

Comprehensive analysis of biochemical responses in a soil collembola (*Cyphoderusjavanus* borner, 1906) exposed to lead-contaminated garden soil: Implications for soil health and ecological risk assessment

Priyanka Sarangi¹, Partha Pratim Chakravorty^{2*}, Bhabatosh Das³

¹ Research Scholar, Department of Zoology, Raja Narendralal Khan Women's College (Autonomous), Gope Palace, Medinipur (Affiliated to Vidyasagar University), West Bengal, India

² Associate Professor, Department of Zoology, Raja Narendralal Khan Women's College (Autonomous), Gope Palace, Medinipur (Affiliated to Vidyasagar University), West Bengal, India

³ Professor, Department of Biotechnology, Translational Health Science and Technology Institute, NCR Biotech Cluster, 3rd Milestone, Faridabad-Gurugram, Haryana, India

Abstract

This study elucidates the biochemical stress responses of the microarthropod species *Cyphoderusjavanus* Borner when subjected to sublethal doses of lead-treated garden soil under tropical conditions. Specimens were maintained in an environmental chamber at a stable temperature of $28^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$, with distilled water periodically added to ensure consistent moisture levels. The study aimed to investigate the impact of chronic lead exposure on *C. javanus* by assessing biomarkers associated with antioxidant defenses and detoxification processes, specifically measuring levels of glutathione (GSH), glutathione-S-transferases (GST), acetylcholinesterase (AChE), and metallothionein (MT) to determine biochemical stress responses. The findings revealed significant alterations in these biomarkers, suggesting that lead exposure induced substantial toxicity in *C. javanus*. These findings draw attention to the possible impacts of heavy metal pollution in soil ecosystems, highlighting the damaging consequences on soil collembola, which are essential for preserving soil health and ecosystem functionality. The multi-biomarker approach provides a comprehensive assessment of the environmental stress imposed by lead contamination, underscoring the importance of using multiple indicators to evaluate ecological risks.

Keywords: Soil collembola, heavy metal pollution, antioxidant enzymes, bioindicator, biomarker

Introduction

Heavy metal contamination of soil is a significant global concern due to its persistence and non-biodegradable nature, leading to the accumulation of toxic levels over time (Tang *et al.*, 2019) [45]. These metals persist in the soil for many years as stress factors and can cause long-term harm to the ecological environment (Khan *et al.*, 2010; Xiao *et al.*, 2017; Yang *et al.*, 2016; Tang *et al.*, 2019) [20, 53, 56, 45]. Among these contaminants, soil contamination with lead (Pb) is particularly challenging due to its extended retention in the environment, with estimates ranging from 150 to 5,000 years (Kumar *et al.*, 1995; Luo *et al.*, 2014b; Ding *et al.*, 2021) [22, 29, 81]. One of the most common constituents of metal-contaminated sites, lead (Pb) represents a hazard to both human and ecological receptors in terrestrial and aquatic ecosystems (Baghurst *et al.*, 1992; Rogivalet *et al.*, 2006; Lanno *et al.*, 2019) [2, 37, 24]. When lead (Pb) enters agricultural soil, it poses significant biological toxicity risks, including reducing crop yields and the potential for plants to wilt and die (Yang *et al.*, 2001) [55]. Additionally, soil invertebrates like earthworms, springtails, and mites can have their growth and reproduction inhibited by lead (Pb) contamination. These effects include decreased egg-laying, lower hatching and survival rates, weight loss, and other adverse consequences (Xu *et al.*, 2009) [54].

Ecotoxicological assays are important tools for assessing the impact of soil pollution. These tests are frequently used as supplemental tools alongside soil chemical analysis to assess the toxic effects of chemicals on living organisms (Bur *et al.*, 2012) [5]. Soil invertebrates, which may respond

to many compounds at various soil concentrations, have been exploited as useful bioindicators of soil health. The widespread interest in developing soil invertebrate tests to evaluate the ecotoxicological effects of chemicals has led to several proposals to standardize experiments involving invertebrates (Fountain and Hopkin, 2001) [12].

Collembola are considered ideal test organisms for ecotoxicological studies (ISO, 2004; OECD, 2009; Liu *et al.*, 2018) [16, 33, 26], because of their extensive presence in various soil types, high population density, and essential functions in decomposing organic matter, they regulate microbial activity and facilitate nutrient cycling (Fountain and Hopkin, 2001; Bur *et al.*, 2010; Luo *et al.*, 2014a) [12, 4, 28]. Numerous studies have explored Collembola sensitivity and resistance to environmental contaminants, particularly potentially toxic metals (Nursita *et al.*, 2005; Sørensen and Holmstrup, 2005; Liu *et al.*, 2018) [32, 43, 26]. The springtail *Folsomia candida* is often used as a biological indicator of pollution due to its widespread application in assessing soil health (Fountain and Hopkin, 2001; Bur *et al.*, 2010; Luo *et al.*, 2014b) [12, 4, 29].

Despite the considerable research on the effects of heavy metals on Collembola, it is important to note that most of these studies have been carried out mainly in European soil contexts (Addison *et al.*, 2003; Syrek *et al.*, 2006; Xu *et al.*, 2009; Kayiranga *et al.*, 2023) [1, 44, 54, 19]. This focus on temperate regions has resulted in a considerable vacuum in our knowledge of Collembola's response to heavy metal contamination in tropical soils.

The study examines the impact of lead on *Cyphoderus javanus* concerning biomarkers associated with antioxidant defense and detoxifying enzymes to understand the biochemical responses of *C. javanus* to heavy metal pollution under tropical conditions.

Materials method

Test organism

C. javanus was collected from organic-rich soil and cultured in 4 cm × 5.5 cm polythene vials containing 7 g of moist garden soil (Sahana *et al.*, 2014) [39]. The organisms were kept at 28°C ± 0.5°C in an environmental chamber, with baker's yeast used as food and water added to maintain moisture (Chakravorty *et al.*, 2015) [7]. Juveniles were reared separately after being transferred from the adult vials. Toxicity tests were conducted using 12-to 15-day-old juveniles.

