

International Journal of Scientific Research and Reviews

Effect of Various Wastes on Biochemical Profile of Earthworm, *Eisenia Fetida*

Sharma Suman¹ and Kaur Harsimran^{1*}

Department of Zoology & Environmental Sciences Punjabi University, Patiala-147002, Punjab

ABSTRACT

The present study was conducted to explore the deleterious effects of fly ash, *Parthenium hysterophorus* and milk processing industry sludge on the health of earthworm, *Eisenia fetida*. Earthworms were allowed to grow in the mixture of cow dung: fly ash (60:40), cow dung: *Parthenium hysterophorus* (75:25) and cow dung: milk processing industry sludge (60:40) for 60 days. The biochemical parameters i.e glycogen, cholesterol and protein content were assessed after 15, 30, 45 and 60 day of exposure. The results revealed that growth and cocoon production was significantly retarded with the exposure of different waste. The significant reduction in glycogen, cholesterol and protein content of earthworm was observed in different treatments. Herein we report that the biochemical parameters act as good biomarker for the evaluation of ecotoxicological studies.

KEYWORDS: *Eisenia fetida*, fly ash, milk processing industry sludge, *Parthenium hysterophorus*, biochemical parameters.

***Corresponding Author:**

Harsimran Kaur

Department of Zoology and Environmental Sciences,
Punjabi University, Patiala-147002, Punjab.

Phone no- +91-9814745885

Email: simrankaur0905@gmail.com

INTRODUCTION

A large quantity of organic waste all over the world poses major environmental nuisance such as offensive odors, contamination of ground water and soil and disposal problems¹. Large scale urbanization, industrialization as well as population growth have affected the strong relationship between nature and man². Living organisms exposed to polluted environments can have significant deleterious consequences³.

Fly ash (FA) is a product of coal and lignite combustion⁴. During electricity generation that enters the flue gas stream⁵. Fly ash is a complex and anthropogenic material, to lead to serious environmental problems like air, soil, water pollution and cause disturbance in various ecological cycles⁶. As compare to other countries, India generates higher amount of fly ash but its lower percentage is utilized. Therefore, maximum portion of fly ash is disposed in ash ponds located near the power plants that occupies more than 65,000 acres of land^{7, 8}. Accumulation and leaching of inorganic and organic toxic compounds from fly ash is of vital environmental concern and known to have serious adverse impact such as bioaccumulation of heavy metals, oxidative stress, and DNA damage.^{7, 9-11}.

Parthenium hysterophorus is one of the top ten troublesome weeds of the world¹². *P. hysterophorus* is an invasive alien weed that has a global significance¹³. The trichomes of leaves and stems of parthenium contains sesquiterpene lactones namely parthenin and coronopilin that are responsible for causing serious allergies like hay fever, contact dermatitis, bronchitis and asthma in human beings^{13, 14}. *P. hysterophorus* is toxic to livestock when it is ingested or are continually in contact with the weed¹⁵.

In India, milk-based product processing industries are one of the major food processing industries. The liquid and solid wastes generated from these industries can cause various health issues and other pollution problems^{16, 17}. The solids and wastewater generated from milk processing industry pose serious problems like disposal of treated and/or untreated wastewater solids and safe management¹⁶. The disposal methods such as open dumping and land filling practices of various wastes generated from industries are very expensive and unsafe for environment also¹⁸. The open dumping of wastewater sludge pollutes the natural resources surrounding that area and also provides shelter to various disease-vectors and disease causing agents¹⁹.

Stress induced by toxic environment activates the production of reactive oxygen species (ROS) which are generated as side products of tissue respiration in organisms²⁰. Due to their unstable nature, these ROS interact rapidly and aggressively with polyunsaturated fatty acids, DNA and proteins and affect biomolecules^{21, 22}. Other reactive oxygen species and free radicals lead to

oxidative stress^{23, 24}. The organisms counteract to environmental stress by developing vital potential to prevent this stress. The biochemical alterations occurring in the body give initial indication of oxidative stress. During stress conditions, an organism requires adequate energy, which is supplied from reserve materials i.e. glycogen, proteins and lipids. In low stress conditions, stored glycogen is used as energy source, but during excessive stress conditions, the energy stored in lipid and protein may also be used²⁵.

