Journal of Hazardous Materials 215–216 (2012) 191–198

Contents lists available at SciVerse ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Effect of fly ash on biochemical responses and DNA damage in earthworm, *Dichogaster curgensis*

Vijaykumar L. Markad, Kisan M. Kodam*, Vikram S. Ghole

Division of Biochemistry, Department of Chemistry, University of Pune, Pune 411007, India

ARTICLE INFO

Article history:
Received 22 August 2011
Received in revised form 18 February 2012
Accepted 20 February 2012
Available online 28 February 2012

Keywords:
Fly ash
Dichogaster curgensis
Biochemical markers
Comet assay
DNA–protein crosslinks
Neutral red retention assay

ABSTRACT

Fly ash is receiving alarming attention due to its hazardous nature, widespread usage, and the manner of disposal; leading to environmental deterioration. We carried out bio-monitoring and risk assessment of fly ash in earthworms as a model system. *Dichogaster curgensis* were allowed to grow in presence or absence of fly ash (0–40%, w/w) for 1, 7, and 14 d. The biochemical markers viz. catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPx), glutathione S-transferase (GST), and malondialdehyde (MDA) level were measured. The comet and neutral red retention assays were performed on earthworm coelomocytes to assess genetic damages and lysosomal membrane stability. The results revealed increased activities of SOD, GPx, GST, and MDA level in a dose–response manner while GR activity was decreased with increasing concentrations of fly ash. No obvious trend was observed in the CAT activity and fly ash concentration. Lysosomal membrane destabilization was noted in the earthworms exposed to 5% and more fly ash concentration in a dose and time dependent manner. The comet assay demonstrated that the fly ash induced DNA damage and DNA–protein crosslinks in earthworm coelomocytes.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

About 75% of the electricity in India is generated from coal-based thermal power plants. However, these power plants produce vast amounts (over 100 million tons/year) of fly ash [1]. Disposal and management of this fly ash is a major environmental concern. Although, 38% of the total fly ash is utilized in agriculture as a soil amendment or in manufacturing of cement and concrete bricks, most of it is disposed in ash ponds near the power plants [2–4]. Heavy metals (Cu, Zn, Cd, Pb, Ni, Cr, etc.) and polyhalogenated compounds [5–7] in fly ash have adverse effects on terrestrial and aquatic ecosystems [8–10]. Repetitive application of fly ash as soil amendment, may lead to soil contamination. Therefore, bio-monitoring and risk assessment is necessary before utilization of fly ash as soil amendment. Earthworms have been extensively used to evaluate biological responses of pesticides, polychlorinated biphenyls, polycyclic hydrocarbons, and heavy metals [11–14]. Few studies have been carried out on the ecotoxicology of fly ash [15,16]; yet, there is paucity of data regarding the antioxidant and genotoxic responses of fly ash on earthworms.

Antioxidant enzymes protect the cells from various reactive oxygen species (ROS) and hence considered as biomarkers for assessing the environmental impact of contaminants. Enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST), and lipid peroxidation (LPO) [14,17–20] have been studied as biomarkers of environmental pollution. The lysosomal membrane stability is a sensitive cellular biomarker widely used in ecotoxicology, provides useful information on cellular damage, and was evaluated using the neutral red retention assay. It has been used to assess the effects of Cu, Cd, Ni, and Zn on earthworm coelomocytes [21–25].

The comet assay or single cell gel electrophoresis is yet another effective tool to measure the DNA damage in individual cells, widely used in the area of ecotoxicology [1]. It measures single and double strand breaks, alkali labile sites, oxidative DNA damages, DNA–DNA/DNA–protein/DNA–drug cross-linking, and DNA repair. Some modifications in the standard protocol of comet assay have been proposed for sensitive detection of DNA-crosslinks [26–29]. The comet assay has been demonstrated to be effective in measuring the DNA damage by various genotoxins in earthworm coelomocytes [1,30–32].

