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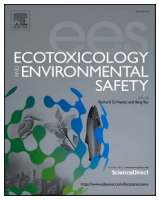

The joint toxicity effect of glyphosate and cadmium in a concentration-dependent manner on nematode *Caenorhabditis elegans*

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ABSTRACT

The co-occurrence of glyphosate (GPS), a commonly used organophosphorus herbicide, and cadmium (Cd), a neurotoxic metal, in agricultural environments prompts concerns about their combined toxic effects on ecosystems. This study explores the combined effects of GPS and Cd on the model organism *Caenorhabditis elegans* (*C. elegans*), to understand their cumulative effects in organismal living environments. We investigated the interaction between GPS and Cd over 24 hours using a comprehensive approach that included a variety of toxicity endpoints as well as the novel Automated Recognition and Statistics Tool (NCLE) for body bend measurement. Our data show a concentration-dependent interplay in which antagonistic effects at lower concentrations reduce phenotypic damage while synergistic effects emerge at higher concentrations, particularly at GPS's LC50. Transcriptome analysis under antagonistic conditions revealed significant downregulation of Cd toxicity-related genes and identified *Y22D7AL.16*, which has a C2H2-type zinc finger domain, as a novel gene involved in metal stress response, implying an alternative Cd-resilience mechanism. The expression profile of this gene shows that it plays a larger role in both development and metal stress adaption. These findings highlight the complexities of compound pollutant interactions, emphasizing the importance of including such dynamics in environmental risk assessments and control techniques.

1. Introduction

Many environmental contaminants, including herbicides and heavy metals, are present in mixtures, contributing significantly to pollution and endangering plant growth and human health (Li et al., 2022). Cadmium (Cd), one of these pollutants, is a highly hazardous heavy metal (Wang et al., 2021a). Cadmium concentrations of higher than 0.3 mg/kg in soil greatly inhibited plant growth and development (Wang et al., 2019). The ability of cadmium to bioaccumulate in organs and tissues of organisms can result in a variety of negative health impacts, including oxidative stress, DNA damage, enzyme inhibition, and altered calcium metabolism. Long-term exposure to high Cd levels has been linked to severe health issues, including kidney damage, hypertension, and an increased risk of cancer (Branca et al., 2020; Chen et al., 2022). Furthermore, Cd toxicity is established for its adverse effects on the reproductive and developmental processes of numerous species (Thompson and Bannigan, 2008). Despite this, Cd usage is increasing due to industrial development. In response to Cd exposure, organisms upregulate detoxification genes such as metallothioneins (mtl-1, mtl-2) and cadmium-responsive genes (cdr-1) to mitigate toxicity.

Glyphosate (GPS), a widely used herbicide, inhibits protein synthesis and growth in plants by targeting the enzyme EPSPS in plants, disrupting protein synthesis and impairing growth (Moser et al., 2022; Milesi et al., 2021; Duke and Powles, 2008 Apr). Its persistence in the environment raises concerns about soil and water contamination (Bai

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and Ogbourne, 2016). The combined presence of Cd and GPS in ecosystems is plausible, although their joint effects are poorly understood. This includes potential chemical interactions that could result in additive, synergistic, or antagonistic effects (Ramakrishnan et al., 2011; Kováčik et al., 2020).

Caenorhabditis elegans, a free-living soil nematode, serves as an excellent model for toxicological studies due to its short lifecycle, transparent body, robust reproductive system, and visible phenotypes indicating toxic exposure (Long et al., 2023; Tejeda-Benitez and Olivero-Verbel, 2016). Its genetic pathways and functional homologies to humans make it an ideal model for studying molecular stress responses and diseases (Markaki and Tavernarakis, 2020; Grishok, 2005; Braendle et al., 2008). The behavior phenotypes of *C. elegans*, such as body bending (Fang-Yen et al., 2009; Gems and Riddle, 2000; Luo et al., 2008), head thrashing (Zhang et al., 2022a), and pharyngeal pumping (Zhang et al., 2022b), are closely linked to environmental toxicity and can be quantified through advanced techniques for comprehensive toxicity screening. Our use of the Nematode Central Line Extraction (NCLE) technique for analyzing nematode movement patterns significantly enhances data accuracy and collecting efficiency. NCLE stands as a testament to the ingenuity applied in harnessing *C. elegans* for toxicological advancements, affirming its status as an indispensable tool in environmental and health-related research.

We propose that the combined exposure to cadmium and glyphosate has additive or synergistic toxic effects on *C. elegans*, impacting both acute lethality and sublethal endpoints. Our goals are to assess the individual and combined toxic effects of Cd and GPS on *C. elegans* survival and behavior, analyze changes in gene expression related to detoxification pathways, and investigate the molecular mechanisms underlying the observed toxic effects. This study aims to bridge the knowledge gap on the combined effects of Cd and glyphosate, providing insights into their potential risks to environmental and human health.

2. Materials and methods

2.1. Chemicals and reagents

Cadmium powder was purchased from Sigma-Aldrich (CAS number: 7440–43–9). The herbicide used was water-based glyphosate isopropyl amine salt with a 30 % active component content purchased from China Fuhua Tongda Pesticide Technology Co., Ltd (Product no: 13192). The experimental setup used K-Medium (3.04 g NaCl, 2.39 g KCl, and 1 L ddH_2O) as the dissolving medium for cadmium to form treatment solutions. To maintain consistency and reproducibility of experimental results, all other chemicals and reagents used were of analytical grade and utilized as received, with no further purification.

2.2. Maintenance and experimental setup for Caenorhabditis elegans

Wild-type *C. elegans* strain N2 was obtained from the Caenorhabditis Genetics Center (University of Minnesota) and was used for all analyses. Nematodes were grown on NGM (nematode growth medium) agar plates (3 g NaCl, 2.5 g BactoPeptone, 20 g Agar and 1 L ddH_2O) seeded with *Escherichia coli* strain OP50 at 20°C unless otherwise noted. The nematodes were synchronized using the conventional bleach procedure, which included distilled water, 5 M NaOH, and 10 % sodium hypochlorite (NaClO).

The experimental design includes four distinct groups: a control group that received no pollutants, a cadmium-only group that was exposed to varying concentrations of cadmium ions, a glyphosate-only group that was exposed to varying concentrations of glyphosate, and a mixed group that was exposed to varying concentrations of both cadmium ions and glyphosate. Each experimental condition was repeated three times to ensure robustness.

2.3. Acute toxicity

The acute lethal toxicity of the two tested chemicals, Cd and GPS, individually and in mixtures (Cd + GPS), was assessed using a 24-hour lethality assay. The chemical stock solutions were formulated with a Cd concentration of 20 mg/ml and a GPS concentration of 300 mg/ml before the toxicity test. Experiments were conducted in 96-well plates with L4-stage age-synchronized worms. LC50 values were established using five different working concentrations of Cd and GPS plus a control. Cadmium and glyphosate concentrations were tested in four duplicate wells with 300 μl of test solution. Nematodes were subsequently transferred to a 96-well plate containing K-medium, ensuring an average density of 10–30 nematodes per well. Exposure was conducted at 20 °C for 24 hours without food. Worm mortality was examined under a dissecting microscope. Detailed working concentrations are provided in Supplementary Material Table S1.

For the mixture test, the exposure procedure was similar to the tests on individual chemicals, except for the concentrations used. Cadmium and glyphosate were co-administered in specific ratios to determine their collective LC50 values and interaction types, using concentrations of 1/5 LC50, 1/2 LC50, and LC50 according to the method described earlier (Wang et al., 2017).

2.4. Sublethal exposure test

Sublethal exposure tests were conducted at concentrations of 1/1000, 1/100, and 1/10 LC50 for cadmium, glyphosate, and their mixtures using the method described earlier (Wang et al., 2017). After a 24-hour incubation period at 20°C, body bending, head thrashing, pharyngeal pumping, and reproductive rate were measured. Head thrash counts were performed on 15 randomly selected nematodes on NGM plates containing a few drops of M9 buffer, with each back-and-forth movement counted as one thrash. Body bends were identified as one complete sinusoidal movement. Pharyngeal pumping rates were measured on NGM plates seeded with OP50 *Escherichia coli*, with each pump counted. The reproduction rate was determined by counting the offspring of three randomly selected worms every 24 hours after exposure.