Test medium

Soil collected from a natural garden site was used as the test medium for the bioassay studies. The following physicochemical properties (Table 1) of the test medium were determined: soil texture using the international Piper method (Piper, 1942) [36], soil pH with a pH meter (Systronics model 512SE), soil organic carbon using the rapid titration method (Walkey and Black, 1934) [48], and soil water holding capacity following the procedures of Lal (1977) [23] and Viji and Rajesh (2012) [47]. The soil was properly defaunated before being used in the bioassay (Wiles and Frampton, 1996) [51].

Table 1: Physicochemical parameters of the experimental soil

Soil parameters	Results
Texture (g %)	
Clay	17.25
Silt	11.75
Sand	71.00
pH	6.80
Soil Organic Carbon (SOC)	1.45%
Water holding capacity	34.40%

Test chemical

The improper disposal of fly ash, a coal combustion byproduct, poses a significant environmental challenge, particularly in developing countries (Panda & Biswal, 2018) [35]. While fly ash is often used in agriculture for its alkaline properties and mineral content, which improve soil health, it also contains hazardous heavy metals such as arsenic, lead, cadmium, chromium, and mercury, making it a potential environmental threat (Belyaeva & Haynes, 2012; Jambhulkar *et al.*, 2018) [3, 17]. In this study, fly ash-contaminated soil was sampled from the Kolaghat thermal power plant in West Bengal, India (22°25'28.3"N, 87°51'39.4"E), following a randomized block design to ensure systematic collection. Heavy metal content was analyzed by Atomic Absorption Spectroscopy (AAS), with lead, the most abundant metal, selected for further study. Lead acetate trihydrate [(CH₃COO)₂Pb·3H₂O], obtained from Merck Life Science, was used for the experiments.

Table 2: Heavy metal content of fly-ash-contaminated soil

Sl. No.	Heavy Metals	Test Method	Unit	Results
1	Lead as Pb	APHA 23 rd Edition 3111B	mg/kg	6048.30
2	Nickel as Ni	APHA 23 rd Edition 3111B	mg/kg	1692.21
3	Manganese as Mn	APHA 23 rd Edition 3111B	mg/kg	970.01
4	Zinc as Zn	APHA 23 rd Edition 3111B	mg/kg	176.54
5	Copper as Cu	APHA 23 rd Edition 3111B	mg/kg	137.00
6	Chromium as Cr	APHA 23 rd Edition 3111B	mg/kg	131.56
7	Cadmium as Cd	APHA 23 rd Edition 3111B	mg/kg	114.20
8	Arsenic as As	APHA 23 rd Edition 3111B	mg/kg	6.00

Acute toxicological test

The current study examined the acute toxicity of lead acetate on *C. javanus* using standard protocols with some changes (ISO, 2004; OECD, 2016) [16, 34]. Different doses of lead acetate were administered into the test vials concerning the doses found in the fly ash-contaminated soil from the field. The lead solutions were allowed to be distributed evenly throughout the soil before introducing the organisms. Three sets of replicates were created for each dose of lead acetate to determine the concentration that resulted in 50% death and the LC50. Ten *C. javanus* juveniles aged 12 to 15 days were obtained and placed in each jar by timing the egg-laying of the cultured animals. After 24 hours of exposure, the mortality of *C. javanus* was reported.

Biochemical Assays

The further chronic toxicity of lead acetate on different biochemical parameters of the mentioned collembola was assessed following the standard test procedures (OECD, 2016) [34]. The following sub-lethal concentrations were employed for *C. Javanus*: 5933.5, 2966.75, 1977.83, and 1483.37 mg/kg soil, according to the findings of the acute toxicity study.

Preparing homogenates and conducting biochemical analyses

To extract the enzymes, *C. javanus* was cautiously taken out from each replicate jar on days 1, 3, and 7 of the incubation periods. With three replicates for each parameter of lead and control in soil, a total of 60 vessels were made. The estimations were based on the surviving specimens; each replicate comprised about 50 specimens. The chemicals utilized in the experiment were of analytical quality and sourced from Sigma (USA), and E. Merck (India).

Glutathione (GSH) assay

GSH levels were measured using a colorimetric assay where whole-body homogenates were reacted with DTNB, producing a yellow compound that was quantified at 405 nm (Sedlak & Lindsay, 1968) [42]. GSH was expressed in µg per mg of protein.

Glutathione-S-transferase (GST) assay

GST activity was assessed by measuring the formation of a conjugate between GSH and CDNB. The activity was expressed as µmol of DNPG generated per minute per mg of protein (Habig *et al.*, 1974) [14].

Acetylcholinesterase (AChE) activity

The Ellman method was used to test AChE activity, which involves the reaction of thiocholine with DTNB to produce a yellow colour at 412 nm. The results were presented as nmol of thiocholine per minute per mg of protein (Ellman *et al.*, 1961) [11].

Metallothionein (MT) activity

MT activity was determined through a spectrophotometric method with GSH as a standard. MT levels were expressed as µg per mg of protein (Viarengo *et al.*, 1997) [46]. The biuret reaction of protein with alkaline cupric tartrate and the blue color produced by the amino acid tyrosine and tryptophan in the Folin-Ciocalteu reagent was used to determine the total protein content of *X. welchi* tissue (Lowry *et al.*, 1951) [27]. The O.D. was determined at 660 nm with a bovine serum albumin (BSA) standard. The mg per ml value for tissue proteins was reported.

Data presentation and statistical analysis

The SPSS software and Microsoft Excel were used for statistical analysis and graphical depiction of the results. The LC50 (effect on survival) value was calculated using the probit analysis program of SPSS software version 29.0.1.0 (171). The findings about biochemical biomarkers were presented as the mean value along with its corresponding standard deviation (SD). Single-factor

ANOVA was used to examine significant changes between treatments, followed by Tukey's post hoc testing at a 5% probability level.

Results

Acute toxicity of lead acetate on *C. javanus*:

The 24-hour LC50 value of lead acetate for *C. javanus* is recorded as 11,867.13 mg/kg (Table 3).

