ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com
Journal DOI: 10.21474/IJAR01

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

ASSESSMENT OF ALTERATIONS INDUCED BY FLY ASH ON SOME BIOCHEMICAL PARAMETERS IN *EISENIA FETIDA*.

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Manuscript Info

Manuscript History:

Received: 18 March 2016
Final Accepted: 15 April 2016
Published Online: May 2016

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

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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. Besides being a health hazard, fly ash degrades the environment. Earthworms are globally accepted as a model organism in terrestrial ecotoxicology for assessment of environment pollution. This study evaluated the effect of different concentrations of fly ash on biochemical responses in the earthworm, *Eisenia fetida*. Earthworms were allowed to grow in different proportion of fly ash (70-30%, 50-50% and 30-70%) for 1, 7 and 14 day. The biochemical markers viz. catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and malondialdehyde (MDA) level were measured. The activities of superoxide dismutase (SOD), glutathione peroxidase (GPx) and malondialdehyde (MDA) were significantly increased. These results demonstrate that fly ash has adverse biological effects on the indicator organism *Eisenia fetida*.

Introduction:-

Fly ash, a resultant of combustion of coal at high temperature, has been regarded as a problematic solid waste all over the world (Sarojini et al., 2009). Fly ash, which contains silica, aluminum, oxides of iron, calcium, magnesium, arsenic, chromium, lead, zinc, nickel and other toxic metals is a by-product of coal fired electricity generation plants (Gupta et al., 2005).

A massive amount of fly ash (4750 million tons) is generated worldwide from coal-based thermal power plants (Yao et al., 2015). 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 (Maity et al., 2009; Markad et al., 2012). 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 (Pandey and Singh, 2010; Pandey et al., 2011). According to World Bank, by 2015, India will require 1000 km^2 of land for the disposal of coal fly ash (Singh et al., 2010). These ash ponds have become a potential source for contamination of soil and water streams (Mandal and Sengupta, 2006; Pandey et al., 2011; Dragović et al.,2013).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 (Ali et al. ,2004; Chakraborty and Mukherjee, 2009; Grumiaux et al., 2010; Pandey and Singh, 2010).

Earthworms are universally employed as an ecosystem indicator species in eco-toxicological studies on soil contaminants (Marino and Morgan, 1999; Langdon et al., 2001; Reinecke and Reinecke, 2004). Earthworms are sensitive, readily available and easy to handle and chronological data are existing from their use in toxicity investigations (OECD 1984). Biochemical responses in organisms against environmental stress are regarded as early warning indices of pollution in the environment. Antioxidant enzymes are defending the cells from several reactive oxygen species (ROS). Hence, antioxidant enzymes are considered as biomarkers for evaluating the environmental

effect of pollutants. Enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST) and lipid peroxidation (LPO) have been assayed as biomarkers of environmental contaminations (Livingstone et al., 1990; Dallinger, 1993; Saint-Denis et al., 1998; Łaszczyca et al., 2004; Ferreira-Cravo et al., 2009). Accumulation of reactive oxygen species (ROS) such as H_2O_2 and superoxide radical does destruction to cellular components such as DNA, proteins and lipids (López et al., 2006).

The present study was aimed to evaluate the biological effects of fly ash on the earthworm, *Eisenia fetida.* Various biochemical parameters such as CAT (Catalase), SOD (Superoxide dismutase), GPx (Glutathione Peroxidase) and MDA (Malondialdehyde) were evaluated.

Materials and methods:-

Collection of Fly ash and Cow dung:-

Fly ash was procured from a thermal power plant, Rajpura while cow dung obtained from nearby village.

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

T_1 – (70% cow dung + 30% fly ash)
T_2 – (50% cow dung + 50% fly ash)
T_3 – (30% cow dung +70% fly ash)

In each plastic tray, five healthy earthworms were introduced, water was sprinkled daily on trays using an 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:-

Five earthworms were removed from each group at an interval of 1,7,14 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. Biochemical estimations such as catalase (CAT) enzyme was estimated from the rate of decomposition of H_2O_2 by the method of Aebi (1983). SOD activity was determined by the method of Das et al. (2000). GPx activity was estimated by Rotruck et al. (1973). Lipid peroxidation was measured as malondialdehyde a thiobarbutaric acid reacting substance, using the method of Wilbur et al. (1949).

Statistical analysis:-

The data was analyzed by using Student's *t*-test.

Results and discussion:-

Biochemical responses of the earthworms to environmental stress were regarded as early warning system for soil pollution (Łaszczyca et al., 2004). In the present investigation, SOD activity was significantly increased in all the experimental sets at both the intervals as compared to control earthworms. Superoxide dismutase catalyzes dismutation of superoxide anion into oxygen and hydrogen peroxide, while catalase protects the cells by eliminating hydrogen peroxide (Saint-Denis *et al*., 1998). Markad et al (2012) reported a decrease in a activity of SOD in earthworm (*Dichogaster curgensis*) on 14 day exposure as compared to 7 day at all doses of fly ash except 40% fly ash dose (Markad et al., 2012). In present study, no decrease in SOD activity was noted.