Since quite less literature is available on effect of environmental pollutants on biochemical profile of earthworm. The present study has been undertaken with the aim to determine the effect of fly ash, *P.hysterophorus* and milk processing industry sludge on growth, fecundity and biochemical parameters like protein, glycogen and cholesterol content of the earthworm *Eisenia fetida*.

MATERIAL AND METHODS

Collection of substrate material:-

Cow dung was obtained from nearby village. Fly ash was collected from thermal power plant, Rajpura. *Parthenium hysterophorus* was collected from fallow lands of Patiala. Milk processing industry sludge was collected from milk Plant, Mohali.

Collection of Earthworms:-

The earthworms (*Eisenia fetida*) were obtained from Punjab State Council for Science and Technology, Chandigarh.

Experimental Setup:-

The experiments were conducted in plastic trays of 1kg capacity. The cow dung, *P.hysterophorus* and sludge were mixed in different ratios as bedding material: Cow dung: fly ash (60:40), Cow dung: *P.hysterophorus* (75:25), Cow dung: Sludge (60:40). 20 healthy earthworms were introduced in plastic trays. The plastic trays were kept under shade to avoid direct sunlight.

Growth and reproduction:-

The earthworms and cocoons were separated from the feed by hand sorting; they were counted and weighed after washing with water. Biomass gain and cocoon production by worms in each treatment were recorded on 0, 15, 30, 45 and 60 days.

Biochemical assays:-

Eight earthworms were removed from each group at the intervals of 15, 30, 45 and 60 days of exposure, rinsed with distilled water and kept for 48h on moist filter paper in petridishes to deplete their gut content. The earthworms were homogenized in potassium phosphate buffer (0.1M) and centrifuged at 10,000 rpm for 10 min at 4°C. The biochemical analysis was performed using dual beam UV-visible spectrophotometer from Labtronics (LT-2900).

Quantitative estimation of total protein content was determined by the method of Lowery et al., (1951)²⁶. 0.1ml of the supernatant was transferred into a test tube and 4 ml of alkaline copper sulphate reagent was added, followed by 0.4 ml of diluted commercial Folin's reagent. The optical density of the blue color developed was read at 520 nm after 30 minutes of addition of the Folin's reagent.

Total glycogen was determined by the method given by Montgomery (1957)²⁷. 1ml of phenol was added in a test tube to 1ml of supernatant followed by addition of 5 ml of conc. H₂SO₄. The optical density was read at 490 nm.

Total cholesterol was determined by the method of Zlatkis et al., (1953)²⁸. 0.1 ml of supernatant was added to the test tubes followed by the addition of 9.9 ml of Ferric chloride and acetic acid solution. The reagents were incubated at 60°C for 2 minutes. 3ml of H₂SO₄ was added in the test tube. The optical density was read at 560 nm.

Statistical analysis:-

The data analyzed by Student's t-test using graph pad followed by two-way Anova and considering p<0.001 as significant and p>0.05 as non significant. The data was expressed as the mean ± S.E.M.

RESULTS AND DISCUSSION:

➤ Growth and Reproduction:-

The biomass and reproduction rate of the earthworms were recorded on day 0 and at day 15, 30, 45 and 60 of vermicomposting in all the treatments i.e. fly ash, *P.hysterophorus* and milk processing industry sludge. During the study period, no mortality was observed. The rate of biomass gain was almost equal in all the treatments i.e. Fly ash, *P.hysterophorus* and milk processing industry sludge on day 15 of exposure thereafter; a significant difference was observed on 30th day of experiment, followed by a stabilization period during day 45 and 60 for biomass gain in *E. fetida*. The earthworms showed significant elevation in growth rate in fly ash and sludge treatment up to day 60.

In *P.hysterophorus* treatment the earthworms showed elevation up to day 30 followed by sudden decline in growth rate up to day 60 (Fig. 1).

