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Research article

# Effects of fly ash and *P. hysterophorus* on the antioxidant status of earthworm (*Eisenia fetida*)

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**Key words:** Fly ash, *Parthenium hysterophorus, Eisenia fetida* and Antioxidant enzymes.

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**Abstract**

Fly ash is a serious source of air pollution since it remains air borne for a long period of time and causes health hazards. *Parthenium hysterophorus* is one among the most troublesome weeds at the global level. The aim of the present study was to evaluate the effect of fly ash and parthenium weed on antioxidant status of earthworm, *Eisenia fetida*. Earthworms were allowed to grow in the mixture of cow dung: fly ash (60:40) and cow dung: parthenium (75:25) for 60 days. The biochemical markers viz. catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and malondialdehyde (MDA) level were measured at day 15, 30, 45 and 60. The results revealed increased MDA level, while SOD, GPx and CAT activities showed variation in both the treatments. The study indicated that fly ash and *Parthenium hysterophorus* has adverse biological effects on the model organism *Eisenia fetida*.

## Introduction

Large scale industrialization, urbanization and population growth have affected the healthy relationship between man and nature. Various human activities generate huge quantities of solid wastes throughout the world and their management has become a technical and ecological challenge for all. Most of the wastes are disposed in ecologically unsustainable manner by open dumping or burning [1]. Production of large quantities of organic waste all over the world poses major environmental (offensive odors, contamination of ground water and soil) and disposal problems [2].

Fly ash, a resultant of combustion of coal at high temperature, has been regarded as a problematic solid waste all over the world [3]. A massive amount of fly ash (4750 million tons) is generated worldwide from coal-based thermal power plants [4]. Recently it has started receiving alarming attention due to its hazardous nature, wide spread usage, and the manner of disposal; leading to severe environmental pollution [5, 6]. India generates higher amount of fly ash and utilizes lower percentage of fly ash compared to other countries. Therefore, major portion of it is disposed in ash ponds near the power plants occupying more than 65,000 acres of land [7, 8]. These ash ponds have become a potential source for contamination of soil and water streams [7, 9, 10]. Leaching and accumulation of organic and inorganic toxic compounds from fly ash is of major environmental concern and known to have severe adverse impact such as bioaccumulation of metals, oxidative stress, DNA damage and reproduction on terrestrial and aquatic ecosystems [8, 11-13].

*Parthenium hysterophorus* is one of the top ten worst weeds of the world and has been listed in the global invasive species database [14]. *P. hysterophorus* is an invasive alien weed of global significance [15]. The sesquiterpene lactones namely parthenin and coronopilin present in the trichomes of leaves and stems of *Parthenium*, are responsible for causing various allergies like contact dermatitis, hay fever, asthma and bronchitis in human beings [15, 16 ]. *P. hysterophorus* is poisonous to livestock when it is consumed or repeatedly in contact with the weed. Those animals can encounter death, rashes on their body and udders, alopecia, loss of skin pigmentation, allergic skin reactions, dermatitis, diarrhoea, anorexia and pruritus [17]. Several control methods (chemical, biological, mechanical and integrated) are being used and most of the methods are not successful due to rapid re-infestation of the plant [18].

Earthworms are universally employed as an ecosystem indicator species in ecotoxicological studies on soil contaminants [19-21]. Earthworms are sensitive, readily available and easy to handle, and chronological data are existing from their use in toxicity investigations [22]. Environmental conditions greatly affect their vital processes and the diversity of living organisms [23]. Biochemical responses in organisms against environmental stress are regarded as early warning indices of pollution in the environment. Antioxidant enzymes protect the cells from various reactive oxygen species (ROS) and hence considered as biomarkers for

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assessing the environmental impact of contaminants. Enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST), as well as lipid peroxidation (LPO) [24-28] have been studied as biomarkers of environmental pollution. There is lack of data on biochemical responses in *E. fetida* by *P. hysterophorus* and fly ash.

Therefore, the aim of the present study was to understand the biological effects of fly ash and *P.hysterophorus* on the earthworm, *Eisenia fetida* and to provide additional information on their toxicological effects.

## Materials and methods

### Collection of Cow dung

Cow dung was obtained from nearby village.