Table 3: 24 hours LC50 values (mg/kg) of lead acetate on *C. javanus*

Heavy metal	24hours LC50	95% confidence limit
Pb as Lead Acetate	11,867.126	7806.334-14773.270

Effects of lead toxicity on enzyme activities

After 1 day of exposure, the T2, T3, and T4 groups had significantly higher GSH concentrations than the control group ($P < 0.05$), whereas the T1 group did not show a significant difference (Fig 1A). After 3 days, there was no significant difference in GSH concentration between the T1 and T2 groups and the control ($P > 0.05$). However, the T3 and T4 groups showed a substantial increase ($P < 0.05$) (Fig 1B). By day 7, the T3 and T4 groups had significantly increased GSH levels than the control group ($P < 0.05$) (Fig 1C).

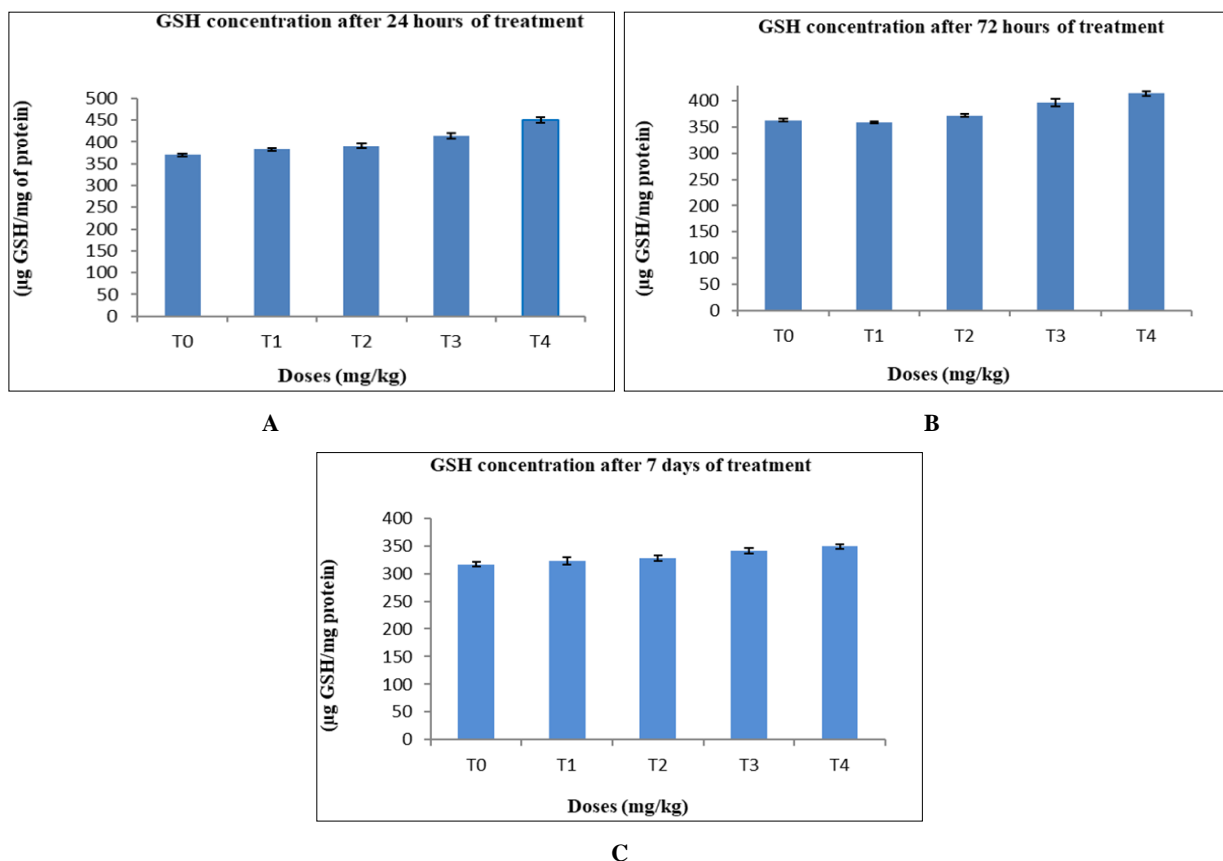


Fig 1: Reduced Glutathione (GSH) concentration of *C.javanus*exposed to sublethal doses (T1, T2, T3, and T4) of lead acetate and the control (T0; without treatment) after 1 (A), 3 (B), and 7 (C) days of exposure. The mean ± standard deviation is used to express the results (n=3)

After 1 day of treatment, the T2, T3, and T4 groups showed a substantial increase in GST activity (µM DNPG/min/mg p µM DNPG/min/mg protein) compared to the control group ($P < 0.05$) (Fig 2A). After 3 days, the same groups (T2, T3, and T4) showed a significant increase in GST activity ($P <$

0.05) compared to the control (Fig 2B). By day 7, only the T3 and T4 groups had a significant rise in GST concentration ($P < 0.05$), whereas the T1 and T2 groups showed no significant change compared to the control ($P > 0.05$) (Fig 2C).