2.5. Sequencing information

Total RNA was extracted from both the 0-hour treated control and 24-hour treated samples, which were subsequently processed for validation before sequencing. To ensure reproducibility, the experiment was repeated three times in triplicates, and only results that were consistent across all three replicates were selected for further analysis. The original RNA-seq readings were processed using Trimmomatic (version 0.39) to filter out low-quality reads and trim adapter sequences (Bolger et al., 2014 Aug 1). The cleaned reads were then aligned with the WBcel235 reference genome using the STAR aligner (version 2.7.5c) (Dobin et al., 2013). RNA sequencing (RNAseq) analysis was performed using the DIANE software platform (Cassan et al., 2021), which runs R version 4.0.0.

The count table generated by DESeq2 was used to perform differential gene expression analysis with DIANE. Low counts were filtered out with a threshold of 15 to ensure statistical robustness in downstream analysis. Differentially expressed genes (DEGs) were identified using a false discovery rate (FDR) of 0.05 and a log2 fold change (logFC) of 1 as cutoffs. The RNAseq data were then further explored to construct gene regulatory networks using the GENIE3 algorithm within the DIANE framework (Huynh-Thu et al., 2010).

2.6. Nematode Central Line Extraction (NCLE)

For the extraction of the central line in nematodes, individual nematodes were imaged in a 384-well plate, with one nematode per well.

The images were annotated using the Labelme library (version 5.4.1) in Python (version 3.7). The collected images were processed using the OpenCV library (version 4.9.0) in Python. This includes median filter denoising, contrast enhancement with histogram equalization, and conversion to 8-bit grayscale images. For model training and optimization, we collected and used images of nematodes in liquid culture media annotated using Labelme as training data, as well as data augmentation techniques. The deep learning model was trained on this data using PyTorch (version 2.2.0), and hyperparameters were optimized using cross-validation and the Hyperopt library.

The trained U-Net model segmented the nematode images to isolate the central line. The segmented line was skeletonized with OpenCV and refined to ensure continuity and eliminate unnecessary branches. The generated centerline was used for subsequent analysis, such as visualizing nematode trajectories.

2.7. Statistical analysis

All assays were performed in triplicate to ensure statistical validity. Data analysis was conducted using GraphPad Prism version 9.0. LC50 values and corresponding 95 % confidence intervals for individual metals were calculated using nonlinear regression in GraphPad Prism. LC50 values for metal mixtures were determined similarly. Data were transformed to a logarithmic scale before calculating LC50 values to achieve normal distribution and homogeneity of variance. The significance of differences between experimental groups was determined using one-way ANOVA to compare means across multiple groups. To discover particular group differences, post-hoc comparisons were performed using the Fisher's Least Significant Difference (LSD) test. A t-test was used for subsequent comparisons of group differences on body bending, head swing, and pharyngeal pumping. Comparisons of toxicity effects on worm reproduction (number of progenies produced by each worm) among individual metals or metal mixtures were conducted using one-way ANOVA followed by post hoc multiple comparisons. A p-value <0.05 was considered statistically significant for all tests, indicating that observed differences were unlikely due to chance.

3. Results

3.1. Combinational toxicity effects of cadmium and glyphosate

This study systematically assessed the acute toxicity of cadmium (Cd) and glyphosate (GPS) on *C. elegans* using a meticulously conducted 24-hour LC50 assay. The assay was performed on nematodes of the L4 developmental stage, utilizing a 96-well plate setup. Specifically, five concentrations of cadmium and five concentrations of glyphosate were tested, with each concentration having a control. Each concentration was tested in quadruplicate, with 150 μL of worm suspension (containing approximately 10–30 worms) and 150 μL of K-M solution added to each well. The worms were exposed for 24 hours at 20°C without feeding, and their mortality was assessed under a microscope to determine the LC50 values for Cd and GPS independently. The 24-hour LC50 values obtained were 71.54 mM for glyphosate (95 % confidence interval [CI]: 70.79, 72.31) and 11.7 mM for cadmium (95 % CI: 9.28, 14.53) (Fig. 1A, B, and Table S1).

To assess the combined toxicity of Cd and GPS, we employed the method described by Wang et al (Wang et al., 2017). This approach involves comparing the observed toxicity in experimental conditions with the toxicity predicted by additive models. For example, when combining 1/5 of the LC50 of Cd (corresponding to 10 % mortality) with 1/2 of the LC50 of GPS (resulting in 25 % mortality), the predicted combined mortality rate is 35 % (10 %+25 %). Contrary to this prediction, at the LC50 concentration of Cd, increasing concentrations of GPS led to lower observed toxicity than expected, suggesting an antagonistic interaction. A similar antagonistic effect was observed when combining Cd at 1/2 and 1/5 of its LC50 with GPS at 1/5 and 1/2 of its LC50 respectively. However, when GPS reached its LC50 concentration, all tested combinations exhibited synergistic effects, indicating

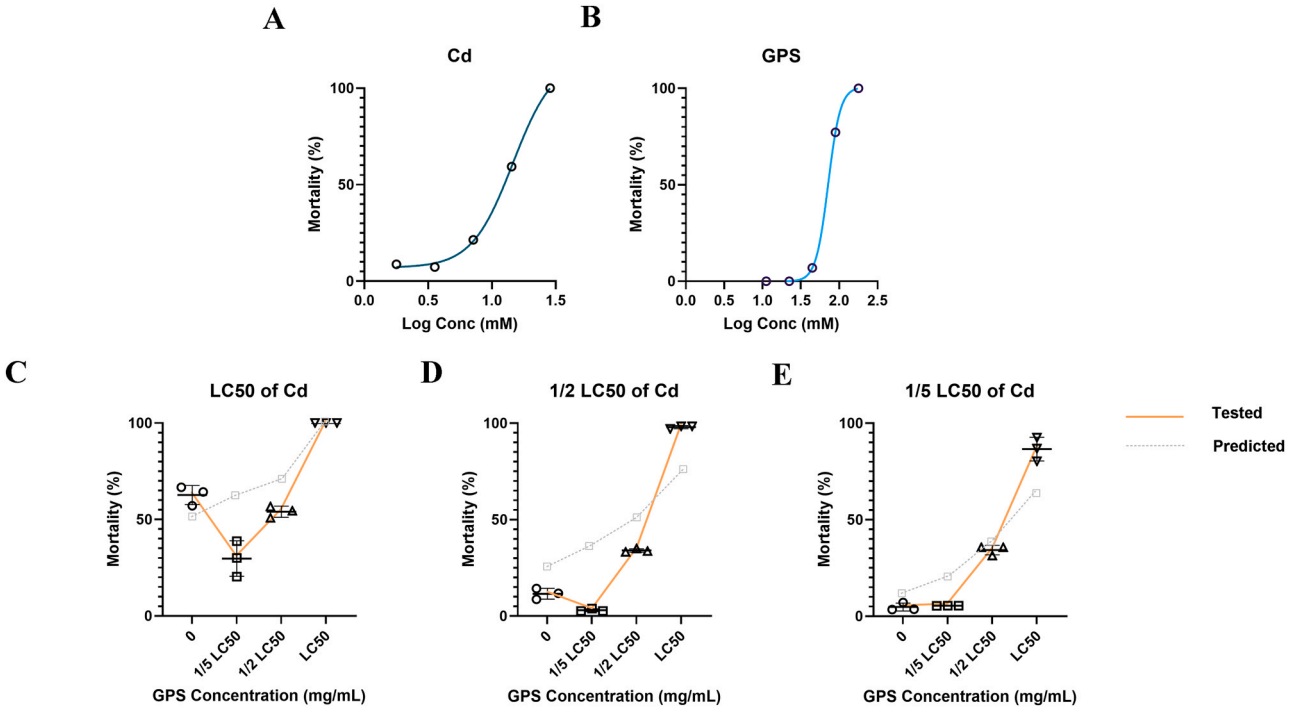

Fig. 1. Concentration-response relationships for the acute and combined toxic effects of glyphosate and cadmium on C. elegans at LC50, 1/2 LC50, and 1/5 LC50. Panels A and B show the 24-hour LC50 values for glyphosate and cadmium in the L4 stage, along with their 95 % confidence intervals (calculated using logistic regression or probit analysis). Panels C-E show the combined treatment of LC50 GPS of 1/5, 1/2 and 1 at Cd concentrations of 1, 1/2 and 1/5 LC50, respectively. Each dilution was tested times. The calculated data represent theoretical outcomes (Gray dotted line) , which are juxtaposed with actual observations to determine whether the effects are additive, synergistic, or antagonistic.

potentiation of toxicity. (Fig. 1 C-E, and Table S2).