### Collection of Fly ash

Fly ash was procured from a thermal power plant, Rajpura.

### Collection of *P. hysterophorus*

*Parthenium hysterophorus* was collected from fallow lands of Patiala.

### Procurement 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, each with a capacity of 1 kg, with a hole at the bottom. The cow dung, fly ash and *P.hysterophorus* were mixed in different ratios as a bedding material:

Cow dung: Fly ash - 60:40

Cow dung: *P.hysterophorus* - 75:25

Total four plastic trays were taken. Thirty healthy earthworms were introduced in plastic trays; water was sprinkled daily on the trays using sprayer to maintain the moisture level of 55-60%. The plastic trays were kept under shade and covered with the gunny bags to avoid direct sunlight.

### Biochemical Analysis

Eight earthworms were removed from each group at the interval 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 enzyme assays were performed using dual beam UV-visible spectrophotometer from Labtronics (LT-2900).

Lipid peroxidation was measured as malondialdehyde (MDA) a thiobarbutaric acid reacting substance, using the method of Wilbur [29]. The reaction mixture contained 0.8 M HCl, 12.5% TCA and 1.23% TBA. The mixture was incubated for 15 minutes at 90℃, after cooling, reaction was measured at 530nm. Values are expressed in μmol $mg^{-1}$ protein using extinction coefficient of $1.56 \times 10^5$ $cm^{-2}$ $mol^{-1}$.

SOD activity was determined as described by Das [30] in a reaction mixture containing phosphate buffer ( pH 7.4), 20 mM α-methionine, 100mM hydroxylamine hydrochloride, 50μ M EDTA, 1% triton X- 100, 100μM riboflavin, 0.1% napthylethylene diamine (NED),1.0% sulphanilamide, 5% orthophosphoric acid and 100μl sample. The activity was measured at 543nm.

Catalase (CAT) was estimated by the method of Aebi [31]. The reaction mixture contained 0.1mM phosphate buffer (pH 7.4) and 30 M $H_2O_2$ and 50μl sample. The rate of decomposition of $H_2O_2$ was measured at 240nm.

GPx activity was measured as described by Rotruck [32]. The reaction mixture contained 0.4 M Tris buffer (pH 7.0),10 mM sodium azide, 2.5 mM hydrogen peroxide, 4mM reduced glutathione, 10% TCA, 0.3M Phosphate solution, 4mM EDTA, 0.04% Ellman's reagent, 1mM reduced glutathione and 100 μl sample. The activity of glutathione was measured at 420nm.

### Statistical analysis

The data was analyzed by Student's *t*-test using graph pad and considering $p<0.001$ as significant and $p>0.05$as non significant.

## Results and discussion

### Results

The effects of fly ash and *P. hysterophorus* on the biochemical responses of *E. fetida* during vermicom posting are shown in Figure 1, 2, 3 and 4. The MDA level showed significant increase at 15, 30, 45 and 60 day as compared to control in fly ash groups. In *P. hysterophorus* treated groups, the same trend was observed i.e. increased MDA level at all intervals in comparison to control.

The SOD activity significantly increased at 15 and 30 day, while the level of SOD was decreased at 45 and 60 day in fly ash and *P. hysterophorus* treated groups as compared to control.

The activities of CAT showed significant elevation at 15 and 30 day, while significant reduction was observed at 45 and 60 day in fly ash treated groups as compared to control. On exposure to *P. hysterophorus,* the same trend was observed i.e. significant increase at 15 and 30 day exposure, while decline in activity of CAT was observed at 45 and 60 day in comparison to control.

A significant increase in Gpx activity was observed on 15 and 30 day, while a significant decrease was noted on 45 and 60 day as compared to control in fly ash and *P. hysterophorus* treated groups.