Therefore, the objective of the present study was to better understand the biological effects of fly ash on the earthworm, *Dichogaster curgensis* and underlying mechanisms in order to provide additional information on their toxicological effects. Various

* Corresponding author. Tel.: +91 20 25691728; fax: +91 20 25691728.
E-mail addresses: kodam@chem.unipune.ac.in, kisankodam@gmail.com (K.M. Kodam).

0304-3894/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2012.02.053

biomarkers viz. biochemical responses, lysosomal membrane stability, and DNA damage in the fly ash exposed earthworms were evaluated.

2. Materials and methods

2.1. Chemicals and fly ash

Glutathione reductase, thiobarbituric acid, low melting agarose, and epinephrine were purchased from Sigma (St. Louis, Mo, USA). Aroclor 1260 (Cat No. 4-8704) and PAHs mix (Cat No. 48905-U) were purchased from Supelco, USA. All other chemicals used were of analytical grade, purchased from Sisco Research Laboratories (India), Himedia (India), and Merck (India). The fly ash sample was collected from the thermal power station, Nashik district (19°30′–20°45′N and 73°15′–74°45′E), Maharashtra, India.

2.2. Animals

A stock culture of earthworm, *D. curgensis* was maintained in laboratory on hand collected, dried, homogenized cattle manure with 12/12 h dark–light cycle, 40–50% humidity, and temperature at 22 ± 2 °C. The adult healthy worms (average weight ~250–300 mg) with well-developed clitellum were used.

2.3. Preparation of experimental sets and exposure of worms

The experiments were carried out under laboratory conditions in polythene culture pots (20 cm × 10 cm × 8 cm). Two types of experimental beds were prepared as follows: (i) control set (cattle manure only); (ii) test set (cattle manure + fly ash). The test set was amended with 2.5, 5, 10, 20, and 40% (w/w) fly ash [3]; moisture content was maintained at 40% and temperature at 22 ± 2 °C. The sets were left for 2 d undisturbed prior to experimentation, for stabilization. The earthworms ($n = 15$) were exposed to different doses of fly ash for 1, 7, and 14 d. All the experiments were carried out in triplicate.

The pH and electrical conductivity of the samples were measured (in 0.01 M $CaCl_2$) as described earlier [33]. The organic matter was determined by combusting the samples in a furnace by heating for 1 h at 200 °C, 1 h at 400 °C, and 6 h at 500 °C. The organic matter was calculated as the loss on ignition [33].

2.4. Biochemical assays

The earthworms ($n = 3$ for each group) were randomly selected at an interval of 1, 7, and 14 d of exposure to fly ash, were rinsed with distilled water and kept for 48 h on moist filter paper in Petri dishes to depurate their gut contents. The earthworms were homogenized in Tris–HCl buffer (100 mM, pH 7.5) for 1 min at 4 °C using Potter-Elvehjem homogenizer and centrifuged at 12,000 × *g* for 20 min. The supernatant was used as an enzyme source and aliquots were stored at −80 °C until further use. The enzyme assays were performed using temperature-controlled dual beam UV–Vis spectrophotometer (Jasco V-630). All the assays were carried out in triplicate.