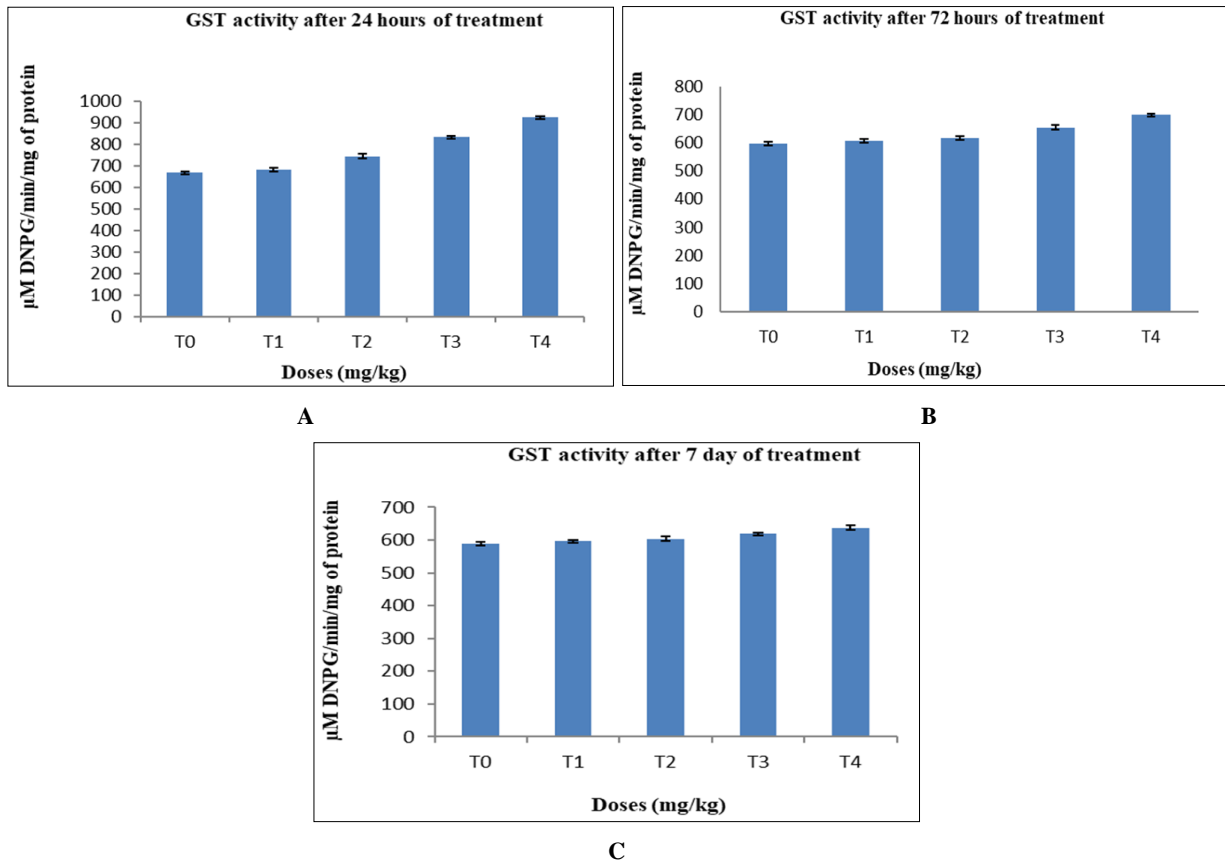


Fig 2: Glutathione-S-transferase (GST) activity of *C. javanus* exposed to sub-lethal doses (T1, T2, T3, and T4) of lead acetate and the control (T0; without treatment) after 1 (A), 3 (B) and 7 (C) days of exposure. The mean ± standard deviation is used to express the results (n = 3)

C. javanus exhibited no significant changes in AChE activity ($P > 0.05$) after day 1 of lead exposure across all treatment groups compared to the control. After 3 days, the T3 and T4 groups had significantly reduced ($P < 0.05$)

AChE activity than the control (Fig 3B). By day 7, all treatment groups, except T1, had significantly reduced AChE activity (Fig 3C).

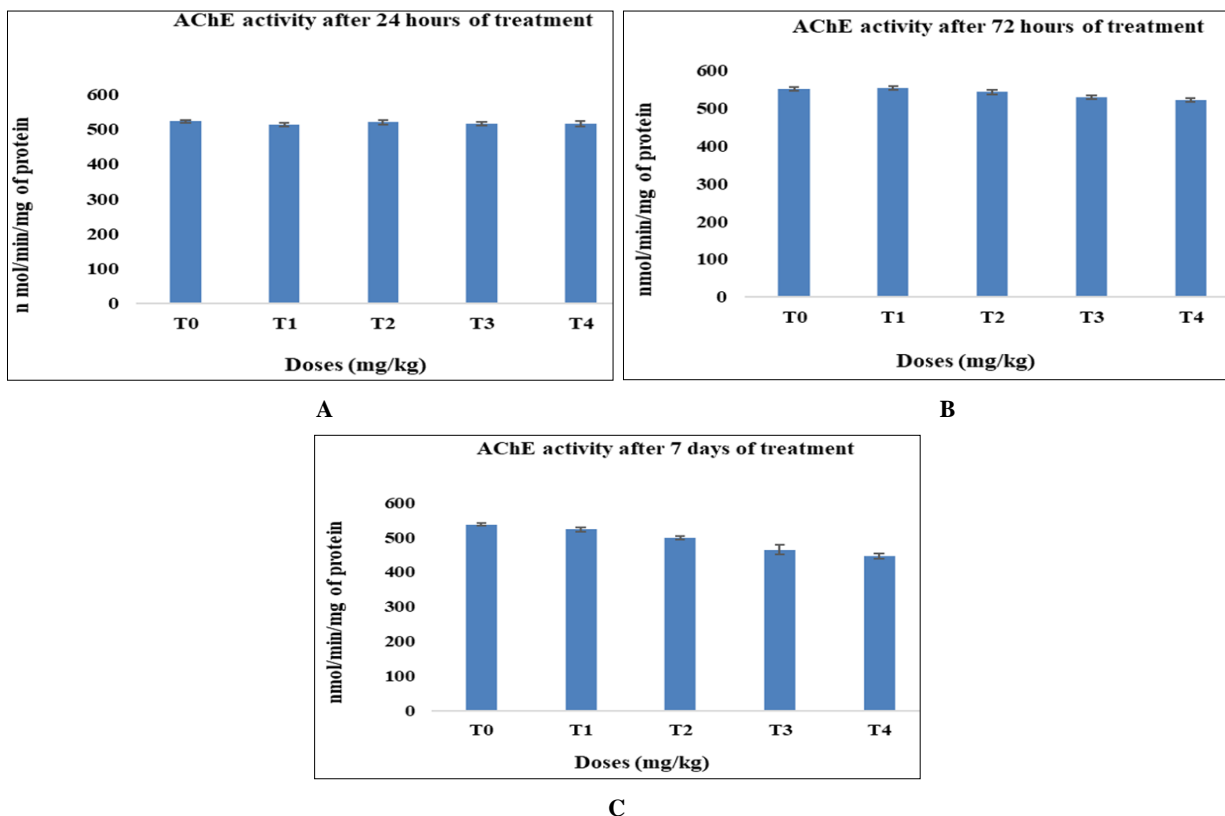


Fig 3: Acetylcholinesterase (AChE) activity of *C. javanus* exposed to sub-lethal doses (T1, T2, T3, and T4) of lead acetate and the control (T0; without treatment) after 1 (A), 3 (B) and 7 (C) days of exposure. The mean ± standard deviation is used to express the results (n = 3)

After 1 day of exposure, there was no significant difference in MT activity between the T1 and T3 groups and the control, whereas the T2 and T4 groups showed a considerably significant increase (Fig 4A). On day 3, both

the T3 and T4 groups showed a significant increase in MT activity compared to the control one (Fig 4B). After 7 days, all treatment groups, except T1, had significantly higher MT activity than the control (Fig 4C).