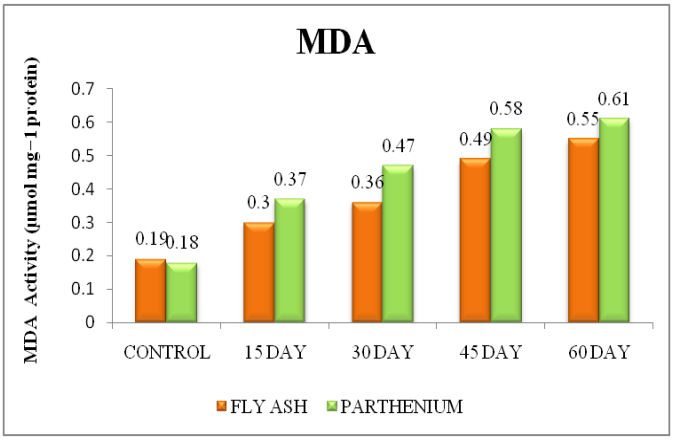


**Figure 1. Effect of fly ash and *P.hysterophorus* on the MDA content of *E. fetida*.**

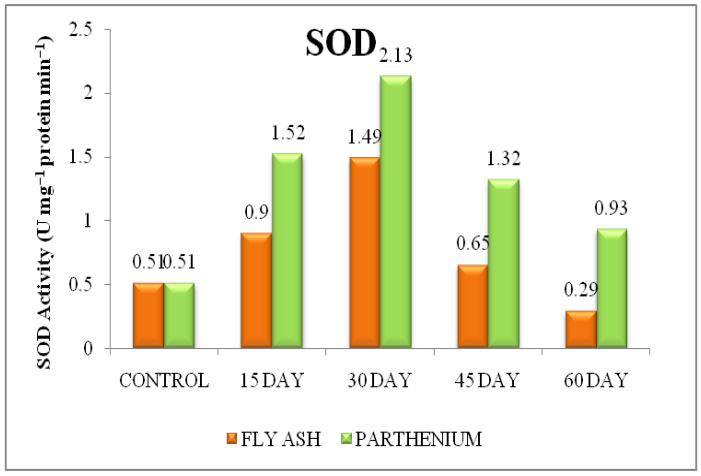


**Figure 2. Effect of fly ash and *P.hysterophorus* on the SOD activity of *E. fetida*.**

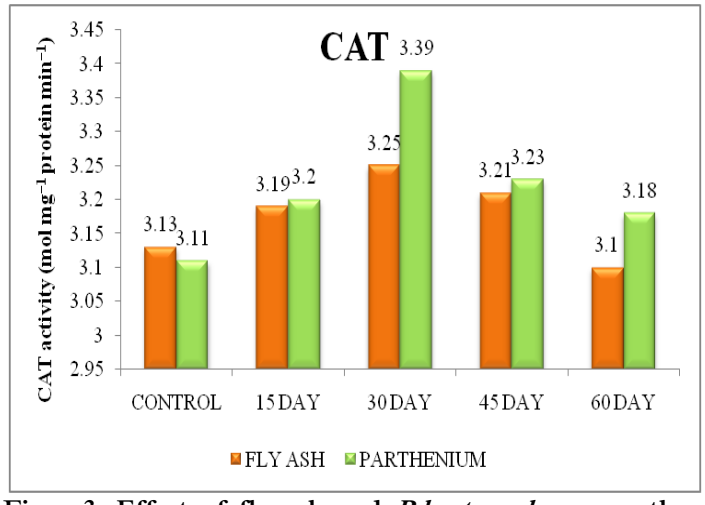


**Figure3. Effect of fly ash and *P.hysterophorus* on the CAT activity of *E. fetida*.**

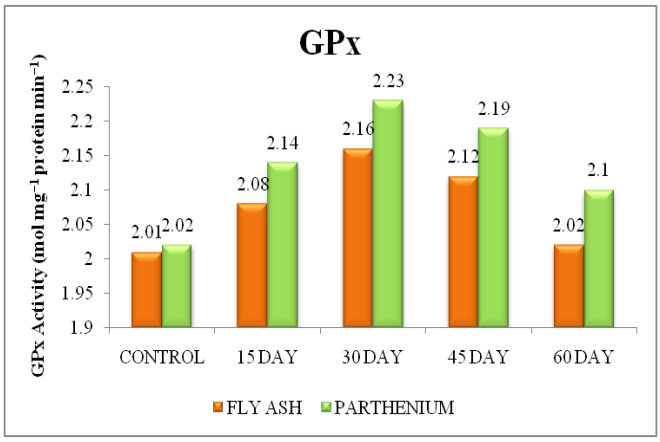


**Figure 4. Effect of fly ash and *P.hysterophorus* on the Gpx activity of *E. fetida*.**

## Discussion

Biological molecular markers are regarded as fast, diagnostic and prognostic early warning system to detect and assess the environmental impact of wide range of contaminants, which cannot be achieved from chemical analysis of environmental samples [26, 33, 34].