3.2. Impact of Cd and GPS on locomotion, reproduction, and physiological responses in C. elegans

Environmental stressors can severely impact locomotion, reproduction, and overall growth in living organisms. In our study, we investigated how simultaneous exposure to Cd and GPS affects these key physiological responses in *C. elegans*. Specifically, we focused on evaluating locomotion (measured by head thrashing and body bending), pharyngeal pumping, and brood size (Table S3), which are critical endpoints for assessing ecotoxicological effects in *C. elegans*.

We first calculated the LC50 for the Cd and GPS mixture, focusing on determining the GPS concentration at 1/5 LC50 of Cd. Following several experimental iterations, the GPS concentration was determined to be 5.3 mg/ml (95 % confidence interval: 5.2–5.4, Figure S1 and Table S4). Following this, we conducted sublethal phenotypic assessments by exposing nematodes to individual and combined doses of Cd and GPS at 1/1000, 1/100, and 1/10 of the determined 24-hour LC50. Our goal was to meticulously examine the effects of these pollutants at sublethal levels, both individually and in mixture, on the physiology of *C. elegans*.

The integrative analysis depicted in Fig. 2 illustrates the complex relationships between these substances and their biological effects. At a concentration of 1/10 LC50, the interaction between Cd and GPS in the mixture was antagonistic, as indicated by the restoration of body bending activity, a crucial locomotive function, to near-normal levels (Fig. 2A). This finding contrasts with the expected cumulative effects of two toxic substances, suggesting a unique interplay at specific concentrations. Additionally, the impact on head thrashing, another measure of nematode locomotion, exhibited a less pronounced but still detectable antagonistic effect at the same concentration (Fig. 2B). The lack of significant changes at lower concentrations (1/1000 and 1/100 LC50) indicates a threshold below which *C. elegans* is resilient to the toxic effects of these substances, which could have profound ecological and environmental implications. Moreover, pharyngeal pumping rates increased significantly at 1/10 LC50 when treated with the combination (Fig. 2C), possibly indicating an adaptive response to maintain homeostasis or an unexpected consequence of the chemicals' interaction affecting neural or muscular control mechanisms. Furthermore, the reproductive rate as determined by brood size was significantly affected. Worm exposure to individual Cd and GPS toxicity resulted in a considerable reduction in brood size. However, the mixture of these substances at 1/10 LC50 resulted in a marked increase in brood size (Fig. 2D), indicating an antagonistic interaction that mitigates the effects observed with individual substance exposures. Additionally, locomotion patterns, illustrated in Fig. 2E, showed distinctive behavioral tracking for nematodes under each treatment condition.

3.3. Utilization of Nematode Central Line Extraction (NCLE) to reveal antagonistic interaction effects

We used the Nematode Central Line Extraction (NCLE) approach to systematically assess body bending in worms subjected to individual and combined Cd and GPS toxicity. This involved measuring the frequency of body bends over 20-second intervals at various sublethal concentrations, ranging from 1/1000–1/10 of the LC50 for each substance and their combination.

Initial experiments to validate our approach before sequencing

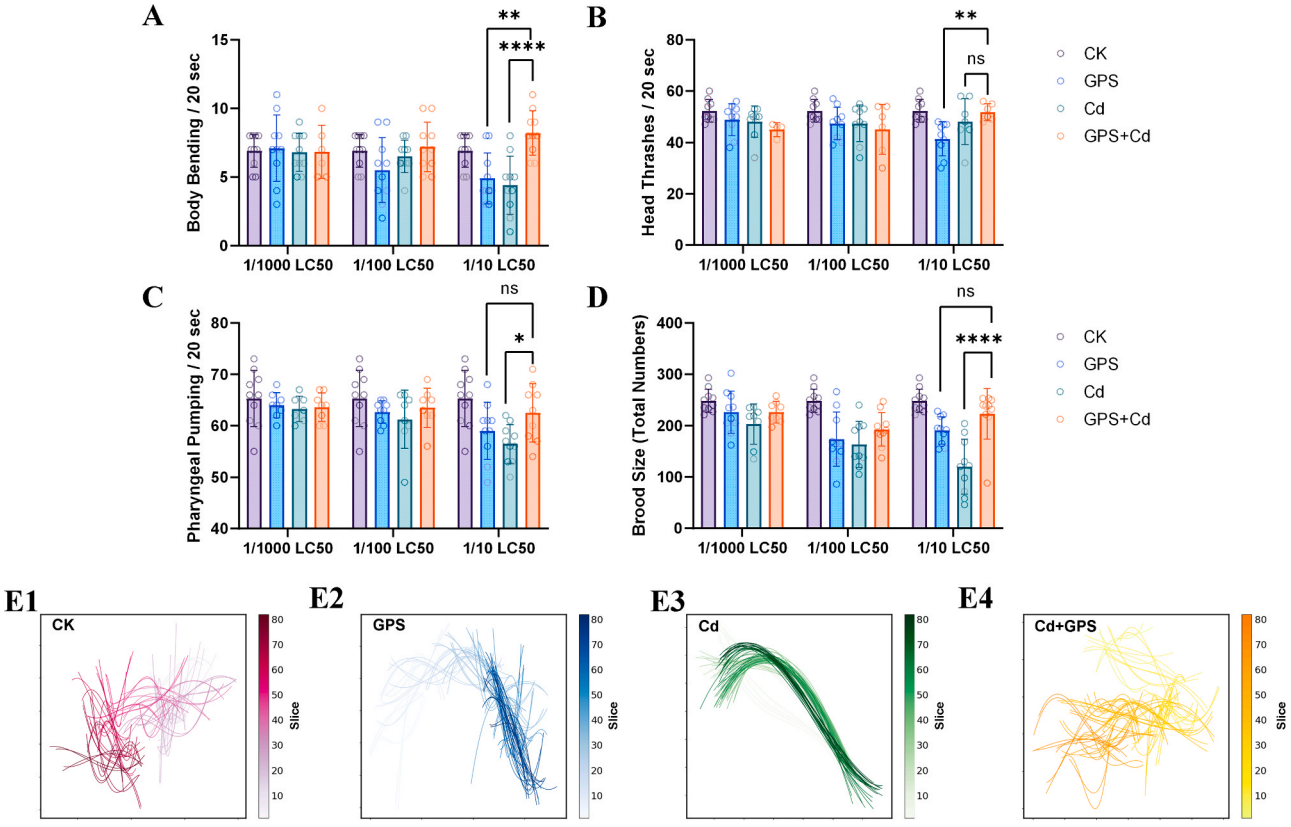

Fig. 2. Impact of Cd, GPS, and Cd+GPS on C. elegans phenotypes under individual or combined exposures. Panels A, B, C, and D show the effects on body bending, head thrashing, pharyngeal pumping, and brood size at concentrations of 1/1000 LC50, 1/100 LC50, and 1/10 LC50, both individually and in combination, respectively. Each test involved 15 nematodes, with results replicated more than three times to ensure reliability. Panel E depicts the behavioral tracking of worms in response to chemical treatments. Individual nematode paths were monitored in a 384-well plate, subjecting each nematode to different conditions: Cd (cadmium), GPS (glyphosate), and a combined treatment of both. Trajectories, delineated by the central line of motion over a 10-second interval, are displayed before and after the application of a smoothing process to clearly illustrate the locomotion patterns.

showed a recovery trend in body bending and head thrash phenotypes at a 1/10 LC50 concentration of the mixture treatment, compared to each substance alone (Figure S2, Table S5). In addition, our studies on pharyngeal pumping showed a significant increase in feeding rate and frequency under mixture treatment, suggesting a potential reduction in toxicity through the combined application of Cd and GPS. Notably, at a 1/10 LC50 concentration of the combined Cd and GPS treatment, there was a statistically significant increase in body bending frequency compared to exposure to either substance alone, indicating an antagonistic effect (Fig. 2A, E1-E4). This antagonistic trend was particularly evident at the 1/10 LC50 concentration for the combined treatment and diminished at lower concentrations, highlighting a concentration-dependent response in nematode motility.