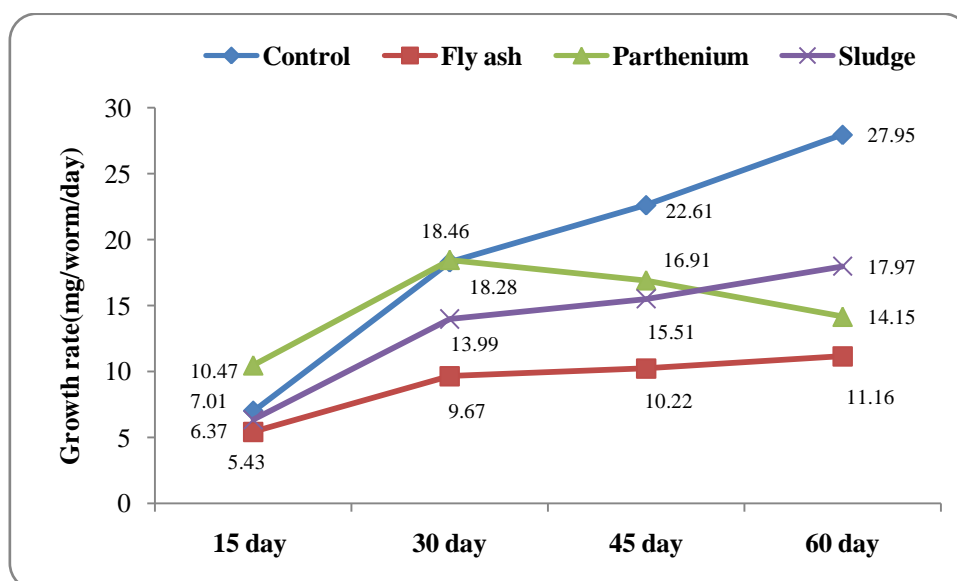


Fig: 1 Growth rate of earthworm (mg/worm/day) in fly ash, *Parthenium hysterophorus* and milk processing industry sludge treatment on 15, 30, 45 and 60 day.

It is well known that the growth and development of earthworm during vermiconversion depends upon the feed quality and growth supporting nutrients present in it²⁹. The variation in growth could be attributed to the degradability of feedstock. Suthar (2008a, b)^{30, 31} also recorded that the pattern of growth in earthworms depends on microbial populations and available nutrients in feeds mixture. Neuhauser et al., (1980)³² investigated that when earthworm received food below a maintenance level, the rate of weight loss, depends upon the nature and quantity of its ingestible food. The variation in weight gain of earthworm among different treatments may be due to quality of food material which directly affects the palatability of feed and rate of assimilation^{33, 34}. In the present study, the observed difference in growth rate among different treatments may be due to diverse food quality.

During the experimental period, all the earthworms reproduced successfully. The earthworms showed different pattern of cocoon production in all the treatments. The cocoon formation was observed on day 45 of the experiment in all the waste mixtures. Initially, rate of cocoon formation was less, but with the termination of experiment, it was enhanced. The number of cocoons produced in sludge treatment was higher than fly ash group. In *P.hysterophorus* treatment the cocoon production was lesser than both the treatments i.e. fly ash and milk processing industry sludge (Fig.2).

