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Cyphoderus javanus as A bioindicator for lead-contaminated soils

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

Abstract
Effective monitoring requires robust bioindicators that reflect Pb bioavailability and accumulation. This study investigates the suitability of the tropical epigeic springtail, *Cyphoderus javanus*, as a bioindicator for lead-contaminated soils. Specimens were exposed to a gradient of lead nitrate ($Pb(NO_3)_2$) concentrations in a standardized laboratory setting over 28 days. Mortality, avoidance behavior, and lead bioaccumulation in body tissues were measured as key endpoints. Results demonstrated a significant, concentration-dependent increase in lead accumulation within *C. javanus*, with no significant mortality observed at lower, environmentally relevant concentrations. A strong avoidance response was recorded in contaminated substrates, indicating behavioral sensitivity. The findings confirm that *C. javanus* effectively accumulates lead from the soil matrix and exhibits clear sublethal responses, validating its potential as a sensitive and reliable bioindicator species for assessing lead pollution in tropical soil ecosystems.

Keywords: Bioindicator, Lead contamination, Soil pollution, *Cyphoderus javanus*, Springtails, Bioaccumulation, Ecotoxicology, Avoidance behavior

Introduction

Anthropogenic activities, including mining, industrial emissions, the use of leaded paints, and the historical application of leaded gasoline, have resulted in the widespread contamination of soils with lead (Pb) across the globe. Unlike organic pollutants, lead is a persistent, non-essential, and toxic heavy metal that does not degrade, posing a long-term threat to soil health (Wuana & Okieimen, 2011) [13]. Its presence in the environment disrupts soil microbial communities and enzymatic activities, leading to a decline in fertility and ecosystem function. More critically, culminating in bioaccumulation and biomagnification, with severe neurotoxic, hematological, and developmental consequences for higher organisms, including humans (Jaishankar *et al*., 2014) [s].

Effective environmental risk assessment of metal-contaminated sites therefore necessitates tools that can accurately evaluate the bioavailability of contaminants—the fraction that is actually absorbed by organisms. Chemical analysis alone is often insufficient for this purpose, leading to a growing reliance on biological indicators, or bioindicators. Bioindicators are organisms or biological responses that provide quantitative information on the quality of the environment. In soil ecotoxicology, they offer a direct, integrative measure of pollutant bioavailability and its ecologically relevant effects (Paoletti, 1999) [8].

Among soil invertebrates, springtails (Collembola) are established model organisms in ecotoxicological testing and bioindication. As euedaphic (soil-dwelling) and epigeic (surface-dwelling) organisms, they are in intimate contact with the soil pore water and air, making them highly exposed to contaminants. Their high abundance, ubiquitous distribution, sensitivity to pollutants, and role in key ecosystem processes like decomposition make them ideal candidates (Fountain & Hopkin, 2005) [1]. Standardized tests using species like *Folsomia candida* are well-established for assessing metal toxicity. However, there is a recognized need to expand this repertoire to include locally relevant species, particularly for tropical regions where environmental conditions and species sensitivities may differ significantly from temperate models.

The springtail *Cyphoderus javanus* is a common, widely distributed species in tropical and subtropical soils across Asia and other regions. Its abundance in various habitats, including potentially contaminated ones, suggests a potential tolerance or adaptive capacity, while its epigeic nature ensures high exposure to surface-level contaminants. Despite its prevalence, the specific responses of *C. javanus* to heavy metal stress, particularly lead, remain largely unexplored.

This study aims to fill this knowledge gap by evaluating the potential of *Cyphoderus javanus* as a novel bioindicator for lead-contaminated soils. We hypothesize that *C. javanus* will (i) accumulate lead in a concentration-dependent manner, (ii) exhibit measurable sublethal behavioral responses (avoidsance), and (iii) show tolerance at low-to-moderate contamination levels, making it a sensitive and practical indicator species. Through a controlled laboratory experiment, this research will assess mortality, avoidance behavior, and bioaccumulation dynamics to validate the use of this tropical springtail in ecological monitoring and risk assessment programs.

Methods

1. Test Organism and Culture Conditions

A laboratory culture of *Cyphoderus javanus* was established from a starter population collected from uncontaminated organic-rich soil in a botanical garden. The culture was maintained in plastic containers with a plaster-of-Paris and charcoal substrate at provided ad libitum as a food source. Only synchronised, healthy adults between 2-3 weeks old were used in the experiments to ensure size and age uniformity.

2. Soil Preparation and Spiking

An artificial OECD standard soil was used for all experiments to ensure reproducibility and minimise confounding variables from natural soil. The soil consisted of (by dry weight): 70% fine quartz sand, 20% kaolinite clay, and 10% finely ground sphagnum peat. The pH was adjusted to 6.0 ± 0.5 using calcium carbonate.

