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

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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.

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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 problems[1]. Large scale urbanization, industrialization as well as population growth have affected the strong relationship between nature and man[2]. Living organisms exposed to polluted environments can have significant deleterious consequences[3].

Fly ash (FA) is a product of coal and lignite combustion [4]. During electricity generation that enters the flue gas stream [5]. 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 cycles [6]. 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 world[12]. *P. hysterophorus* is an invasive alien weed that has a global significance [13]. 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 weed[15].

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 management [16].The disposal methods such as open dumping and land filling practices of various wastes generated from industries are very expensive and unsafe for environment also[18]. The open dumping of wastewater sludge pollutes the natural resources surrounding that area and also provides shelter to various disease-vectors and disease causing agents[19].

Stress induced by toxic environment activates the production of reactive oxygen species (ROS) which are generated as side products of tissue respiration in organisms [20]. 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 used [25].

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 depurate their gut content. The earthworms were homogenized in potassium phosphate buffer (0.1M) and centrifuged at 10,000 rpm for 10 min at 4^{o}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 Folins reagent. The optical density of the blue color developed was read at 520 nm after 30 minutes of addition of the Folins 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. H_2SO_4. 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^{0}C for 2 minutes. 3ml of H_2SO_4 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).

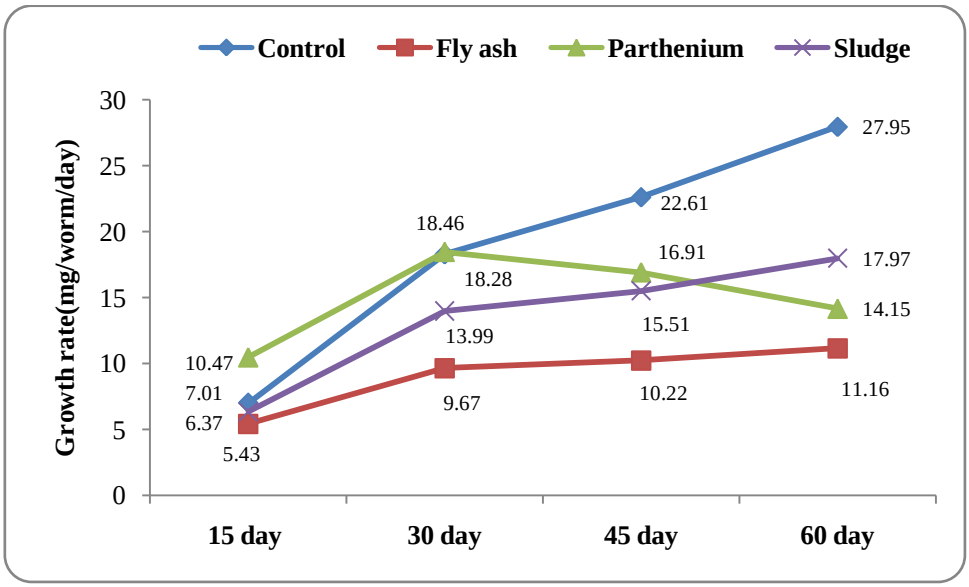

Fig: 1 Growth rate of earthworm (mg/worm/day) in fly ash, *Parthenium hysterophorus* and milk processing industry sludge treatment on 15, 30, 45 and 60 day.

It is well known that the growth and development of earthworm during vermiconversion depends upon the feed quality and growth supporting nutrients present in it [29]. 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).