The control group (CK) exhibited erratic and extensive movements (Figure E1), whereas GPS and Cd individually induced more linear and restricted paths (Figure E2 and E3). Interestingly, the combined treatment resulted in a trajectory that, while not identical to the control, indicated a complex modulation of behavior that could not be described simply as the sum of the effects of the individual treatments (Figure E4).

This analysis, facilitated by the innovative NCLE technique, highlights the nuanced and concentration-dependent antagonistic interaction between Cd and GPS on *C. elegans* locomotion, providing valuable insights into their combined toxicological effects.

3.4. Unraveling the transcriptomic responses of C. elegans to combined glyphosate and cadmium exposure

To better understand the molecular mechanisms underlying the responses of *C. elegans* exposed to cadmium and glyphosate, both individually and in combination, we conducted RNA sequencing to identify actively transcribed genes and their expression levels under these conditions. The RNA sequencing was performed on samples exposed to 1/10 LC50 concentrations of Cd, GPS and the Cd+GPS mixture. This concentration was chosen for sequencing due to its significant impact on phenotypic responses and the observed antagonism between the substances when combined, as detailed in Figure S2 and Table S5.

After 24 hours of cadmium exposure, 97 genes showed significant upregulation, while 35 genes were significantly downregulated. In contrast, exposure to GPS resulted in the significant upregulation of 27 genes and the significant downregulation of 7 genes. We also conducted RNA sequencing on untreated worms as a baseline control. This comparison enabled us to identify transcriptional changes induced by Cd and GPS exposure relative to the untreated state. These changes in gene expression were determined using a threshold of $|\log2FC| \geq 1$ and adjusted P-value <0.05 (Fig. 3A).

The differential gene expression in *C. elegans* due to exposure to Cd and GPS, as shown in the Venn diagrams, reveals distinct and overlapping responses. Cd exposure individually upregulated 74 genes, GPS uniquely upregulated 57 genes, and a mixture of Cd and GPS upregulated 37 genes (Fig. 3B). Regarding downregulated genes, 150 genes were specific to Cd, 155 genes to GPS, and 68 genes were downregulated

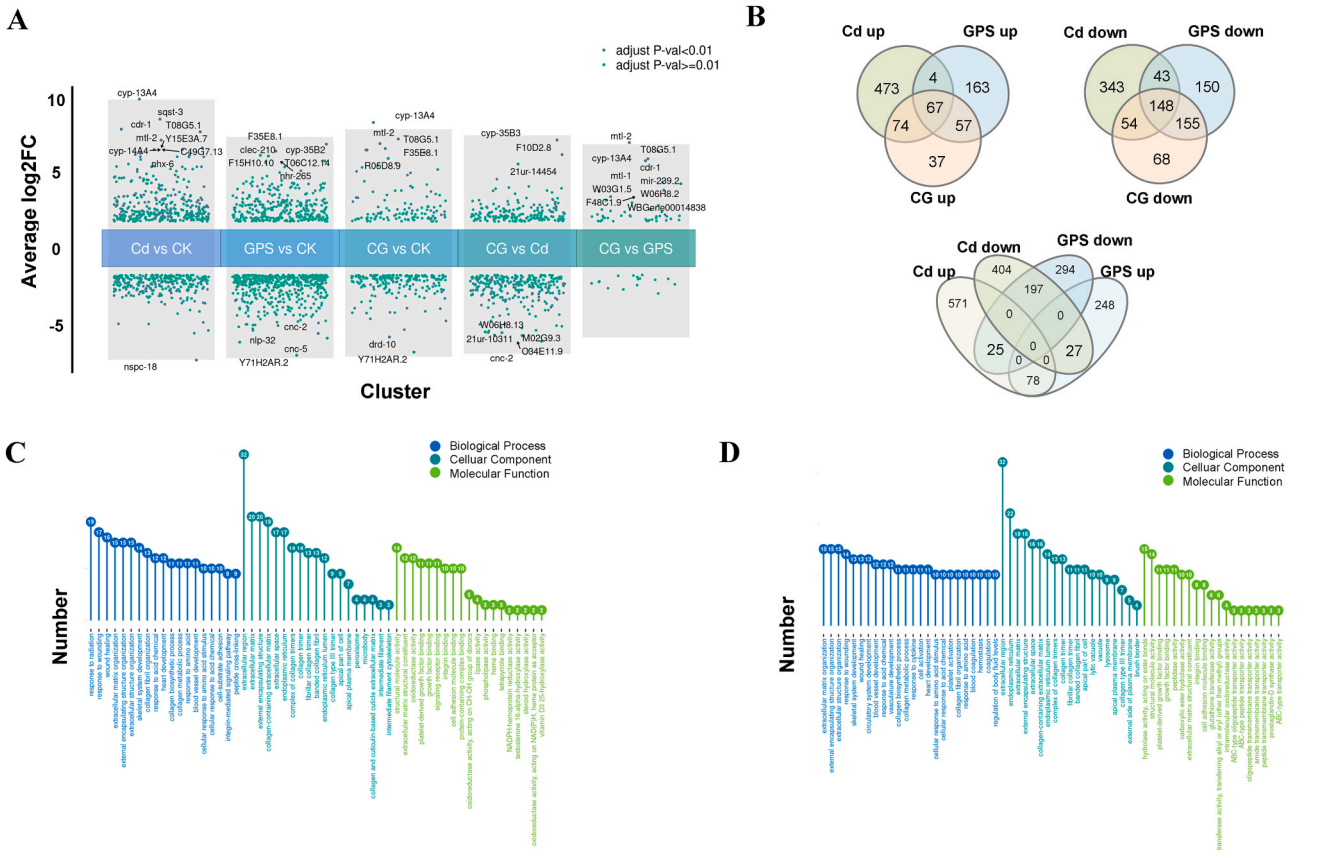

Fig. 3. Gene expression alterations in nematodes after exposure to cadmium (Cd) and glyphosate (GPS) as well as their combined exposure (CG). A. Scatter plots illustrate the average log2 fold change (logFC) in gene expression across different treatment comparisons: Cd versus control (CK), GPS versus CK, CG versus CK, CG versus Cd, and CG versus GPS. Each point represents a gene, with teal points indicating genes with an adjusted P-value ≥ 0.01, and dark blue points denoting genes with a more stringent adjusted P-value of <0.01. Key differentially expressed genes are labeled, with logFC direction reflecting upregulation (positive values) or downregulation (negative values) in comparison to the control. B. Venn diagrams depict the shared and unique differentially expressed genes in nematodes exposed to cadmium (Cd), glyphosate (GPS), and their combination (CG). C. Graphical representation of Gene Ontology (GO) analysis for nematodes treated with Cd2+.D. Bar graph detailing the GO analysis of nematodes after glyphosate (GPS) treatment.

by both Cd and GPS treatments. This indicates that the nematode's gene expression exhibits both unique and overlapping responses to these two stressors.