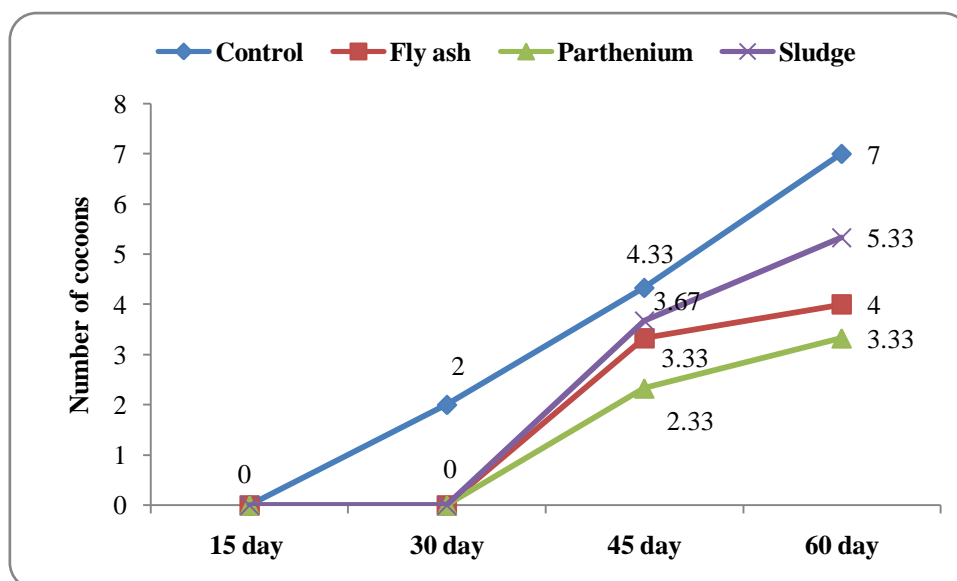


Fig: 2 Number of cocoons produced by earthworms in fly ash, *Parthenium hysterophorus* and milk processing industry sludge treatment on 15, 30, 45 and 60 day.

There was a pivotal relationship between food quality and rate of cocoon production³³. The cocoon production pattern was directly related to the feedstock quality used in bedding mixture. Earthworms showed better reproduction performances with suitable ratio of bedding materials. The difference between cocoon productions may be related to biochemical quality of the feed mixtures, which is one of the crucial factors in determining onset of cocoon production³⁵.

➤ **Biochemical parameters:-**

Glycogen is a reserve food material. It is a main energy source and plays an important role in cellular metabolism by acting as fuel and supplying energy to the body cells. The disturbance in the glycogen profiles is one of the major outstanding biochemical lesions due to the impact of various toxic chemicals³⁶. In the present study, glycogen content was significantly decreased in all the treatments during the experimental period (Fig 3).

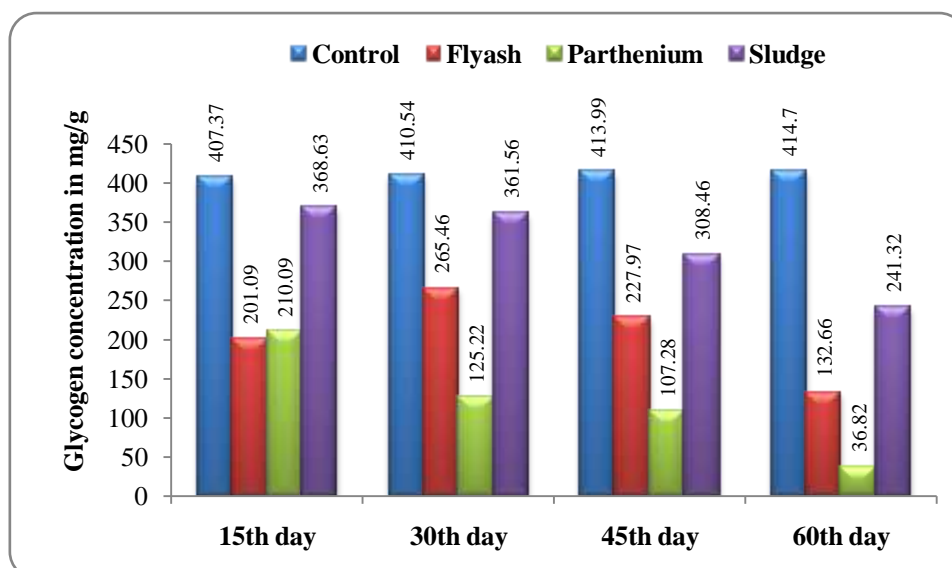


Fig: 3 Glycogen content in earthworm exposed to fly ash, *Parthenium hysterophorus* and milk processing Industry sludge on day 15, 30, 45 and 60 compared with control.

In *P.hysterophorus* treatment, the maximum reduction in glycogen content was observed. The depletion of glycogen level in earthworm *Eisenia fetida* is may be due to energy demand of organism under the stress condition. The other reason of reduction may also be due to activation of a glycogen phosphorylase enzyme which is an important contributing factor to glycogen utilization. These results are supported by Wojdani (1982); Quaglino et al., (1996); Mohrig et al., (1996); Suzuki et al., (1995) and Yahia et al., (2002)³⁷⁻⁴¹ who reported depletion in glycogen content in different earthworm species due to various toxic chemicals.

