



## Antioxidant enzyme activities and markers of oxidative stress in the life cycle of different Earthworm species

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Earthworms play a crucial role in soil fertility through decomposition, nutrient mineralization and water infiltration, and they are used as a standard organism in ecotoxicological testing. However, to use them for evaluations of environmental pollution, knowledge on the age-related variations in antioxidant enzymes within this species as they undergo different environmental conditions such as treatment to heavy metal insecticides, herbicides, salinity and polluted soil, vermifiltration etc. that cause stress. Due to oxidative stress, the growth and reproductive potential of earthworms are affected because of an imbalance between antioxidant enzymes and Reactive oxygen species (ROS). This study provides a fundamental understanding of the antioxidant enzyme activity and oxidative stress in three earthworm species (*Eisenia fetida*, *Eudrilus eugeniae* and *Pheretima posthuma*) at various stages of their lives. Before studying their usage as potential biomarkers, it is necessary to explore the age-related variations in antioxidant enzymes within these species which serve as the quintessential terrestrial invertebrates in evaluations of environmental pollution. The superoxide dismutase (SOD), catalase (CAT), ascorbic peroxidase (APX), and peroxidase (POD), as well as hydrogen peroxide ( $H_2O_2$ ) as an oxidative stress marker, were measured in the total body of juvenile, sub-adult, and adult earthworms. SOD and CAT activities respectively declined and increased with age in all earthworm species and maximum SOD activity (4.86 U/g FW) and CAT activity (12.33 U/g FW) were found in respectively juvenile (EF- J) and adult (EF- A) stages of *E. fetida*. In *P. posthuma*, APX and POD activities rose with age. APX activity was significantly maximum (6.18 U/g FW) and CAT activity (12.33 U/g FW) whereas significant maximum POD activity ( $0.46 \pm 0.01 \Delta OD/min$ ) in EF- J stage.  $H_2O_2$ , an oxidative stress marker, increased with age in all earthworm species. *P. posthuma* adult (PP-A) had the maximum activity (4.06  $\mu mole/g$  FW), and EF- J life stage had the lowest activity (1.35  $\mu mole/g$  FW). In response to increased oxidative stress ( $H_2O_2$ ), the antioxidant enzymes (SOD, CAT, APX, and POD) work together. Overall, *Eisenia fetida* performed better than *E. eugeniae* and *P. posthuma*. This study fills the gaps in antioxidant enzyme activities at the different age stages of earthworms.

**Keywords:** *Eisenia fetida*, *Eudrilus eugeniae*, Oxidative stress marker, *Pheretima posthuma*, Reactive oxygen species (ROS)

antioxidant antioxidant defence system is divided into