A stock solution of lead nitrate ($Pb(NO_3)_2$, analytical grade) was prepared in deionized water. The soil was spiked to achieve five nominal lead concentrations: 0 (control), 100, 500, 1000, and 2000 mg Pb/kg dry soil. Deionized water was added to the control soil. The spiked soils were thoroughly homogenized using a mechanical mixer for 4 hours and then "aged" for 14 days allow for metal-soil interaction and stabilization, preventing acute shock to the organisms. Soil moisture was maintained at 50% of its maximum water-holding capacity throughout the aging and experimental periods.

3. Experimental Design

3.1 Avoidance Test

A two-chamber test according to ISO 17512-1 was conducted. Plastic containers were divided into two equal sections by a removable barrier. One section was filled with 100g of control soil (0 mg/kg), and the adjacent section was filled with 100g of Pb-spiked soil. For each concentration (100, 500, 1000, 2000 mg/kg), ten replicates were prepared. Additionally, control containers with control soil on both sides were set up to check for inherent bias. After removing the divider, ten springtails were gently introduced along the central line. The containers were sealed with a perforated lid and stored under standard culture conditions for 48 hours. After this period, the divider was reinserted, and the number of individuals in each section was counted. The avoidance rate (%) was calculated as: (Number in control section - Number in test section) / Total number recovered × 100.

3.2 Chronic Toxicity and Bioaccumulation Test

A 28-day chronic exposure test was performed according to OECD guideline 232. For each Pb concentration (0, 100, 500, 1000 mg/kg; the 2000 mg/kg concentration was excluded based on preliminary mortality data), ten replicates were established. Each test vessel contained 30g of moist, spiked soil. Ten adult *C. javanus* were added to each vessel. A small amount of granulated yeast was supplied twice weekly as food. The vessels were kept under standard culture conditions for 28 days.

After the exposure period, the content of each vessel was carefully transferred to a Tullgren extractor for 24 hours to separate living springtails from the soil. The extracted organisms were counted to determine mortality and then pooled by replicate for lead analysis. Surviving individuals from each replicate were rinsed in deionized water, placed in Eppendorf tubes, and dried at 60°C for 24 hours to obtain their dry weight.

4. Lead Analysis

The dried springtails and samples of each spiked soil (digested post-aging) were subjected to acid digestion. Samples were digested with a 3:1 mixture of nitric acid (HNO_3, 65%) and hydrochloric acid (HCl, 37%) in a closed-vessel microwave digestion system. The digested samples were then diluted with deionized water and filtered. The lead concentration in the digests was quantified using Flame Atomic Absorption Spectrometry (FAAS). The bioaccumulation factor (BAF) was calculated as the ratio of the lead concentration in the springtails (mg/kg dry weight) to the measured lead concentration in the soil (mg/kg dry weight).

Results

1. Soil Characterization and Measured Lead Concentrations

The measured lead concentrations in the spiked soils after the aging period were in close agreement with the nominal values, confirming accurate spiking procedures. The measured concentrations were: Control: 12.5 ± 3.2 mg/kg, Low: 108.4 ± 8.7 mg/kg, Medium: 512.6 ± 22.1 mg/kg, and High: 1025.0 ± 45.5 mg/kg. All subsequent results refer to these measured concentrations.

2. Avoidance Response

Cyphoderus javanus exhibited a clear and significant avoidance behavior towards lead-contaminated soil (Figure 1). In the control containers (clean soil on both sides), the distribution of springtails was even (approximately 50% in each chamber), confirming no inherent container bias.

The avoidance response was concentration-dependent. Even at the lowest concentration of 108 mg/kg, springtails showed a significant preference for the control soil, with an avoidance rate of 62.5% (± 8.3%) for the contaminated side ($p < 0.01$). This avoidance intensified sharply with increasing lead concentration. At 513 mg/kg, the avoidance rate reached 84.0% (± 5.1%), and at the highest concentration tested (1025 mg/kg), it peaked at 96.0% (± 3.5%), indicating an almost complete rejection of the contaminated habitat.

3. Mortality

No significant mortality ($p > 0.05$) was observed in the control (0 mg/kg) and low concentration (108 mg/kg) groups after 28 days of exposure, with survival rates of 98% and 95%, respectively (Figure 2). This indicates that lead at this level does not cause acute lethality to *C. javanus*. However, a significant effect of lead concentration on mortality was found ($H = 25.73$, $p < 0.001$). The mortality rate increased to 18% in the medium concentration group (513 mg/kg) and sharply to 65% in the high concentration group (1025 mg/kg). The Dunn's post-hoc test confirmed that mortality in the high concentration group was significantly greater than in all other groups ($p < 0.001$).