Cholesterol is the base material for all steroid hormones. During, synthesis of cortisol, a large amount of cholesterol is required⁴². The results of the present study showed that in all the treatments i.e fly ash, *P.hysterophorus* and milk processing industry sludge, the cholesterol content decreases on day 15, 30, 45 and 60 as compared to control (Fig 4).

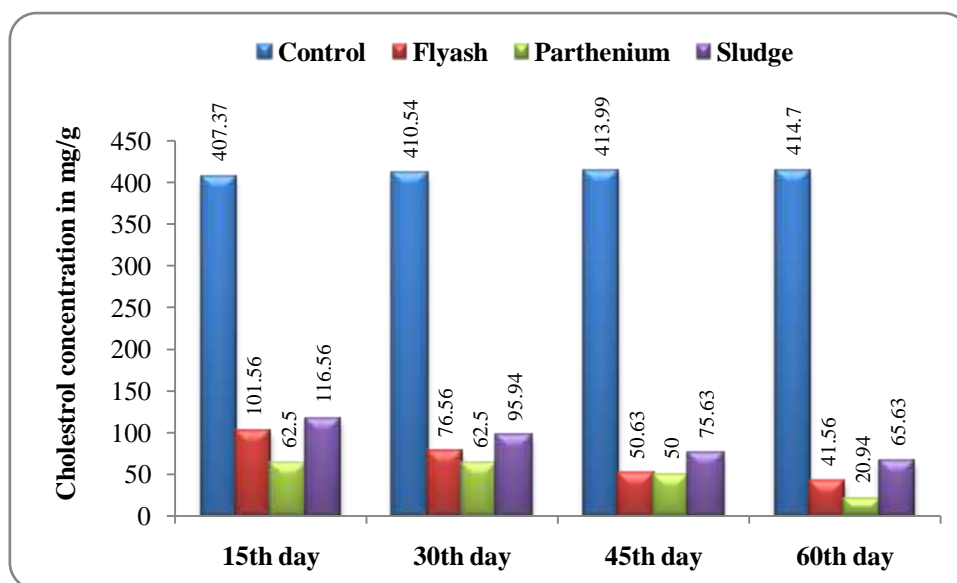


Fig: 4 Cholesterol content in earthworm exposed to fly ash, *Parthenium hysterophorus* and milk processing industry sludge on day 15, 30, 45 and 60 compared with control.

The maximum reduction was observed during *P.hysterophorus* treatment. Therefore, the reduction in the cholesterol content may be due to its utilization in the cortisol synthesis. The increase concentration of cortisol is considered to be a biomarker of oxidative stress. Thus various toxicants used in this study enhance oxidative stress in earthworm's body. These results are in confirmation with the findings of Sorrena et al., (2011)⁴³ who also reported reduction in cholesterol content in grass carp exposed to an herbicide.

Protein content is considered to be a major building material and one of the important groups of macromolecules, which occupy a vital place in dynamic and structural aspects of living matter⁴⁴. A significant depletion in protein content was observed in *E.fetida* in all the treatments i.e fly ash, *P. hysterophorus* and milk processing industry sludge on day 15, 30, 45 and 60 of the experiment as compared to control earthworm (Fig 5).