The CAT activity was determined using procedure described by Saint-Denis et al. [19] and the decomposition of hydrogen peroxide was measured spectrophotometrically at 240 nm. The reaction mixture contained 0.1 M Tris–HCl buffer (pH 7.5), 15 mM H_2O_2, and the sample (30 μl) and a molar extinction coefficient of 40 M^{-1} cm^{-1} was used for activity expression. The SOD activity was determined as described by Misra and Fridovich [34] in a reaction mixture containing 0.1 M carbonate buffer (pH 10.2), 0.2 mM EDTA, 0.34 mM adrenaline, and the sample (30 μl). The rate of adrenaline autooxidation was monitored at 480 nm and degree of inhibition was assessed. Glutathione reductase activity was determined according to Racker [35] in a reaction mixture containing 0.1 M Tris–HCl buffer (pH 7.5), 2 mM oxidized glutathione, 0.1 mM NADPH, and the sample (100 μl). The GPx activity was measured as described by Paglia and Valentine [36] against H_2O_2. The reaction mixture contained 0.1 M Tris–HCl buffer (pH 7.5), 1 mM sodium azide, 1 mM reduced glutathione, 1 U of glutathione reductase, 0.1 mM NADPH, 0.1 mM H_2O_2, and the sample (100 μl). The oxidation of NADPH was monitored at 340 nm and a millimolar extinction coefficient (6.22 mM^{-1} cm^{-1}) was used for calculation of both GR and GPx activities.

The GST activity was measured using the method of Habig et al. [37] in a reaction mixture containing 0.1 M Tris–HCl buffer (pH 7.0), 1 mM 1-chloro 2,4-dinitrobenzene, 2 mM reduced glutathione, and the sample (30 μl) and reaction was followed in terms of absorbance at 340 nm. A millimolar extinction coefficient of 9.6 mM^{-1} cm^{-1} was used for activity determination. All enzyme activities were assayed at 25 °C keeping appropriate blanks for non-enzymatic reaction. Lipid peroxidation was estimated spectrophotometrically as described by Livingstone et al. [17]. The reaction mixture contained sample (200 μl), 20% trichloroacetic acid (800 μl), and 0.67% thiobarbituric acid (2 ml). The reaction mixture was incubated at 100 °C for 15 min and the formation of thiobarbituric acid reactive substances was quantified in terms of malondialdehyde (MDA) equivalents at 532 nm. The MDA concentration was presented as μmol of MDA produced per mg protein using a molar extinction coefficient of 1.56×10^5 M^{-1} cm^{-1}. Protein concentration was estimated by the Lowry method [38] using bovine serum albumin as a standard.

2.5. Determination of metals and organic matter

The total metal content of fly ash, cattle manure, and fly ash amended test beds were performed as described earlier [39]. The samples were digested with concentrated nitric and perchloric acid and the digests were diluted to 25 ml with Milli-Q water. Similarly, a pool of 4 earthworms (depurated for 48 h, freeze killed) was acid digested and diluted to 25 ml with Milli-Q water. The metal contents were estimated using Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES, ARCOS, Spectro, Germany).

The PAH and PCB concentrations were determined as described earlier [12]. The samples (fly ash and cattle manure) were Soxhlet extracted with hexane–acetone (2:1) mixture, while freeze-dried earthworms were extracted with hexane–dichloromethane (2:1) mixture. The extracts were pre-cleaned with anhydrous Na_2SO_4, evaporated to 1 ml, and further purified by solid phase extraction for separation of PAHs and PCBs as described earlier [12]. The PAHs and PCBs were estimated by gas chromatography mass spectroscopy (GCMS-QP 5050, Shimadzu).

2.6. Cytotoxic and genotoxic studies

2.6.1. Coelomocytes harvesting

The earthworm coelomocytes were obtained by simple, non-invasive technique described by Eyambe et al. [40]. Three earthworms were randomly selected from each test group on 1, 7, and 14 d of exposures. An individual adult earthworm was washed with distilled water and placed in a glass vial containing chilled extrusion medium (NaCl, 71.2 mM; ethylene glycol tetra acetic acid, 5 mM; and guaicol glycerol ether, 50.4 mM; pH 7.5). The extruded coelomocytes were washed thrice with phosphate buffered saline (PBS) (100 mM, pH 7.3) to remove mucous. The cell viability was checked using trypan blue exclusion method and final cell density was adjusted to 1×10^6 cells ml^{-1} with PBS.

Download English Version:

https://daneshyari.com/en/article/578147

Download Persian Version:

https://daneshyari.com/article/578147

Daneshyari.com