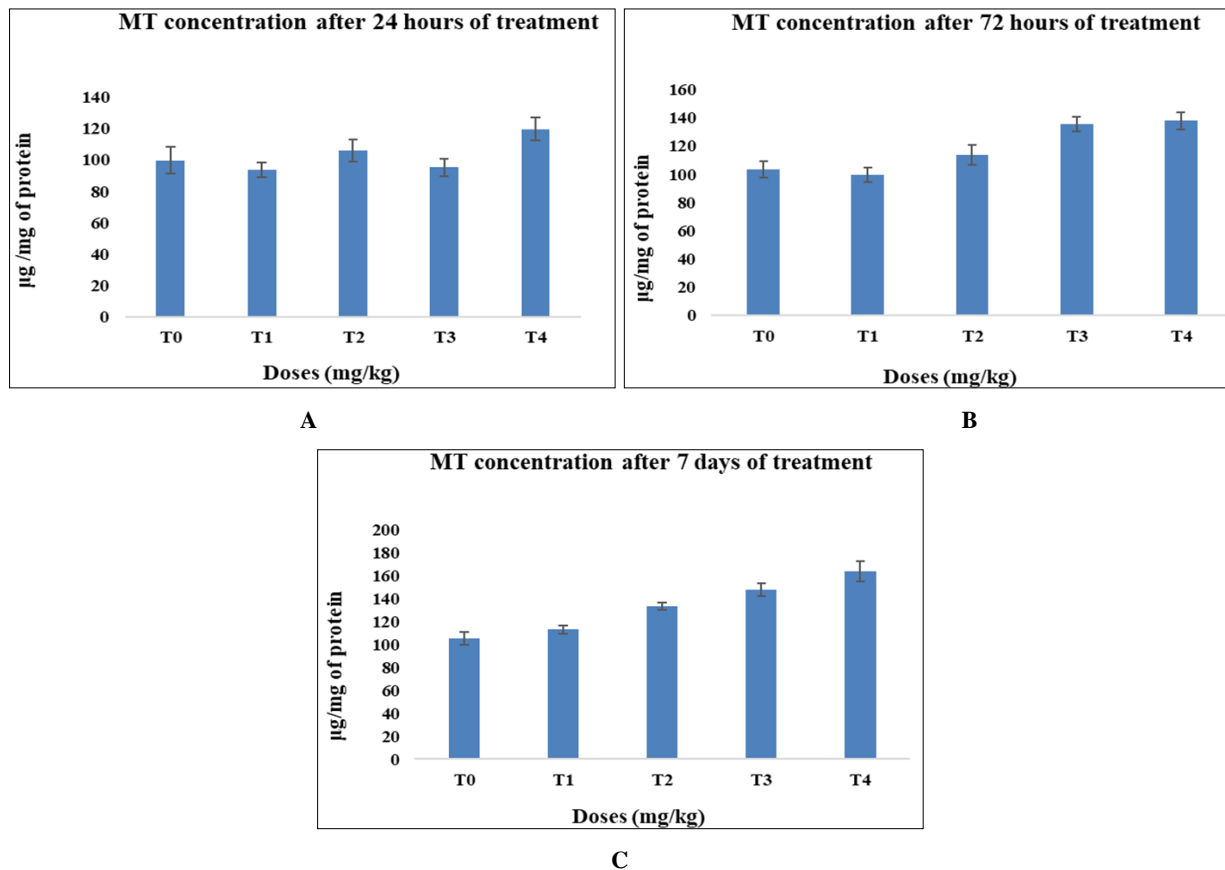


Fig 4: Metallothionein (MT) concentration of *C. javanus* exposed to sub-lethal doses (T1, T2, T3, and T4) of lead acetate and the control (T0; without treatment) after 1 (A), 3 (B) and 7 (C) days of exposure. The mean \pm standard deviation is used to express the results (n = 3)

Discussion

The evaluation of multiple biomarkers has proven to be a dependable method and has become an essential alternative for monitoring various model organisms globally (Wang *et al.*, 2019) [49]. The current study shows that lead (Pb) concentration and exposure duration significantly altered some biochemical parameters of *C. javanus* over 1, 3, and 7 days of culture experiments.

Effects of enzyme activities in *C. javanus* exposed to lead-

This study shows that in *C. javanus*, glutathione (GSH) levels increased with higher concentrations of lead (Pb) in the soil. GSH plays a vital role in the cellular defense against reactive oxygen species (ROS) and toxic substances, contributing to the detoxification process by binding to electrophilic xenobiotics through the action of glutathione S-transferase (GST). This finding supports numerous other studies that have demonstrated the activation of antioxidant enzymes, such as GSH, in *F. candida* by various metals, including cadmium (Cd), copper (Cu), lead (Pb), and zinc (Zn) (Dai *et al.*, 2017) [10]. Our results are consistent with those of Gudbrandsen *et al.*, (2007) [13] and Lawson and Yu (2003) [25], who observed elevated GSH levels in the earthworm *Eisenia fetida* under oxidative stress. Whereas Sahana *et al.*, (2014) [39], reported a decrease in GSH content in *C. javanus* due to Pb and Cd exposure. The observed rise in GSH content in the present study can be

attributed to oxidative stress induced by Pb exposure. Prolonged exposure to Pb could potentially lead to even more severe oxidative stress in collembola, highlighting the organism's response mechanism to prolonged metal-induced stress (Du *et al.*, 2015) [9].