This upregulation extended to well-characterized families of related genes, encompassing those encoding phase I and phase II detoxification proteins, innate immunity proteins, and ATP-binding cassette (ABC) transporter proteins. However, in the combined exposure to cadmium (Cd2+) and glyphosate (GPS), some genes exhibited inverse changes. Specifically, a subset of responsive genes showed a marked decrease compared to the group exposed to Cd2+ alone. The upregulation extended to well-characterized families of related genes, including those encoding phase I and phase II detoxification proteins, innate immunity proteins, and ATP-binding cassette (ABC) transporter proteins. However, in the combined exposure to cadmium (Cd2+) and glyphosate (GPS), some genes exhibited inverse changes. Specifically, a subset of responsive genes showed a marked decrease compared to the group exposed to Cd2+ alone. Gene Ontology (GO) enrichment analysis revealed that after 24 hours of exposure to Cd or GPS, categories such as cellular transport, fatty acid metabolism, and cell wall metabolism were enriched with downregulated genes. This suggests that cadmium toxicity may compromise various cellular functions. Additionally, GO molecular function analysis indicates that many of these genes are involved in metal ion binding and catalytic activities (Fig. 3C, D).

To identify co-regulated gene groups and gain insights into the potential functional consequences of exposure to Cd and GPS, as well as the intricate dynamics of gene regulation under combined environmental stresses, we classified the differentially expressed genes (DEGs) into nine discrete clusters based on their expression profiles and functional annotations, as illustrated in Fig. 4A. This classification unveiled distinct transcriptional responses among the clusters to the different treatments. Clusters 1 through 9 represent unique patterns of gene expression in response to cadmium (Cd2+), the combination of cadmium and glyphosate (CG), and the control group at 0 hours (CK0). The expression profiles are displayed as box plots, showing the normalized mean counts of genes within each treatment group.

Clusters 1, 2, 3, 6, and 9 include genes that are highly sensitive to cadmium. Interestingly, these genes show a downward expression trend after the introduction of glyphosate, suggesting a potential attenuating effect of glyphosate on cadmium toxicity. This includes genes such as *mtl-1*, *mtl-2*, *cdr-1*, and others involved in encoding cytochrome P450 enzymes, glutathione S-transferases, and UDP-glucuronosyltransferases. Cluster 4 consists of genes specifically downregulated in response to cadmium, indicating an inhibitory effect of this metal on their expression. Cluster 5 highlights genes that are more responsive to glyphosate exposure, with further decreases in expression under combined exposure conditions. Finally, Cluster 7 contains genes associated with the developmental changes in the nematodes, providing insights into the potential impacts of these treatments on nematode growth and development (Fig. 4A, Table S6).

3.5. *Identification of a new gene that may represent a new regulatory network associated with cadmium resistance in C. elegans*

In *C. elegans*, the upregulation of known gene families such as *mtl-1*, *mtl-2*, *cdr-1*, and *ttm-1* which are target genes of the p38 MAPK pathway involved in toxin regulation has been established as a response to cadmium exposure (Sharma et al., 2023; Ma et al., 2022), indicating their functional role in conferring resistance to cadmium toxicity. However, the biological significance of increased transcriptional levels in other cadmium-responsive genes remains to be elucidated. Additionally, as shown in Table 1, cadmium exposure affects the expression of nuclear receptor genes, including *hizr-1*, *nhr-85*, *nhr-181*, and *nhr-172* to *nhr-12,* suggesting these genes play a crucial role in resistance to metal/-cadmium toxicity (Magner and Antebi, 2008). To further explore these transcriptional responses to cadmium and identify potential regulatory mechanisms, we employed GENIE3 (Gene Network Inference with Ensemble of Trees), which uses a machine learning approach to infer gene regulatory networks from expression data, allowing us to identify key regulatory interactions that may govern the enhanced resistance

Table 1
Summary of Regulatory Genes.

Gene	Group	Description
hizr−1	Regulator	High zinc activated nuclear receptor protein
Y22D7AL.16	Regulator	C2H2-type domain-containing protein
nhr−85	Regulator	Nuclear hormone receptor family member nhr−85
mean_nhr−172-nhr−12	Grouped regulators	NA
npax−2	Regulator	Paired domain-containing protein
nhr−181	Regulator	Nuclear Hormone Receptor family
ets−4	Regulator	Transcription factor ets−4
daf−8	Regulator	Smad protein daf−8

Note: Identified regulatory genes, categorized by label and group, accompanied by descriptions. The "Regulator" group represents individual regulatory genes, whereas "Grouped Regulators" refers to a set of related regulatory entities. Descriptions provide brief functional annotations or protein product information, where available. Entries without a specified function are denoted as "NA" (Not Applicable).

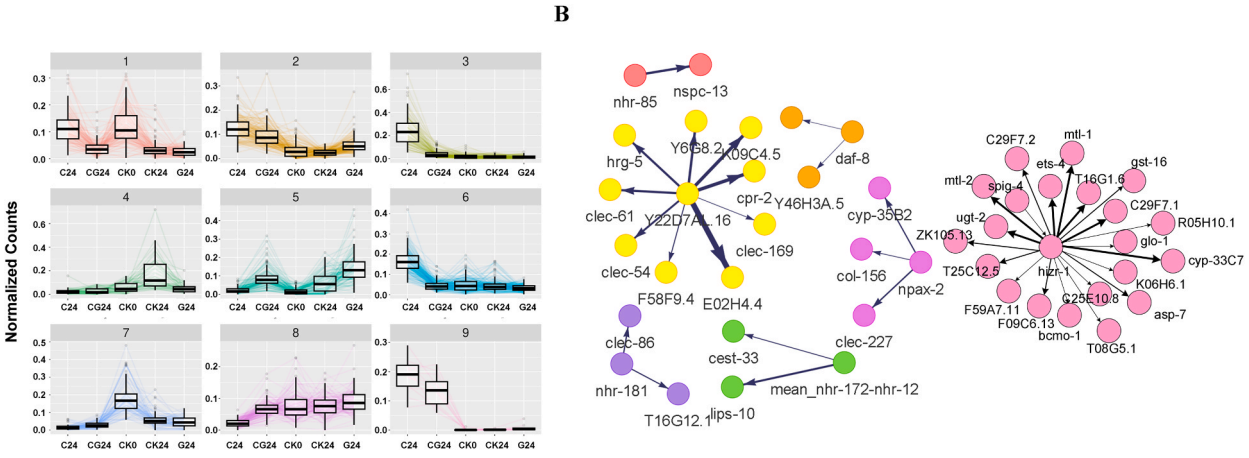

Fig. 4. A. Box plots showing normalized expression levels of genes across treatments: cadmium (Cd2+), combined cadmium and glyphosate (CG), and control (CK24). Gene expressions are connected across treatments within each of the nine clusters. **B.** Network visualization of gene interactions in C. elegans exposed to cadmium, with node sizes indicating regulatory influence and edges showing connections.

mechanisms in *C. elegans* under cadmium stress.

We constructed a gene regulatory network for *C. elegans* following cadmium exposure. As illustrated in Fig. 4B, the network reveals numerous key regulatory genes, including *hizr-1,* known for its role as a high zinc-activated nuclear receptor protein, and members of the nuclear hormone receptor family such as *nhr-85* and *nhr-181*. The nuclear hormone receptor *nhr-172* and *nhr-12* are grouped based on a shared feature, and this group is notable for its possible regulatory role in the gene regulation network constructed for *C. elegans* in response to cadmium exposure.

It has been established that *hizr-1*, a high zinc-activated nuclear receptor transcription factor, plays a pivotal role in mediating responses to zinc homeostasis disruptions (Earley et al., 2021). Previous studies have demonstrated that while *hizr-1* is essential for the activation of certain genes in response to both high zinc and cadmium exposure, other cadmium-activated genes operate independently of *hizr-1 (*Shomer et al., 2019; Warnhoff et al., 2017), suggesting the existence of dual mechanisms for cadmium-responsive transcription.

Consistent with these earlier findings, our data support the idea that cadmium can mimic zinc by activating the high zinc response mechanism through *hizr-1*. We contribute to this body of knowledge by identifying *Y22D7AL.16* (WBGene00021254) as a novel gene potentially involved in the cadmium response network. Our data suggests that *Y22D7AL.16*, a gene containing a C2H2-type zinc finger domain typically associated with transcriptional regulation, is differentially expressed under cadmium stress. The specific upregulation of *Y22D7AL.16* in the presence of cadmium, independent of the *hizr-1* mediated pathway, suggests the possibility of a distinct cadmium-responsive regulatory mechanism. While the exact role of *Y22D7AL.16* remains unclear, we hypothesize that this gene may contribute to an alternate pathway that enables nematodes to cope with cadmium toxicity. Given its expression across various developmental stages, we speculate that *Y22D7AL.16* could be involved in both developmental regulation and metal stress response.