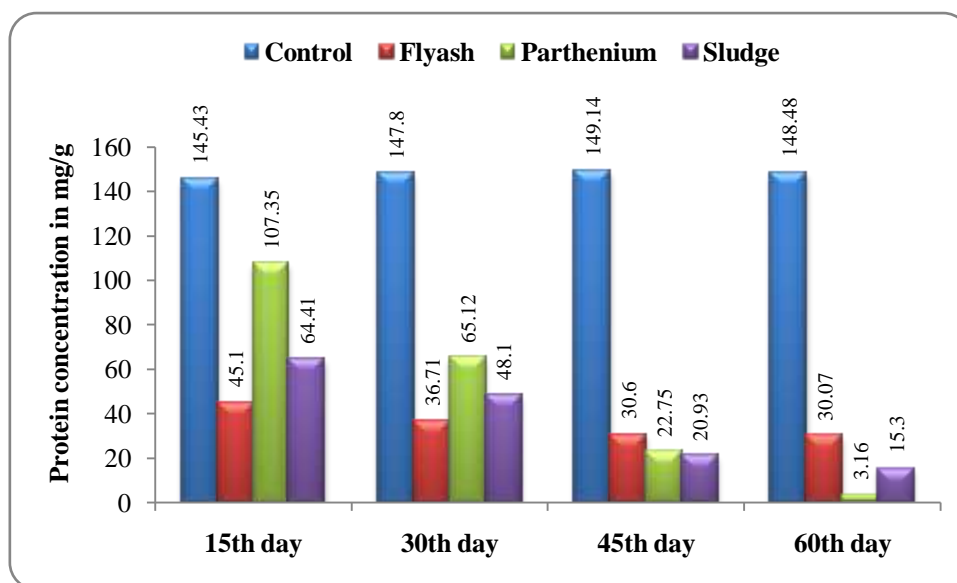


Fig: 5 Protein content in earthworm exposed to fly ash, *Parthenium hysterothorus* and milk processing industry sludge on day 15, 30, 45 and 60 compared with control.

The maximum depletion was found in *P.hysterothorus* treatment. The depletion in protein content may be due to the degradation of proteins into amino acids, which is to be utilized for gluconeogenesis⁴⁵ to combat the stress. Being a part of cell membrane, protein level might be reduced because of its metabolism to release energy during stress conditions⁴⁶. The results of the present study suggested that, in *Eisenia fetida* proteins have been utilized for the production of energy to minimize the stress caused by different wastes. These results are in agreement with findings of several investigators who reported decreased protein content in different organisms under the influence of various stressors⁴⁷⁻⁵².

CONCLUSION

The present study indicates that fly ash, *P.hysterothorus* and milk processing industry sludge cause alterations in the biochemical parameters of earthworm, *Eisenia fetida*. The results clearly indicate that fly ash, *P.hysterothorus* and milk processing industry sludge can detain the growth rate and cocoon production in earthworms. The significant reduction in level of glycogen, cholesterol and protein contents were observed. It might be attributed to the stress which is caused by the intoxication of various wastes in the intermediary metabolism of the earthworm, *Eisenia fetida*.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the facilities provided by Department of Zoology and Environmental Sciences, Punjabi University, Patiala to pursue the research work. Also, the financial

aid extended by UGC in the form of Rajiv Gandhi National Fellowship for minority students is greatly appreciated.

REFERENCES

1. Edwards CA, Bater JE. The use of earthworm in environmental management. *Soil Biol. Biochem.* 1992; 24: 1683–1689.
2. Elvira C, Dominguez J, Sampredo L et al. Vermicomposting for the paper-pulp industry. *Biocycle.* 1995; 36(6): 62–63.
3. Ajani GE. Oxidative stress in fish living in coastal water polluted with sawdust and wood waste along Lagos Lagoon, Nigeria. *Researcher.* 2014; 6(3): 1-5.
4. Kumar V, Singh G, Rai R. Fly ash Utilization Programme (FAUP) TIFAC, DST: New Delhi; 2005.
5. Chattopadhyay GN, Bhattacharya SS. Use of coal ash in agriculture. In: *Proceedings of coal ash utilization, 2010*; 36.
6. Singh RP, Gupta AP, Ibrahim MH et al. Coal fly ash utilization in agriculture: its potential benefits and risks. *Rev Environ Sci Biotechnol.* 2010; 9: 345–358.
7. Pandey VC, Singh N. Impact of fly ash in incorporation in soil systems. *Agr Ecosyst Environ.* 2010; 136: 16–27.
8. Pandey VC, Singh JS, Singh RP et al. Arsenic hazards in coal fly ash and its fate in Indian scenario. *Resour Conserv Recycl.* 2011; 55: 819–835.
9. Ali M, Parvez S, Pandey S et al. Fly ash leachate induces oxidative stress in freshwater fish *Channa punctata* (Bloch). *Environ Int.* 2004; 30: 993–998.
10. Chakraborty R, Mukherjee A. Mutagenicity and genotoxicity of coal fly ash water leachate. *Ecotoxicol Environ Saf.* 2009; 72: 838–842.
11. Grumiaux F, Demuyneck S, Schikorski D et al. Assessing the effects of FBC ash treatments of metal contaminated soils using life history traits and metal bioaccumulation analysis of earthworm *Eisenia andrei*. *Chemosphere.* 2010; 79: 156–161.
12. Callaway RM, Ridenour WM. Novel weapons: invasive success and the evolution of increased competitive ability. *Front Ecol Environ.* 2004; 2(8): 436-443.
13. Kapoor RT. Awareness related survey of an invasive alien weed, *Parthenium hysterophorus* L. in Gautam Budh Nagar district, Uttar Pradesh, India. *J Agri Technol.* 2012; 8(3): 1129-1140.