According to the current study, GST activity in *C. javanus* rises with soil lead (Pb) concentration. These results align with the findings of Wilczek *et al.*, (2004) [50], who observed that the levels of parameters like the activity of glutathione-dependent detoxifying enzymes were elevated in organisms residing in more polluted areas compared to those in cleaner environments. A study by Kovačević *et al.*, (2023) [21] revealed that during the initial days of azoxystrobin treatment, GST activity in *F. candida* increased at certain concentrations. As the treatment continued, GST activity significantly decreased over time. A study by Jeyanthi *et al.*, (2016) [18] reported a significant increase in glutathione-S-transferase (GST) activity in *Eudriluseugeniae* and *Perionyx excavatus* when exposed to lead (Pb). Similarly, the GST enzyme activity was elevated in *E. eugeniae* and *Perionyx ceylanensis* under carbaryl-induced stress. However, in contrast, a study by Hammad *et al.*, (2017) [15] found that treatments with imidacloprid, glyphosate, and methiocarb did not result in an increase in GST activity compared to the control group. In our study, the activity profiles of GSH and GST reflected the tolerance capacity of Collembola after chronic exposure to metal-contaminated soil. Furthermore,

the antioxidant enzymes in Collembola play a crucial role in their adaptive response, helping to neutralize free radicals and reactive oxygen species (ROS), thereby supporting their survival under stress (Jeyanthi *et al.*, 2016)^[18].

In this study, exposure to lead over seven days significantly altered AChE activity in *C. javanus* across various sub-lethal doses. Acetylcholine (ACh), a key neurotransmitter involved in nerve impulse transmission, is affected by heavy metal exposure, which can interfere with normal synaptic functions. Lead disrupts cholinesterase (ChE) enzymes by binding to their active sites, inhibiting the breakdown of ACh in the synaptic cleft. This inhibition results in ACh accumulation, causing excessive neuronal stimulation and potential neurotoxic effects in collembolan species (Sanyal *et al.*, 2024)^[38]. Similar patterns have been observed in other studies, such as Chakravorty *et al.*, (1995)^[6], who reported reduced AChE activity in *C. javanus* following exposure to methyl parathion and carbaryl, both organophosphate and carbamate insecticides. In addition, Kovačević *et al.*, (2023)^[21] reported significant reductions in AChE activity in *F. candida* exposed to azoxystrobin across various doses and time points. In fly ash-treated soil the AChE of collembola is compromised within 7 days of exposure to heavy metals (Sahana & Joy, 2014)^[40].

In this study, metallothionein (MT) concentrations significantly increased in the T2, T3, and T4 treatment groups after 7 days, compared to the control group. This result is consistent with findings from Maria *et al.*, (2014)^[31], who reported elevated MT levels in *Folsomia candida* after 6 days of exposure to heavy metals such as copper (Cu) and cadmium (Cd). Similarly, Maity *et al.*, (2011)^[30] observed MT induction in earthworm tissues in response to lead (Pb) exposure. Metallothioneins, small proteins rich in cysteine residues, play a crucial role in regulating metal ions and neutralizing reactive oxygen species (ROS) (Wong *et al.*, 2004)^[52]. The increase in MT levels observed in this study suggests an enhanced capacity for ROS detoxification, providing potential protection against oxidative stress. Moreover, the sustained rise in MT concentrations over time points to an adaptive cellular response to prolonged heavy metal exposure, indicating a cumulative effect of the treatment.

Conclusion

Lead exposure induces substantial biochemical changes in *C. javanus*, an important bioindicator species for soil health. The multi-biomarker approach in this study offers a thorough and integrative evaluation of the ecological risks associated with lead contamination, emphasizing its potential as an effective tool for monitoring polluted soils. The findings underscore the sensitivity of soil ecosystems, particularly in tropical environments, to heavy metal pollution and stress the urgent need for enhanced conservation and remediation efforts. Protecting soil health in these regions is critical to maintaining overall ecosystem stability and biodiversity, further reinforcing the importance of implementing long-term strategies for soil pollution management.

Acknowledgments

The authors are grateful for the facilities provided by the Principal, Raja Narendra Lal Khan Women's College (Autonomous), Natural and Applied Sciences Research Centre, Paschim Medinipur, West Bengal, India. PS is

grateful for Junior Research Fellowship from the University Grant Commission (UGC) to conduct the research work, and PPC is grateful to DST-CURIE for providing infrastructure assistance.

Conflict of interests

The authors declare that they have no competing interests.