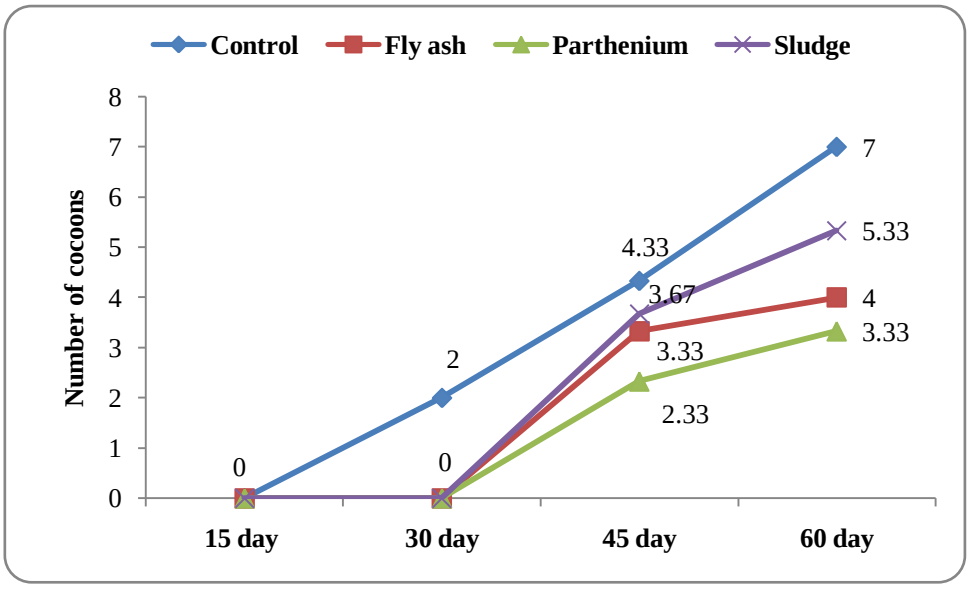

Fig: 2 Number of cocoons produced by earthworms in fly ash, *Parthenium hysterophorus* and milk processing industry sludge treatment on 15, 30, 45 and 60 day.

There was a pivotal relationship between food quality and rate of cocoon production [33]. 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 production[35].

- ***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 chemicals [36]. In the present study, glycogen content was significantly decreased in all the treatments during the experimental period (Fig 3).

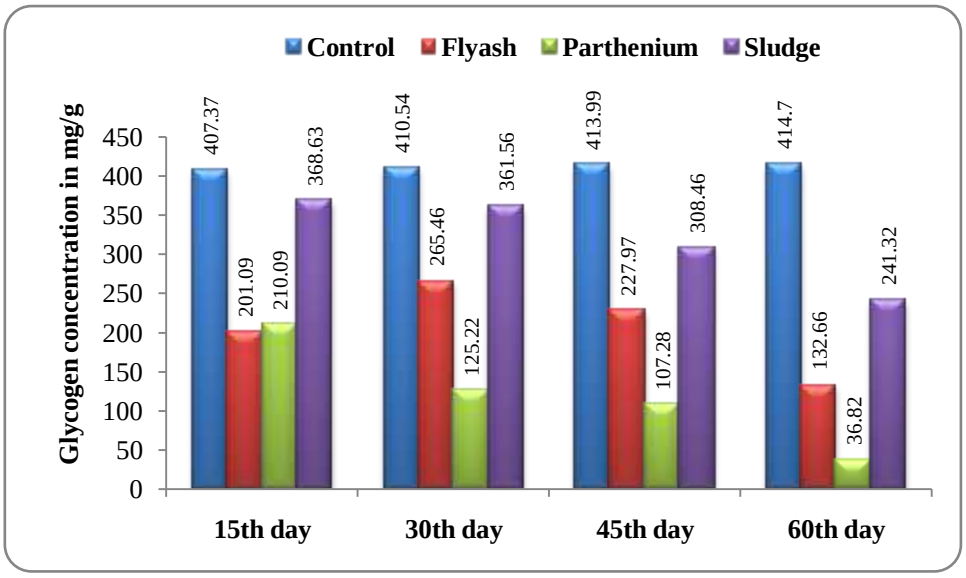

Fig: 3 Glycogen content in earthworm exposed to fly ash, *Parthenium hysterophorus* and milk processing Industry sludge on day 15, 30, 45 and 60 compared with control.

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 required [42]. 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).