4. Discussion

The pervasive use of pesticides and heavy metals in agriculture has emerged as a pressing issue with profound implications for environmental sustainability and human health (Cai et al., 2023; Cordeiro et al., 2023 Jul; Liu et al., 2022). These pollutants not only contaminate soil and water sources but also infiltrate the food chain, posing serious health risks (Alengebawy et al., 2021; Pei et al., 2022; Wang et al., 2021b). Recent research has elucidated various pollutants' intricate interactions and cumulative effects, even at low concentration (Tchounwou et al., 2012; Zhou et al., 2024). Although existing studies, such as those by Zhou et al. and Ramstedt et al (Zhou et al., 2004; Ramstedt et al., 2005)., have investigated the geochemical interactions of cadmium and glyphosate on soil and mineral surfaces, their potential interactions with other toxic metals, as well as the effects of pollutant mixtures are poorly understood. As a result, further investigation into the toxicological effects and interactions of various environmental pollutants is critical.

Exposure to chemical mixtures can lead to dose-additive, interactive (synergistic or antagonistic), or independent effects (Rai et al., 2010). In this study, we examined the toxic effects and interactions between cadmium and glyphosate using *C. elegans*, a well-established model organism in toxicology research. Our results revealed the individual toxicological effects of cadmium and glyphosate on *C. elegans*, shedding light on their deleterious effects when administered independently. However, it is worth noting that the LC50 values for cadmium and glyphosate obtained in our study differed from those previously reported (Wang et al., 2017; Lu et al., 2018), likely due to variations in treatment methods and experimental conditions. Although Cadmium (Cd) is a systemic toxicant that affects multiple cellular processes in humans, its specific toxicity properties in *C. elegans* may result in distinct interactions with glyphosate (GPS). Notably, our study observed antagonistic effects when organisms were exposed to a mixture of cadmium and glyphosate. This finding is particularly intriguing as it represents the first report of antagonistic toxicity effects from a Cd-GPS mixture in *C. elegans*.

Toxicity interactions between chemicals affect several key processes that determine the resultant toxicity of a chemical to an organism. These processes include but are not limited to bioavailability, uptake and transport to the target site, binding at the target site, excretion (Cedergreen, 2014; Vig et al., 2003), as well as the Chelation mechanism (Tsui et al., 2005). In our study, combined exposure to cadmium and glyphosate reduced the overall toxicity, which is consistent with previous reports suggesting a chelating interaction between the two chemicals (Zhou et al., 2014). However, as the concentration of glyphosate increased, the interaction transitioned from antagonistic to synergistic. This suggests that the interaction between Cd and GPS is complex, and their combined toxic effects vary depending on their relative concentrations, with both antagonistic and synergistic interactions occurring under different conditions. It also highlights the importance of regulating glyphosate concentrations in the environment to prevent adverse effects.

Following cadmium (Cd2+) exposure, significant upregulation was observed in several detoxification and stress response genes including *mtl-1*, *mtl-2*, and *cdr-1*, etc. as shown in Table 2, this is conformation with work reported by others (Cui et al., 2007; Liao et al., 2002). Particularly, the *mtl-1* gene, regulated by metal ions, plays a crucial role in reducing the toxic impact of cadmium by binding to metal ions, thus facilitating nematodes' adaptation to contaminated environments (Swain et al., 2004). This suggests that the interaction between Cd and GPS may reduce cadmium toxicity. The study of metallothionein mechanisms, especially through the *mtl-1* gene, provides valuable insights into the processes of anti-oxidative stress and metal detoxification. It also unveils the adaptive mechanisms of nematodes to environmental stress and toxins exposure, contributing to a deeper understanding of organismal resilience and survival mechanisms.

Our regulatory network analysis reveals that cadmium may mimic zinc, thereby triggering the activation of the *hizr-1* high zinc response pathway (Warnhoff et al., 2017). Additionally, the identification of *Y22D7AL.16* as a novel gene with a C2H2-type zinc finger domain and differential expression under cadmium stress within the cadmium response network suggests an alternative pathway for mitigating cadmium toxicity that is independent of the hizr-1-mediated response. The gene's association with nematode development implies its role in developmental processes and stress responses, warranting further investigation to elucidate its role across various developmental stages. Therefore, our findings suggest that the interaction between cadmium and glyphosate significantly affects their combined toxicity. This interaction should be taken into account when evaluating the overall impact of these pollutants on the ecosystem. Specifically, our results emphasize that it is crucial to consider how multiple pollutants interact with each other, rather than assessing the toxicity of individual pollutants in isolation. This approach provides a more comprehensive understanding of their potential ecological impacts.

The primary mechanism behind heavy metal toxicity is the presence of free metal ions. Glyphosate, containing coordination groups such as -COOH, -NH2, and -PO4, has a strong ability to bind with heavy metal cations. When glyphosate interacts with Cd2+, it forms a Cd-GPS complex. This complex can be either toxic or even non-toxic, but importantly, it effectively reduces the concentration of free Cd2+ ions. Since the toxicity of heavy metals generally results from free or active ions, the chelation of these ions by glyphosate into inactive forms considerably limits their potential toxicity or effectiveness. In addition, the binding affinity of glyphosate with metal ions varies with the ion type and valence, being strongest with trivalent metals, followed by bivalent and monovalent ions (Chui-fan et al., 2014; Tsui and Chu, 2003; Abate et al., 1999; Wang et al., 2004). This interaction underscores the potential of

Table 2
Expression Profiles of Key Detoxification Genes Across Treatment Clusters.

Gene	CK	Cd	GPS	Cd+GPS	Proposed Function	Cluster
ugt−13	1.17	5.24	1.89	2.73	glucuronosyltransferase	1
gst−4	9.7	41.86	6.85	16.03	Glutathione S-transferase 4	1
gst−12	0.98	8.62	3.28	6.33	Glutathione S-Transferase	2
gst−14	0.88	7.15	3.2	5.52	Glutathione transferase	2
gst−16	1.25	6.35	1.85	4.33	Glutathione S-Transferase	2
cdr−1	0.31	84.44	0.41	31.01	CaDmium Responsive	3
cyp−13A4	0.02	19.21	0.18	6.41	Putative cytochrome P450 CYP13A4	3
cyp−14A4	0.07	5.92	0.27	2.29	CYtochrome P450 family	3
gst−38	0.51	8.17	1.12	1.47	Glutathione transferase	6
mtl−1	47.77	3460.41	126.38	2859.06	Metallothionein−1	9
mtl−2	18.05	2610.38	19.75	2737.73	Metallothionein−2	9

Note: Expression levels (measured in FPKM) of selected key detoxification genes under four treatment conditions: Cadmium (Cd), Cadmium with GPS co-treatment (Cd+GPS), Control (CK), and GPS treatment (GPS).

glyphosate to mitigate heavy metal toxicity in the environment. Furthermore, several studies have shown that the interaction between pollutants can shift from antagonism to synergy with increasing mixture concentrations. For example, benz[*a*]pyrene and 2-amino-1--methyl-6-phenylimidazo[4,5-*b*]pyridine exhibit antagonistic effects at lower concentrations but become synergistic effects at higher concentrations (David et al., 2016; Glaab et al., 2000; Bukowska et al., 2022). Similarly, heavy metals such as copper and cadmium, as well as polycyclic aromatic hydrocarbons (PAHs), exhibit antagonistic effects at lower concentrations but synergistic effects at higher concentrations (Meynard et al., 2021; Babu et al., 2014; Kumar et al., 2010). These findings indicate that various factors, including pollutant concentrations, exposure period, and exposure pathways, can influence the interactions between pollutants.