14. Wiesner M, Taye T, Hoffmann A et al. Impact of the pan-tropical weed *Parthenium hysterophorus* L. on human health in Ethiopia, Utilization of diversity in land use systems: Sustainable and organic approaches to meet human needs. Tropentag: Witzzenhausen; 2007.
15. Bezuneh TT. Phytochemistry and antimicrobial activity of *Parthenium hysterophorus* L.: a review. Sci J Anal Chem. 2015; 3(3): 30–38.
16. Suthar S. Vermistabilization of wastewater sludge from milk processing industry. Ecol. Eng. 2012; 47: 115-119.
17. Suthar S, Mutiyar PK, Singh S. Vermicomposting of milk processing industry sludge spiked with plant wastes. Bioresour Technol. 2012; 116: 214-219.
18. Slater RA, Frederickson J. Composting municipal waste in the UK: some lessons from Europe. Resour Conserv Recycling. 2001; 32: 359-374.
19. Gómez-Brandón M, Lazcano C, Lores M et al. Short-term stabilization of grape marc through earthworms. J Hazard Mater. 2011; 187: 291-295.
20. Valavanidis A, Vlachogianni T, Fiotakis K. 8-Hydroxy-2'-deoxyguanosine (8-OHdG): A critical biomarker of Oxidative stress and carcinogenesis. J Environ Sci and Health C Environ Carcinog Ecotoxicol. 2009; 27: 120-139.
21. Halliwell B, Gutteridge JM. Oxygen toxicity, oxygen radicals, transition metals and diseases. Biochem J. 1984; 219: 1–14.
22. Damien C, Chantal VH, Pirouz S et al. Cellular impact of metal trace elements in terricolous lichen *Diploschistes muscorum* (Scop.)R. Sant.–identification of oxidative stress biomarkers. Water Air Soil Pollut. 2004; 152:55–69.
23. Guyton KZ, Kensler TW. Oxidative mechanisms in cancerogenesis. Brit Med Bull. 1993; 49(3): 523–44.
24. Lawrence JM. Oxiradicals and DNA damage. Carcinogenesis. 2000; 21(3): 361–370.
25. Mark, E.M. Report of the secretary's commission on pesticides and their relationships to environmental health: U. S. Dept. of Health Education and Welfare Washington: 1961; 269.
26. Lowery OH, Roseburg NJ, Farr AL et al. Protein measurement with the folin-phenol reagent. J Biol Chem. 1951; 193: 265-275.
27. Montgomery R. Determination of glycogen. Arch Biochem Biophys. 1957; 67: 378-381.
28. Zlatkis A, Zak B, Boyle AJ. A new method for the direct determination of serum cholesterol. J Lab Clin Med. 1953; 41: 486-492.
29. Suthar, S., Pandey, B., Gusain, R et al. Nutrient changes and biodynamics of *Eisenia fetida* during vermicomposting of water lettuce (*Pistia* sp.). Environ Sci Poll Res. 2017; 24(1): 199-207.