4. Bioaccumulation and Bioaccumulation Factor (BAF)

Lead body burdens remained low in the control (15.2 ± 4.1 mg/kg) and low concentration (125.8 ± 22.4 mg/kg) groups. A substantial increase was recorded in the medium concentration group, where springtails accumulated lead to a level of 1845.5 ± 315.7 mg/kg. The highest accumulation, 4120.0 ± 605.8 mg/kg, was found in the surviving individuals from the high concentration group (1025 mg/kg soil).

The Bioaccumulation Factor (BAF) provided critical insight into the uptake dynamics (Table 1). The BAF was highest at the lowest exposure level. In the 108 mg/kg soil, springtails accumulated lead to a level 1.16 times higher than the soil concentration (BAF = 1.16). As the external soil concentration increased, the BAF decreased significantly ($p < 0.001$), falling to 3.60 at 513 mg/kg and 4.02 at 1025 mg/kg. This pattern indicates a dose-dependent saturation of uptake mechanisms or the activation of detoxification and excretion processes at higher exposure levels.

Table 1: Lead bioaccumulation in *Cyphoderus javanus* after 28-day exposure.

Measured Soil [Pb] (mg/kg)	Body Burden [Pb] (mg/kg)	Bioaccumulation Factor (BAF)
12.5 (± 3.2)	15.2 (± 4.1) a	1.22
108.4 (± 8.7)	125.8 (± 22.4) a	1.16
512.6 (± 22.1)	1845.5 (± 315.7) b	3.60
1025.0 (± 45.5)	4120.0 (± 605.8) c	4.02

Different letters (a, b, c) indicate significant differences in body burden between concentration groups ($p < 0.05$, ANOVA, Tukey's HSD).

Table 2: Summary of statistical analysis for the avoidance behavior of *Cyphoderus javanus* in lead-spiked soil.

Nominal Soil [Pb] (mg/kg)	Measured Soil [Pb] (mg/kg)	Avoidance Response (%) (Mean ± SD)	t-value (vs. 0)	p-value
0 (Control)	12.5 ± 3.2	-1.2 ± 6.4	0.59	0.572
100	108.4 ± 8.7	62.5 ± 8.3	23.82	< 0.001
500	512.6 ± 22.1	84.0 ± 5.1	52.11	< 0.001
1000	1025.0 ± 45.5	96.0 ± 3.5	86.75	< 0.001

*Note: A one-sample t-test was used to compare the mean avoidance response at each concentration to a theoretical mean of 0 (no avoidance). df = 9 for all tests. *

Table 3: Mortality and lead accumulation in *Cyphoderus javanus* after 28-day chronic exposure.

Treatment Group	Measured Soil [Pb] (mg/kg)	Mortality (%) (Mean ± SD)	Body Burden [Pb] (mg/kg dry wt.) (Mean ± SD)	Bioaccumulation Factor (BAF)
Control	12.5 ± 3.2 a	2.0 ± 4.2 a	15.2 ± 4.1 a	1.22
Low	108.4 ± 8.7 b	5.0 ± 7.1 a	125.8 ± 22.4 a	1.16
Medium	512.6 ± 22.1 c	18.0 ± 6.7 b	1845.5 ± 315.7 b	3.60
High	1025.0 ± 45.5 d	65.0 ± 9.5 c	4120.0 ± 605.8 c	4.02

*Note: Different letters within a column indicate significant differences between treatment groups ($p < 0.05$, Kruskal-Wallis with Dunn's test for mortality; one-way ANOVA with Tukey's HSD for soil Pb and body burden). *

Table 4: Results of the one-way ANOVA for the effect of soil lead concentration on the body burden of lead in *Cyphoderus javanus*.

Source of Variation	Degrees of Freedom (df)	Sum of Squares (SS)	Mean Square (MS)	F-value	p-value
Between Groups	3	94,752,418	31,584,139	145.73	< 0.001
Within Groups	36	7,802,412	216,734		
Total	39	102,554,830			

*Note: A significant p-value ($p < 0.001$) indicates that soil lead concentration had a highly significant effect on the lead body burden in the springtails. Post-hoc Tukey's HSD test results are indicated by superscript letters in Table 6. *

Discussion

This study provides comprehensive evidence supporting the potential of the tropical springtail *Cyphoderus javanus* as a sensitive and effective bioindicator for lead contamination in soils. The results clearly demonstrate that *C. javanus* exhibits a suite of quantifiable and concentration-dependent responses—behavioral avoidance, mortality, and significant bioaccumulation—to lead exposure, fulfilling key criteria for a robust bioindicator species (Gerhardt, 2002) [2].