References

- Addison JA, Trofymow JA, Marshall VG. Abundance, species diversity, and community structure of Collembola in successional coastal temperate forests on Vancouver Island, Canada. *Applied Soil Ecology*,2003;24:233–246.
- Baghurst PA, McMichael AJ, Wigg NR, Vimpani GV, Robertson EF, Roberts RJ, Tong SL. Environmental exposure to lead and children's intelligence at the age of 7 years—The Port-Pirie Cohort study. *N England J Med*,1992;327:1279–1284.
- Belyaeva NO, Haynes JR. Comparison of the effects of conventional organic amendments and biochar on the chemical, physical and microbial properties of coal fly ash as a plant growth medium. *Environmental Earth Sciences*,2012;66:1987-1997.
- Bur T, Probst A, Bianco A, Gandois L, Crouau Y. Determining cadmium critical concentrations in natural soils by assessing Collembola mortality, reproduction and growth. *Ecotoxicology and Environmental Safety*,2010;73:415–22.
- Bur T, Crouau Y, Bianco A, Gandois L, Probst A. Toxicity of Pb and of Pb/Cd combination on the springtail *Folsomia candida* in natural soils: Reproduction, growth and bioaccumulation as indicators. *Science of The Total Environment*,2012;414:187–197. doi: <https://doi.org/10.1016/j.scitotenv.2011.10.029>.
- Chakravorty PP, Bose S, Joy VC, Bhattacharya, S. Biomonitoring of anticholinesterase pesticides in the soil: usefulness of soil Collembola. *Biomedical and Environmental Sciences: BES*, 1995.
- Chakravorty PP, Haque A, Sanyal S, Dasgupta R. Effect of herbicides on *Cyphoderus javanus* (Hexapoda: Collembola) under laboratory conditions. *Journal of Entomology and Zoological Studies*,2015;3(1):220–223.
- Ding Y, Li Z, Ke X, Wu L, Zuo S. Toxicity of lead pollution to the collembolan *Folsomia candida* in Ferri-Udic Cambosols. *Pedosphere*,2021;31:627–637.
- Du L, Li G, Liu M. *et al.* Biomarker responses in earthworms (*Eisenia fetida*) to soils contaminated with di-*n*-butyl phthalates. *Environmental Science and Pollution Research*,2015;22:4660–4669. doi: <https://doi.org/10.1007/s11356-014-3716-8>.
- Dai Wencai, *et al.* Antioxidant Enzyme Activities of *Folsomia Candida* and Avoidance of Soil Metal Contamination. *Environmental Science and Pollution Research*,2017;25:(3):2889–2898. doi: <https://doi.org/10.1007/s11356-017-0489-x>.
- Ellman GL, Courtney KD, Andres V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*,1961;7(2):88–95.
- Fountain MT, Hopkin SP. Continuous monitoring of *Folsomia candida* (Insecta: Collembola) in a metal

- exposure test. *Ecotoxicology and Environmental Safety*,2001:48:275–286.
13. Gudbrandsen M, Sverdrup LE, Aamodt S, Stenersen J. Short-term preexposure increases earthworm tolerance to mercury. *European Journal of Soil Biology*,2007,43(s):261–267.
 14. Habig WH, Pabst MJ, Jakoby WB. Glutathione-S transferase. The first enzymatic step in mercaptyuric acid formation. *Journal of Biological Chemistry*,1974:249:7130 – 7139.
 15. Hammad AMA, Abbo ASH, Abdelbagi AO, Laing, M. First record of collembolans carboxylesterase and glutathione-s-transferase activities exposed to several pesticides. *Journal of Basic and Applied Research International*,2017:21(2):99–105.
 16. ISO. Soil Quality – Inhibition of Reproduction of Collembola (*Folsomia candida*) by Soil Pollutants. International Organization for Standardization, 2004-1999, 11267.
 17. Jambhulkar HP, Shaikh SMS, Kumar MS. Fly ash toxicity, emerging issues and possible implications for its exploitation in agriculture; Indian scenario: A review. *Chemosphere*, 2018. doi: 10.1016/j.chemosphere.2018.09.045
 18. Jeyanthi V, Paul JAJ, SelviBK. *et al.* Comparative Study of Biochemical Responses in Three Species of Earthworms Exposed to Pesticide and Metal Contaminated Soil. *Environmental Process*,2016:3:167–178. doi: <https://doi.org/10.1007/s40710-016-0131-9>.
 19. Kayiranga A, Li Z, Isabwe A, Ke X, Simbi CH, Ifon BE, *et al.* The Effects of Heavy Metal Pollution on Collembola in Urban Soils and Associated Recovery Using Biochar Remediation: A Review,2023:20:3077.
 20. Khan S, Hesham AB, Qiao M, Rehman S, He JZ. Effects of Cd and Pb on soil microbial community structure and activities. *Environmental Science and Pollution Research*,2010:17:288–296.
 21. Kovačević M, Stjepanović N, Zelić L, Lončarić Ž. Temporal Dynamics of Biomarker Response in *Folsomia candida* Exposed to Azoxystrobin. *Agriculture*,2023:13(7):1443.
 22. Kumar PBAN, Dushenkov V, Motto H, Raskin I. Phytoextraction: The use of plants to remove heavy metals from soils. *Environ Sci Technol*,1995:29:1232–1238.
 23. Lal R. Physical properties and moisture retention characteristics of some Nigerian soils, 1978. *cgspace.cgiar.org*. [online] Available at: <https://hdl.handle.net/10568/81259> [Accessed 11 May 2024].
 24. Lanno RP, Oorts K, Smolders E, Albanese K, Chowdhury MJ. Effects of Soil Properties on the Toxicity and Bioaccumulation of Lead in Soil Invertebrates. *Environ Toxicol Chem*,2019:38(7):1486–1494. doi: 10.1002/etc.4433.
 25. Lawson PB, Yu MH. Fluoride inhibition of superoxide dismutase (SOD) from the earthworm *Eisenia fetida*. *Fluoride*,2003:36:143–151.
 26. Liu MP, Xu J, Krogh PH, Song J, Wu LH, Luo YM, *et al.* Assessment of toxicity of heavy metal-contaminated soils toward Collembola in the paddy fields supported by laboratory tests. *Environmental Science and Pollution Research*,2018:25:16969–16978.
 27. Lowry OH, Rosenbrough NJ, Farr AL, Randal RJ. Protein measurement with Folin phenol reagent. *Journal of Biological Chemistry*,1951:193:265–275.
 28. Luo W, Verweij R A, van Gestel CAM. Assessment of the bioavailability and toxicity of lead polluted soils using a combination of chemical approaches and bioassays with the Collembolan *Folsomia Candida*. *Journal of Hazardous Materials*,2014a:280:524–530.
 29. Luo W, Verweij R A, van Gestel CAM. Contribution of soil properties of shooting fields to lead bioavailability and toxicity to *Enchytraeus crypticus*. *Soil Biology and Biochemistry*,2014b:76:235–241.
 30. Maity S, Roy S, Bhattacharya S, Chaudhury S. Metallothionein Responses in the Earthworm *Lampito Mauriti* (Kinberg) Following Lead and Zinc Exposure: A Promising Tool for Monitoring Metal Contamination. *European Journal of Soil Biology*,2011:47(1):69–71.
 31. MariaVL, Ribeiro MJ, Amorim MJB. Oxidative stress biomarkers and metallothionein in *Folsomia candida* - responses to Cu and Cd. *Environmental Research*,2014:133:164–169. doi: <https://doi.org/10.1016/j.envres.2014.05.027>.
 32. Nursita AI, Singh B, Lees E. The effects of cadmium, copper, lead, and zinc on the growth and reproduction of *Proisotomaminuta Tullberg* (Collembola). *Ecotoxicology and Environmental Safety*,2005:60:306–314.
 33. OECD. Guidelines for the testing of chemicals. Test no. 232, Collembolan reproduction test in soil. Organisation for Economic Cooperation and Development, Paris, 2009.
 34. OECD. Comparative Ecotoxicity of Chlorantraniliprole to Non-target Soil Invertebrates, Test No. 232: Collembolan Reproduction Test in Soil,2016:159:473–479.
 35. Panda RB, Biswal T. Impact of Fly Ash on Soil Properties and Productivity. *International Journal of Agriculture Environment & Biotechnology*,2009:11(2):275–283.
 36. Piper CS. Soil and plant analysis. Hans Publ., Bombay, 1942, 368.
 37. Rogival D, Scheirs J, De Coen W, Verhagen R, Blust R. Metal blood levels and hematological characteristics in wood mice (*Apodemus sylvaticus* L.) along a metal pollution gradient. *Environ Toxicol Chem*,2006:25:149–157.
 38. Sanyal S, Chakravorty PP, Saha R. Evaluation of changes in reproductive and biochemical parameters in indigenous earthworm *Perionyx excavatus* (Perrier, 1872) against impact of selected pesticides. *International Journal of Entomology Research*,2024:9(6):47-52.
 39. Sahana A, Agarwal S, Bhattacharya S, Joy VC. Short-term oxidative stress responses in *Cyphoderus javanus* Börner (Collembola), as biomarkers of heavy metal pollution in lateritic soil. *Pollution Research*,2014:33:201-206.
 40. Sahana A, Joy VC. Temporal changes of Collembola population and alterations of life history parameters and acetylcholinesterase and superoxide dismutase activities in *Cyphoderus javanus* (Collembola) as biomarkers of fly ash pollution in lateritic soil. *Toxicological and Environmental Chemistry*,2014:95:1359-1368.