30. Suthar S. Development of a novel epigeic- anecic based polyculture vermireactor for efficient treatment of municipal sewage water sludge. *Int J Environ Waste Manage.* 2008a; 2 (1–2): 84–101.
31. Suthar S. Metal remediation from partially composted distillery sludge using composting earthworm *Eisenia fetida*. *J Environ Monit.* 2008b; 10:1099–1106.
32. Neuhauser EF, Hartenstein R, Kaplan DL. Growth of the earthworm *Eisenia foetida* in relation to population density and food rationing. *OIKOS.*1980; 35: 93–98.
33. Suthar S. Nutrient changes and biodynamics of epigenic earthworm *Perionyx excavatus* (Perrier) during recycling of some agriculture wastes. *Bioresour Technol.* 2007; 98: 1608–1614.
34. Singh D, Suthar S. Vermicomposting of herbal pharmaceutical industry waste solids. *Ecol Eng.* 2012; 39:1–6.
35. Flack FM, Hartenstein R. Growth of the earthworm *Eisenia foetida* on microorganisms and cellulose. *Soil Biol Biochem.*1984; 16: 491–495.
36. De-Bruen A. Biochemical toxicology of environmental agent, Elsevier/north-Holland, Biochemical press: Amsterdam.1976.
37. Wojdani A, Stein EA, Lemmi CA et al. Agglutinins and proteins in the earthworm, *Lumbricus terrestris*, before and after injection of erythrocytes, carbohydrates and other materials. *Dev Comp Immunol.* 1982; 6: 613– 624.
38. Quaglino D, Cooper EL, Salvioloi S et al. Earthworm coelomocytes in vitro: cellular features and granuloma formation during cytotoxic activity against the mammalian tumor cell target K 562. *Eur J Cell Biol.*1996; 70:278– 288.
39. Mohrig W, Eue I, Kauschke E et al. Crossreactivity of hemolytic and hemagglutinating proteins in the coelomic fluid of lumbricidae (Annelida). *Comp Biochem Physiol.* 1996; 115:19– 30.
40. Suzuki MM, Cooper EL. Spontaneous cytotoxic earthworm leukocytes kill K562 tumor cells. *Zool Sci.* 1995; 12: 443– 451.
41. Yahia Y, Mosleh Saad MM, Mohamed T et al. Comparative toxicity and biochemical responses of certain pesticides to the mature earthworm *Aporrectodea caliginosa* under laboratory condition. *Env Toxicol.* 2002; 18: 338-346.
42. Kazemi R, Pourdehghani M, Yousefi Jourdehi A et al. Cardiovascular system physiology of aquatic animals and applied techniques of fish hematology. Shabak published book; 2010: 194.

43. Soorena A, Ayoub YJ, Rezvanollah K et al. Effects of Atrazine (Herbicide) on Blood Biochemical Indices of Grass Carp (*Ctenopharyngodon idella*). Journal of the Persian Gulf (Marine Science). 2011; 2 (5): 51-56.
 44. Harper HA, Rodwell VW, Mayees PA. In: Review of physiological chemistry, 16 edn. Lonmedical Publication: California; 1997: 337-344.
 45. Begum BD, Dharani G. Effect of pesticide on biochemical composition of the fresh water beetle *Cybister brevis*. J Ecotoxicol Environ Monit. 1996; 6(2):101.
 46. Chaudhary RB, Kulkarni AB and Magare SR. Effect of monochrotophos on the protein metabolism of a terrestrial snail, *Zootecusinsularis*. J Ecotoxicol Environ Monit. 1993; 3(1):157-159.
 47. Li J. Bacterial toxins. Curr Opin Struct Biol. 1992; 2: 545– 556.
 48. Liu CC, Walsh CM, Young J D. Perforin: structure and function. Immunol Today. 1995; 16: 194– 201.
 49. Berke G. Killing mechanisms of cytotoxic lymphocytes. Curr Opin Hematol. 1997; 4: 32– 40.
 50. Horta MF. Pore-forming proteins in pathogenic protozoan parasites. Trends Microbiol. 1997; 5: 363– 366.
 51. Cho JH, Park CB, Yoon YG et al. Lumbricin I: a novel proline-rich antimicrobial peptide from the earthworm: purification, cDNA cloning and molecular characterization. Biochem Biophys Acta. 1998; 1408: 67– 76.
 52. Homa J, Niklinska M, Polyteycz B. Effect of heavy metal on the earthworm *Allolophora chlorotica*: Abstract of 7th International symposium on earthworm ecology, 2002:164-165.
-