Therefore, when conducting environmental toxicology assessments, it is imperative to consider these factors and simulate real environmental conditions as precisely as possible to effectively assess the impacts of various pollutants on the ecosystem. Additionally, more research is needed to understand the mechanisms and effects of combined cadmium and glyphosate exposure, as well as their existence and potential risks in agricultural environments. This research will benefit both the natural environment and human health. Expanding on these findings provides an opportunity for a deeper exploration of their specific implications and broader significance in both scientific and societal contexts. And will help to improve pollution management measures, enhance ecological resilience, and ultimately promote the sustainable coexistence of human activities with natural ecosystems. While our study focused on a specific model organism, its relevance to other species and its implications for pollutant management strategies in the environment become critical perspectives to consider. Furthermore, exploring the adaptive responses, such as the upregulation of *mtl-1* and the discovery of *Y22D7AL.16* reveals novel resistance pathways that may be applicable or inspire similar studies across different biological models. These findings not only deepen our understanding of organismal responses to environmental stressors but also offer potential avenues for the development of innovative approaches to address pollution-related challenges.

In conclusion, our study reveals a concentration-dependent dynamic interaction between glyphosate and cadmium, particularly with antagonistic effects at lower concentrations that mitigate phenotypic damage in *C. elegans*. Using comprehensive toxicity assessments and transcriptome analysis, we identified key molecular pathways and mechanisms affected by this interaction. These findings underscore the importance of considering combined pollutant exposures in environmental risk assessments, particularly in agricultural settings where such co-occurrence is common. Future research should explore interactions with other pollutants and environmental conditions to fully understand their broader implications. Additionally, exploring potential mitigation strategies, such as bioremediation or pollutant degradation, could offer practical solutions to reduce the environmental and health risks posed by these combined pollutants exposures.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2024.117081.

References

Li, H., Zeng, L., Wang, C., Shi, C., Li, Y., Peng, Y., Chen, H., Zhang, J., Cheng, B., Chen, C., Xiang, M., Huang, Y., 2022. Review of the toxicity and potential molecular mechanisms of parental or successive exposure to environmental pollutants in the model organism Caenorhabditis elegans. Environ. Pollut. 311, 119927. Oct 15.
Wang, M., Chen, Z., Song, W., Hong, D., Huang, L., Li, Y., 2021a. A review on cadmium exposure in the population and intervention strategies against cadmium toxicity. Bull. Environ. Contam. Toxicol. 106 (1), 65–74 (Jan).
Wang, P., Chen, H., Kopittke, P.M., Zhao, F.J., 2019. Cadmium contamination in agricultural soils of China and the impact on food safety. Environ. Pollut. 249, 1038–1048 (Jun).
Branca, J.J.V., Fiorillo, C., Carrino, D., Paternostro, F., Taddei, N., Gulisano, M., Pacini, A., Becatti, M., 2020. Cadmium-induced oxidative stress: focus on the central nervous system. Antioxidants 9 (6), 492. Jun 5.
Chen, X.X., Xu, Y.M., Lau, A.T.Y., 2022. Metabolic effects of long-term cadmium exposure: an overview. Environ. Sci. Pollut. Res Int 29 (60), 89874–89888 (Dec).
Thompson, J., Bannigan, J., 2008. Cadmium: toxic effects on the reproductive system and the embryo. Reprod. Toxicol. 25 (3), 304–315 (Apr).
Moser, V.C., Morris-Schaffer, K., Richardson, J.R., Li, A.A., 2022. Glyphosate and neurological outcomes: a systematic literature review of animal studies. J. Toxicol. Environ. Health B Crit. Rev. 25 (4), 162–209. May 19.
Milesi, M.M., Lorenz, V., Durando, M., Rossetti, M.F., Varayoud, J., 2021. Glyphosate herbicide: reproductive outcomes and multigenerational effects. Front Endocrinol. (Lausanne) 12. Jul 7.
Duke, S.O., Powles, S.B., 2008 Apr. Glyphosate: a once-in-a-century herbicide. Pest Manag Sci. 64 (4), 319–325.
Bai, S.H., Ogbourne, S.M., 2016. Glyphosate: environmental contamination, toxicity and potential risks to human health via food contamination. Environ. Sci. Pollut. Res Int 23 (19), 18988–19001 (Oct).