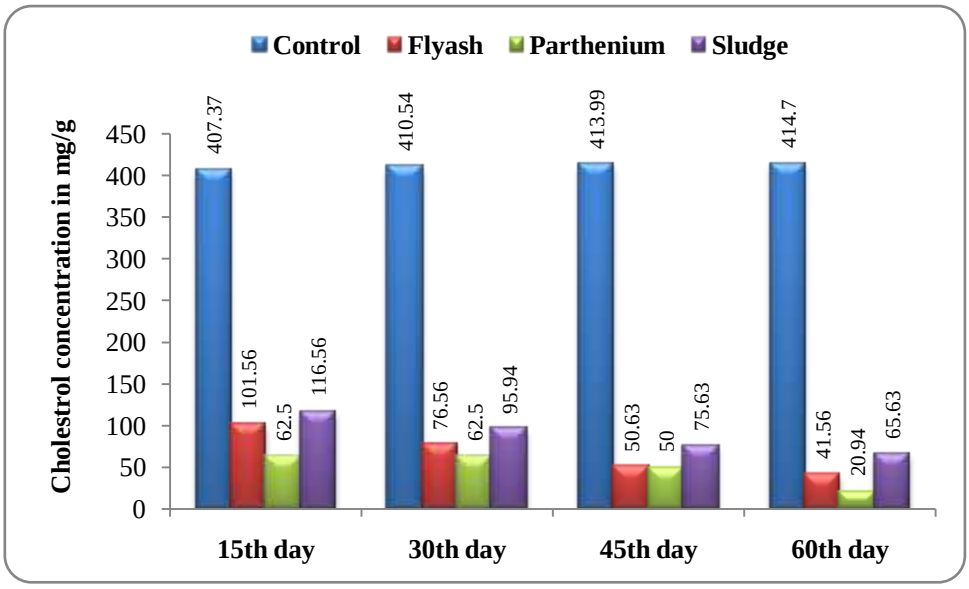

Fig: 4 Cholestrol content in earthworm exposed to fly ash, *Parthenium hysterophorus* and milk processing industry sludge on day 15, 30, 45 and 60 compared with control.

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 matter [44]. 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).

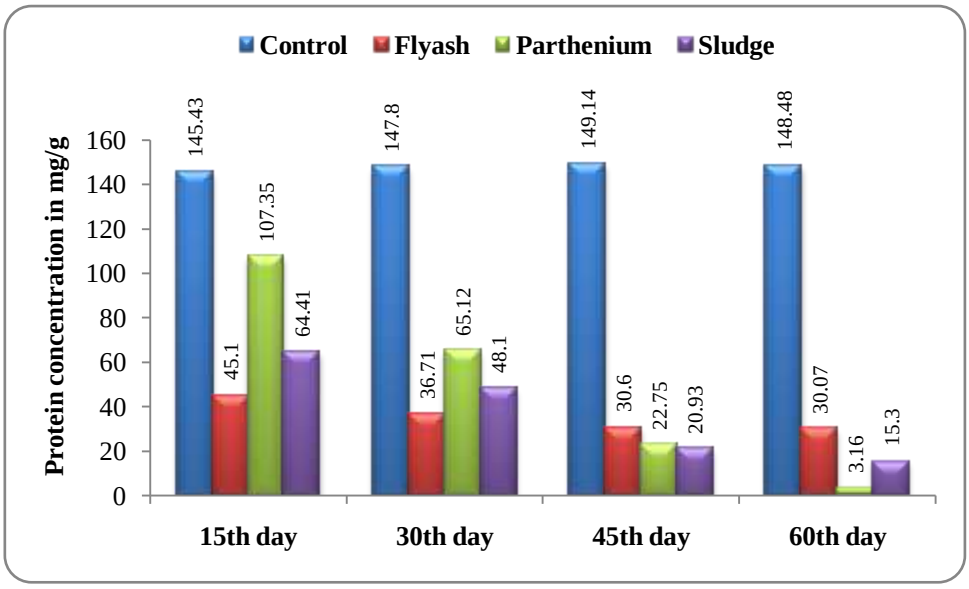

Fig: 5 Protein content in earthworm exposed to fly ash, *Parthenium hysterophorus* and milk processing industry sludge on day 15, 30, 45 and 60 compared with control.

The maximum depletion was found in *P.hysterophorus* treatment. The depletion in protein content may be due to the degradation of proteins into amino acids, which is to be utilized for gluconeogenesis [45] to combat the stress. Being a part of cell membrane, protein level might be reduced because of its metabolism to release energy during stress conditions [46]. 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 stressors [47-52].

CONCLUSION

The present study indicates that fly ash, *P.hysterophorus* and milk processing industry sludge cause alterations in the biochemical parameters of earthworm, *Eisenia fetida*. The results clearly indicate that fly ash, *P.hysterophorus* 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.*

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