41. Santos MJG, Ferreira NG, Soares AMV, Loureiro S. The use of biomarkers to evaluate the toxicity of metaldehyde and methiocarb baits to the terrestrial isopod *Porcellionides pruinosus* Brandt, 1833. *Interdisciplinary Studies on Environmental Chemistry Biological Responses to Contaminants*, 2010, 139–147.
42. Sedlak J, Lindsay RH. Estimation of total, protein bound, and non-protein sulfhydryl groups in tissue with Ellman's reagent. *Analytical Biochemistry*, 1968;25:192 - 205.
43. Sørensen TS, Holmstrup M. A comparative analysis of the toxicity of eight common soil contaminants and their effects on drought tolerance in the collembolan *Folsomia candida*. *Ecotoxicology and Environmental Safety*, 2005;60:132–139.
44. Syrek D, Weiner WM, Wojtylak M, Olszowska G, Kwapis Z. Species abundance distribution of collembolan communities in forest soils polluted with heavy metals. *Applied Soil Ecology*, 2006;31:239–250.
45. Tang J, Zhang J, Ren L, Zhou Y, Gao J, Luo L, *et al.* Diagnosis of soil contamination using microbiological indices: A review on heavy metal pollution. *Journal of Environmental Management*, 2019;242:121–130. doi: <https://doi.org/10.1016/j.jenvman.2019.04.061>.
46. Viarengo A, Ponzano E, Dondero F, Fabbri R. A simple spectrophotometric method for metallothionein evaluation in marine organisms: an application to Mediterranean and Antarctic molluscs. *Marine Environmental Research* [online], 1997;44(1):69–84.
47. VijiR, Rajesh PP. Assessment of water holding capacity of major soil series of *Lalgudi, Trichi*. *Indian Journal of Environmental Research and Development*, 2012;7(1A):393-398.
48. Walkey A, Black I. An examination of Degtjareff method for determining organic carbon in soils: effect of variations in digestion conditions and of inorganic soil constituents. *Soil Science*, 1934;63:251-263.
49. Wang X, Zhu X, Peng Q, Wang Y, Ge J, Yang G, *et al.* Multi-level ecotoxicological effects of imidacloprid on earthworm (*Eisenia fetida*). *Chemosphere*, 2019;219:923–932.
50. Wilczek G, Babczyńska A, Augustyniak M, Migula P. Relations between metals (Zn, Pb, Cd and Cu) and glutathione-dependent detoxifying enzymes in spiders from a heavy metal pollution gradient. *Environmental Pollution*, 2004;30(90):1–10:453–461.
51. Wiles JA, Frampton GK. A field bioassay approach to assess the toxicity of insecticide residue on soil to Collembola. *Journal of Pesticide Science*, 1996;47:273-285.
52. Wong HL, Sakamoto T, Kawasaki T, Umemura K, Shimamoto K. Down-Regulation of Metallothionein, a Reactive Oxygen Scavenger, by the Small GTPase OsRac1 in Rice. *Plant Physiology*, 2004;135(3):1447–1456.
53. Xiao XY, Wang MW, Zhu HW, Guo ZH, Han XQ, Zeng P. Response of soil microbial activities and microbial community structure to vanadium stress. *Ecotoxicology and Environmental Safety*, 2017;142:200–206
54. Xu J, Ke X, Krogh PH, Wang Y, Luo YM, Song J. Evaluation of growth and reproduction as indicators of soil metal toxicity to the Collembolan, *Sinellacurviseta*. *Insect Science*, 2009;16:57–63.
55. Yang YG, Patersoln E, Campbell C. Accumulation of heavy metals in urban soils and impacts on microorganisms. *Environ Sci (in Chinese)*, 2001;22:44–48.
56. Yang JS, Yang FL, Yang Y, Xing GL, Deng CP, Shen YT, *et al.* A proposal of “core enzyme” bioindicator in long-term Pb-Zn ore pollution areas based on topsoil property analysis. *Environmental Pollution*, 2016;213:760–769.