CAT activity was significantly increased in the earthworms on 7 day exposure in all the experiments. However on 14 day, CAT activity was significantly decreased as compared to 7 day and increased as compared to control. This decrease may be due to inhibition of enzyme by high cellular stress or inactivation of singlet oxygen, peroxyl radicals and superoxide radical (Kono and Fridovich, 1982; Escobar et al., 1996)

GPx activity was significantly increased in all the experimental sets at 7 day and 14 day interval as compared to control earthworms. 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. GPx activity showed an increase with an increase in the concentration of fly ash and the duration of exposure.

The MDA is an oxidized product of cellular lipid membranes and could be used as a sensitive biomarker of cell injury (Saint-Denis et al., 2001; Livingstone et al., 1990). Earthworms are particularly susceptible to peroxidation of lipids due to high content of polyunsaturated fatty acids: various contaminants like metals are known to induce lipid peroxidation through the formation of ROS (Krauss et al., 2000; Saint-Denis et al., 2001). Lipid peroxidation is a sensitive indicator of oxidative stress and various pollutants are known to induce lipid peroxidation through the formation of ROS. In the present study, a significant increase in MDA level was observed on 7 day and 14 day of exposure of the earthworms in all experimental groups as compared to control. Similar observation were reported in *Eisenia fetida andrei* and Polyachaeta *Laenereis acuta* exposed to heavy metals Pb and Cu(Saint-Denis et al., 2001; Ferreira-Cravo et al., 2009) .This may be attributed to time-dependent enhancement in ROS generation. Further it can be emphasized that the reduction in CAT activity may favour lipid peroxidation due to accumulation of H_2O_2, a precursor of hydroxyl radical that trigger lipid peroxidation (Sandrini et al., 2006)