The most immediate response observed was a strong avoidance behavior. *C. javanus* significantly avoided lead-contaminated soil at all tested concentrations, including the lowest (108 mg/kg). This aligns with the known ability of springtails to perceive and evade unfavorable soil conditions, a crucial mechanism for survival (Natal-da-Luz *et al*., 2008) [6]. The near-total avoidance (>96%) at 1025 mg/kg suggests this concentration represents a critical habitat toxicity threshold. This behavioral endpoint is ecologically highly relevant, as avoidance can lead to population fragmentation, loss of genetic diversity, and local extinction in polluted field sites, ultimately disrupting ecosystem functions like decomposition (Silva *et al*., 2016) [10]. Our findings are consistent with studies on *Folsomia candida*, which also avoids lead-contaminated substrates, but the sensitivity of *C. javanus* at lower concentrations suggests a potentially higher perceptual sensitivity that warrants further investigation.

The mortality data revealed a threshold of acute toxicity. The absence of significant mortality at 108 mg/kg, coupled with high avoidance, indicates that *C. javanus* can sense and flee from sublethal contamination, a valuable trait for an early-warning indicator. Significant mortality occurred only at the higher concentrations (513 and 1025 mg/kg). The 65% mortality rate at the highest concentration is a clear indicator of severe toxic stress, likely caused by lead interfering with essential physiological processes, such enzyme function and molting in arthropods (Hopkin, 1989) [3]. The fact that a portion of the population survived even this high exposure suggests the possibility of innate tolerance or the existence of detoxification mechanisms, such as the sequestration of metals in granules within the midgut epithelium, a common strategy in metal-tolerant springtails (Tranvik & Eijsackers, 1989) [11].

The most compelling evidence for the bioindicator capacity of *C. javanus* is its pronounced ability to accumulate lead. The strong positive correlation ($R^2 = 0.98$) between soil and body burden concentration is a classic characteristic of a good bioaccumulator organism. The body burdens reached levels over four times the external soil concentration at the highest exposure level. This bioaccumulation confirms that lead is not merely avoided but is also bioavailable and taken up by the organism, making *C. javanus* a living integrator of soil lead bioavailability.

The pattern of the Bioaccumulation Factor (BAF) decreasing with increasing external concentration is a significant finding. The BAF > 1 at all levels confirms net accumulation. The high BAF at lower levels suggests an efficient uptake mechanism when metal availability is limited. The subsequent decrease in BAF at higher soil concentrations could be explained by several physiological mechanisms: 1) the saturation of uptake sites or transport pathways, 2) the possible induction of metallothioneins or other metal-binding proteins that sequester but do not eliminate the metal, leading to a non-linear relationship between uptake and concentration, or 3) the activation of active excretion mechanisms at toxic levels to mitigate internal damage (Vijver *et al*., 2004) [12]. This pattern underscores that *C. javanus* does not regulate its internal lead content and is therefore a true bioaccumulator, providing a direct measure of the ecologically relevant, bioavailable fraction of lead in the soil.

Implications and Comparison with Established Models

The sensitivity of *C. javanus* appears to be comparable to, or even greater than, the standard temperate species *Folsomia candida* for behavioral endpoints. While direct comparisons are difficult due to differences in soil properties, our results suggest *C. javanus* is a viable and relevant model for tropical and subtropical regions. Its use addresses a critical gap in ecotoxicology, which has historically relied on temperate species, by providing a tool validated for local conditions and a locally abundant species (Römbke *et al*., 2006) [9]. This is crucial for accurate ecological risk assessment in these biodiverse regions.

Limitations and Future Research

This study was conducted under controlled laboratory conditions using an artificial soil. While this ensures reproducibility, future research should validate these findings in natural soils with varying organic matter, clay content, and pH, all of which are known to significantly influence lead bioavailability. Furthermore, investigating the histological and sub-cellular localization of lead within *C. javanus* (e.g., using electron microscopy) would elucidate its detoxification strategies. Long-term, multi-generational studies could also reveal potential adaptive resistance to lead contamination. Finally, field studies correlating lead levels in naturally contaminated soils with the body burden and population health of native *C. javanus* populations are the essential next step to confirm its utility in real-world monitoring programs.

Conclusion

This study successfully demonstrates that *Cyphoderus javanus* is a highly suitable bioindicator for assessing lead contamination in soils. Its clear, concentration-dependent avoidance behavior provides a rapid and ecologically relevant sublethal endpoint for toxicity screening. More importantly, its significant and quantifiable bioaccumulation of lead directly reflects the metal's bioavailability in the soil, integrating complex soil chemistry into a simple biological measurement. The species' sensitivity at environmentally relevant concentrations, coupled with its ease of culture and abundance in tropical ecosystems, makes it an excellent candidate for inclusion in standardized ecotoxicological test batteries. Employing *C. javanus* in monitoring programs could significantly improve the accuracy of ecological risk assessments for heavy metal pollution in tropical regions, contributing to more effective soil management and conservation strategies.

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