Various contaminants like metals are known to induce lipid peroxidation through the formation of ROS and earthworms are particularly susceptible to peroxidation of lipids due to high content of polyunsaturated fatty acids [35, 36]. The MDA is an oxidized product of cellular lipid membranes and could be used as a sensitive biomarker of cell injury [23, 36]. In the present study, MDA levels were found to be increased in *E.fetida* after exposure to fly ash and *P.hysterophorus* at all intervals, indicating the formation of ROS. These results are in confirmation with the findings of Saint-Denis [36] and Ferreira-Cravo [28] who exposed *Eisenia fetida andrei* and polychaeta *Laenereis acuta* to Pb and Cu. Similar results were reported in *Dichogaster curgensis* exposed to fly ash [5].

The enzyme activities varied with duration of exposure. Superoxide dismutase catalyzes dismutation of superoxide anion into $O_2$ and $H_2O_2$ [26]. It is a primary remover of $O^{2-}$ radical and can play an important role in defending against accumulation of toxic activated oxygen species (AOS). The enhanced SOD activity resulted in increased $H_2O_2$ [37]. In present study, SOD activity showed elevation at day 15 and 30 as compared to control indicated that the earthworms exposed to fly ash and *P.hysterophorus* may induce the formation of O2- that resulted in the synthesis of superoxide dismutase to protect the cells from oxidant damage [38]. These results are in accordance with the findings of the study done by Łaszczyca [27] in which the earthworms were exposed to heavy metal contaminated soils. The SOD activity was decreased on day 45 and 60 in fly ash and *P.hysterophorus* treated groups. This may be due to the removal of high reactive superoxide or inactivation of

SOD by singlet oxygen, hydrogen peroxide and peroxyl radicals [39-44].

Catalase protects the cells by eliminating $H_2O_2$ [26]. In the present study, the catalase activity showed significant elevation at day 15 and 30 while significant reduction was observed at 45 and 60 day when treated with fly ash and *P.hysterophorus* The changes in catalase activity were consistent with the activity of superoxide dismutase. The catalase elevated activity at 15 and 30 day of treatment is may be due to increase in the substrate concentration produced to maintain the hydrogen peroxide level [45]. Thus, an elevation in catalase activity may be an indicator to the oxidative stress [46]. The reason for the reduction of CAT activity at 45 and 60 day exposure might be due high cellular stress or the presence of high levels of ROS [47]. Ribera [39] used CAT as a biomarker for the study of biochemical responses in earthworm (*E. fetida andrei*) exposed to carbaryl.

Glutathione peroxidase eliminates $H_2O_2$ by using reduced glutathione as a hydrogen donor, while glutathione reductase reduces oxidized glutathione to maintain the cellular antioxidant status. The increase in GPx activity may be a compensatory mechanism between GPx and GR [27] or NADPH availability and/or inactivation of GR by binding of metals to biomolecules [37]. The GPx activity was found to be increased at 15 and 30 day in fly ash and *P.hysterophorus* treated groups might be due to the increased demand for organism to manage peroxidative damage by reducing the level of peroxides [48]. The reduced GPx activity at 45 and 60 day may be due to some detoxification and phenotypic adaptive mechanisms [49].

## Conclusion

The study reports on the effects of fly ash and *P. hysterophorus* on the antioxidant status of earthworm (*Eisenia fetida*). Toxicological effects of fly ash and parthenium weed on *E. fetida* were assessed by using biomarkers. During management and risk assessment of fly ash and parthenium weed it was found that fly ash and parthenium induces harmful effects such as oxidative stress to earthworm *E. fetida*. Therefore, use of fly ash as soil amendment and *P. hysterophorus* as feed may lead to harmful environmental hazards.

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