2008 organized by Pad. Dr. D.Y. Patil A. C. S. College, Pimpri, Pune.

7. National Seminar cum-Workshops on “ALTERNATIVES TO ANIMAL USE IN MEDICAL AND NON-MEDICAL STUDIES” held on 20<sup>th</sup> and 21<sup>st</sup> Sept. 2010, organized by Department of Zoology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad-431004.

8. 21<sup>st</sup> “ALL INDIA CONGRESS OF ZOOLOGY” National Seminar on “Biodiversity Conservation with special Reference to Fisheries and its Management for Food, Livelihoods and Environmental Security” organized by, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, on 21<sup>st</sup>-23<sup>rd</sup> Dec. 2010.

9. National Seminar on ‘Advances in Aquatic Biology, Bio-diversity Conservation and Livelihood’ held on 12<sup>th</sup>-13<sup>th</sup> March 2011 organised by Department of Zoology Bundelkhand University, Jhansi