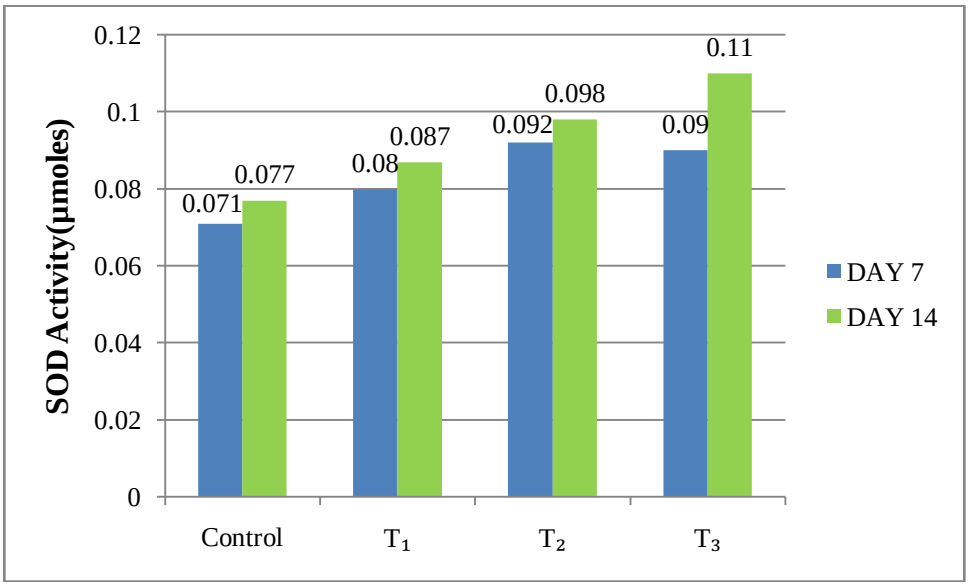

Fig.1 SOD activity in control and treated groups on 7 and 14 d of exposure

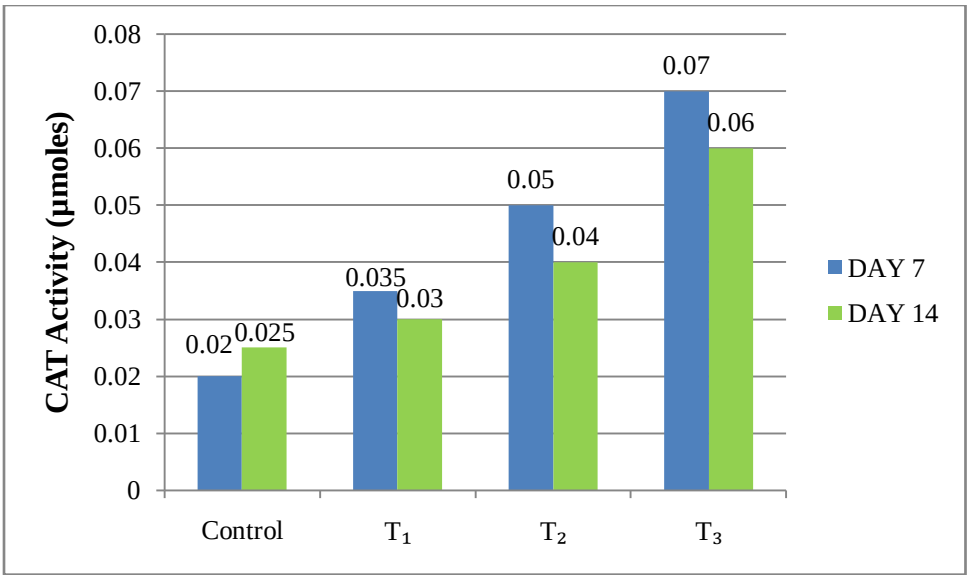

Fig.2 CAT activity in control and treated groups on 7 and 14 d of exposure.

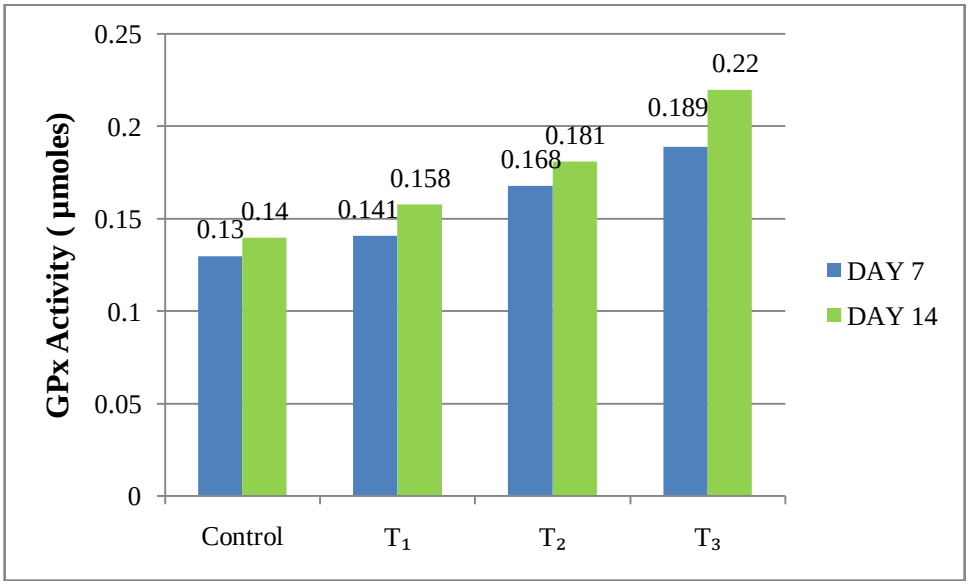

Fig.3 GPx activity in control and treated groups on 7 and 14 d of exposure.

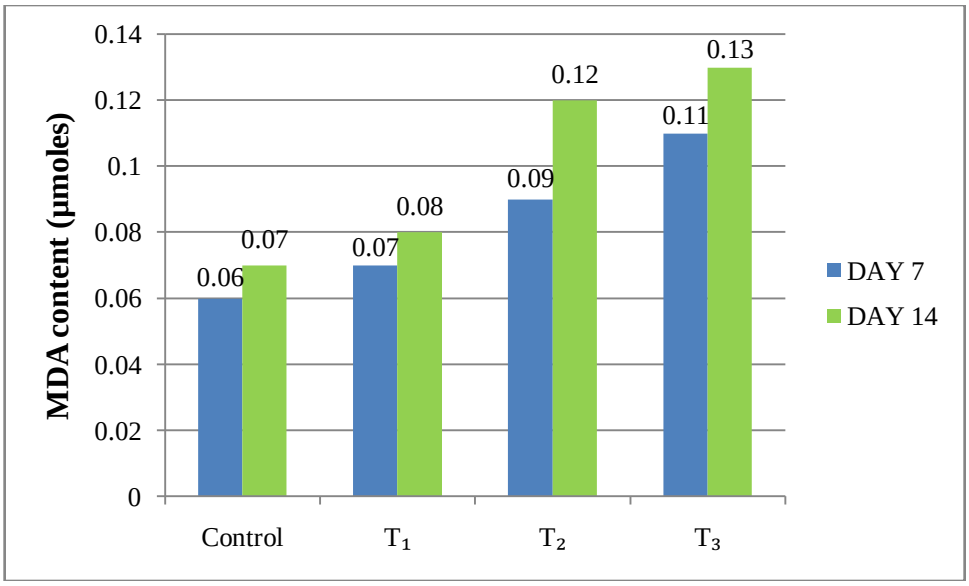

Fig.4 MDA activity in control and treated groups on 7 and 14 d of exposure.

Acknowledgment:-

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.

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