Ramakrishnan, B., Megharaj, M., Venkateswarlu, K., Sethunathan, N., Naidu, R., 2011. Mixtures of environmental pollutants: effects on microorganisms and their activities in soils. Rev. Environ. Contam. Toxicol. 211, 63–120.
Kováčik, J., Novotný, V., Bujdoš, M., Dresler, S., Hladký, J., Babula, P., 2020. Glyphosate does not show higher phytotoxicity than cadmium: cross talk and metabolic changes in common herb. J. Hazard Mater. 383, 121250. Feb 5.
Long, N.P., Kang, J.S., Kim, H.M., 2023. Caenorhabditis elegans: a model organism in the toxicity assessment of environmental pollutants. Environ. Sci. Pollut. Res Int 30 (14), 39273–39287 (Mar).
Tejeda-Benitez, L., Olivero-Verbel, J., 2016. Caenorhabditis elegans, a biological model for research in toxicology. Rev. Environ. Contam. Toxicol. 237, 1–35.
Markaki, M., Tavernarakis, N., 2020. Caenorhabditis elegans as a model system for human diseases. Curr. Opin. Biotechnol. 63, 118–125 (Jun).
Grishok, A., 2005. RNAi mechanisms in Caenorhabditis elegans. FEBS Lett. 579 (26), 5932–5939. Oct 31.
Braendle, C., Milloz, J., Félix, M.A., 2008. Mechanisms and evolution of environmental responses in Caenorhabditis elegans. Curr. Top. Dev. Biol. 80, 171–207.
Fang-Yen, C., Avery, L., Samuel, A.D., 2009. Two size-selective mechanisms specifically trap bacteria-sized food particles in Caenorhabditis elegans. Proc. Natl. Acad. Sci. USA 106 (47), 20093–20096. Nov 24.
Gems, D., Riddle, D.L., 2000. Longevity and resistance to stress in nematodes. Ann. Rev. Gerontol. Geriatr. 20, 37–57.
Luo, L., Gabel, C.V., Ha, H.I., et al., 2008. Unique features of the *C.elegans* cholinergic neuromuscular junction revealed by differential proteomics. HFSP J. 2 (6), 389–399.
Zhang, H., Gao, S., Chen, W., 2022a. Automated recognition and analysis of head thrashes behavior in *C. elegans*. BMC Bioinforma. 23 (1), 87. Mar 7.
Zhang, W., Zhang, N., Zheng, S., Zhang, W., Liu, J., He, L., Ezemaduka, A.N., Li, G., Ning, J., Xian, B., Gao, S., 2022b. Effects of commercial beverages on the neurobehavioral motility of Caenorhabditis elegans. PeerJ 10, e13563. Jul 14.
Wang, Y., Ezemaduka, A.N., Li, Z., Chen, Z., Song, C., 2017. Joint toxicity of arsenic, copper and glyphosate on behavior, reproduction and heat shock protein response in Caenorhabditis elegans. Bull. Environ. Contam. Toxicol. 98 (4), 465–471 (Apr).
Bolger, A.M., Lohse, M., Usadel, B., 2014 Aug 1. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30 (15), 2114–2120.
Dobin, A., Davis, C.A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., Gingeras, T.R., 2013. STAR: ultrafast universal RNA-seq aligner. Bioinformatics 29 (1), 15–21. Jan 1.
Cassan, O., Lèbre, S., Martin, A., 2021. Inferring and analyzing gene regulatory networks from multi-factorial expression data: a complete and interactive suite. BMC Genom. 22 (1), 387. May 26.
Huynh-Thu, V.A., Irrthum, A., Wehenkel, L., Geurts, P., 2010. Inferring regulatory networks from expression data using tree-based methods. PLoS One 5 (9), e12776. Sep 28.
Sharma, A.K., Finney, L., Vogt, S., Vatamaniuk, O.K., Kim, S., 2023. Cadmium alters whole animal ionome and promotes the re-distribution of iron in intestinal cells of Caenorhabditis elegans. Front Physiol. 14, 1258540. Sep 26.
Ma, X., Hou, M., Liu, C., Li, J., Ba, Q., Wang, H., 2022. Cadmium accelerates bacterial oleic acid production to promote fat accumulation in Caenorhabditis elegans. J. Hazard Mater. 421, 126723. Jan 5.
Magner, D.B., Antebi, A., 2008. Caenorhabditis elegans nuclear receptors: insights into life traits. Trends Endocrinol. Metab. 19 (5), 153–160 (Jul).
Earley, B.J., Cubillas, C., Warnhoff, K., Ahmad, R., Alcantar, A., Lyon, M.D., Schneider, D.L., Kornfeld, K., 2021. Cadmium hijacks the high zinc response by binding and activating the HIZR-1 nuclear receptor. Proc. Natl. Acad. Sci. USA 118 (42), e2022649118. Oct 19.
Shomer, N., Kadhim, A.Z., Grants, J.M., Cheng, X., Alhusari, D., Bhanshali, F., Poon, A.F., Lee, M.Y.Y., Muhuri, A., Park, J.I., Shih, J., Lee, D., Lee, S.V., Lynn, F.C., Taubert, S., 2019. Mediator subunit MDT-15/MED15 and Nuclear Receptor HIZR-1/HNF4 cooperate to regulate toxic metal stress responses in Caenorhabditis elegans. PLoS Genet 15 (12), e1008508. Dec 9.
Warnhoff, K., Roh, H.C., Kocsisova, Z., Tan, C.H., Morrison, A., Croswell, D., Schneider, D.L., Kornfeld, K., 2017. The nuclear receptor HIZR-1 uses zinc as a ligand to mediate homeostasis in response to high zinc. PLoS Biol. 15 (1), e2000094. Jan 17.
Cai, H., Bao, Y., Cheng, H., Ge, X., Zhang, M., Feng, X., Zheng, Y., He, J., Wei, Y., Liu, C., Li, L., Huang, L., Wang, F., Chen, X., Chen, P., Yang, X., 2023. Zinc homeostasis may reverse the synergistic neurotoxicity of heavy metal mixtures in Caenorhabditis elegans. Sci. Total Environ. 868, 161699. Apr 10.
Cordeiro, L.M., Soares, M.V., da Silva, A.F., Dos Santos, L.V., de Souza, L.I., da Silveira, T. L., Baptista, F.B.O., de Oliveira, G.V., Pappis, C., Dressler, V.L., Arantes, L.P., Zheng, F., Soares, F.A.A., 2023 Jul. Toxicity of copper and zinc alone and in combination in Caenorhabditis elegans model of Huntington's disease and protective effects of rutin. Neurotoxicology 97, 120–132.
Liu, L., He, S., Tang, M., Zhang, M., Wang, C., Wang, Z., Sun, F., Yan, Y., Li, H., Lin, K., 2022. Pseudo toxicity abatement effect of norfloxacin and copper combined exposure on Caenorhabditis elegans. Chemosphere 287 (Pt 1), 132019 (Jan).
Alengebawy, A., Abdelkhalek, S.T., Qureshi, S.R., Wang, M.Q., 2021. Heavy Metals and Pesticides Toxicity in Agricultural Soil and Plants: Ecological Risks and Human Health Implications. Toxics 9 (3), 42. Feb 25.
Pei, C., Sun, L., Zhao, Y., Ni, S., Nie, Y., Wu, L., Xu, A., 2022. Enhanced uptake of arsenic induces increased toxicity with cadmium at non-toxic concentrations on *Caenorhabditis elegans*. Toxics 10 (3), 133. Mar 10.
Wang, Y., Liu, S.S., Huang, P., Wang, Z.J., Xu, Y.Q., 2021b. Assessing the combined toxicity of carbamate mixtures as well as organophosphorus mixtures to Caenorhabditis elegans using the locomotion behaviors as endpoints. Sci. Total Environ. 760, 143378. Mar 15.
Tchounwou, P.B., Yedjou, C.G., Patlolla, A.K., Sutton, D.J., 2012. Heavy metal toxicity and the environment. Exp. Suppl. 101, 133–164.
Zhou, H., Yang, Y., Kang, Y., Guo, T., Zhou, Y., Zhang, Y., Ma, L., 2024. Synergistic toxicity induced by the co-exposure of tenuazonic acid and patulin in Caenorhabditis elegans: Daf-16 plays an important regulatory role. Ecotoxicol. Environ. Saf. 270, 115871. Jan 15.
Zhou, D.M., Wang, Y.J., Cang, L., Hao, X.Z., Luo, X.S., 2004. Adsorption and cosorption of cadmium and glyphosate on two soils with different characteristics. Chemosphere 57, 1237–1244.
Ramstedt, M., Norgren, C., Shchukarev, A., Sj€oberg, S., Persson, P., 2005. Coadsorption of cadmium(II) and glyphosate at the water–manganite (g-MnOOH) interface. J. Colloid Inter. Sci. 285, 493–501.
Rai, A., Maurya, S.K., Khare, P., Srivastava, A., Bandyopadhyay, S., 2010. Characterization of developmental neurotoxicity of As, Cd, and Pb mixture: synergistic action of metal mixture in glial and neuronal functions. Toxicol. Sci. 118, 586–601.
Lu, C., Svoboda, K.R., Lenz, K.A., Pattison, C., Ma, H., 2018. Toxicity interactions between manganese (Mn) and lead (Pb) or cadmium (Cd) in a model organism the nematode C. elegans. Environ. Sci. Pollut. Res Int 25 (16), 15378–15389 (Jun).
Cedergreen, N., 2014. Quantifying synergy: a systematic review of mixture toxicity studies within environmental toxicology. PLoS One 9 (5), e9658.
Vig, K., Megharaj, M., Sethunathan, N., Naidu, R., 2003. Bioavailability and toxicity of cadmium to microorganisms and their activities in soil: A review. Adv. Environ. Res 8, 121–135.
Tsui, M.T.K., Wang, W.X., Chu, L.M., 2005. Influence of glyphosate and its formulation (RoundupR) on the toxicity and bioavailability of metals to Ceriodaphnia dubia. Environ. Pollut. 138, 59–68.
Zhou, C.F., Wang, Y.J., Sun, R.J., Liu, C., Fan, G.P., Qin, W.X., Li, C.C., Zhou, D.M., 2014. Inhibition effect of glyphosate on the acute and subacute toxicity of cadmium to earthworm Eisenia fetida. Environ. Toxicol. Chem. 33 (10), 2351–2357 (Oct).
Cui, Y., McBride, S.J., Boyd, W.A., Alper, S., Freedman, J.H., 2007. Toxicogenomic analysis of Caenorhabditis elegans reveals novel genes and pathways involved in the resistance to cadmium toxicity. Genome Biol. 8 (6), R122.
Liao, V.H., Dong, J., Freedman, J.H., 2002. Molecular characterization of a novel, cadmium-inducible gene from the nematode Caenorhabditis elegans. A new gene that contributes to the resistance to cadmium toxicity. J. Biol. Chem. 277 (44), 42049–42059. Nov 1.
Swain, S.C., Keusekotten, K., Baumeister, R., Stürzenbaum, S.R., 2004. *C. elegans* metallothioneins: new insights into the phenotypic effects of cadmium toxicosis. J. Mol. Biol. 341 (4), 951–959. Aug 20.
Chui-fan, Zhou, Li Jing-wen, Li Ying, Wang Yu-jun and Zhou Dong-mei. "Inhibitory Effect of Glyphosate on Toxicity of Heavy Metals: A Case Study on Photobacterium phosphoreum T3." (2014).
Tsui, M.T., Chu, L.M., 2003. Aquatic toxicity of glyphosate-based formulations: comparison between different organisms and the effects of environmental factors. Chemosphere 52 (7), 1189–1197 (Aug).
Abate, L., Stefano, C., Foti, C., Sammartano, S., 1999. Binding of glyphosate by open-chain polyammonium cations. Environ. Toxicol. Chem. 18 (10), 2131–2137 (Oct).
Wang Yujun, Zhou Dongmei, Sun Ruijuan, Chen Huaiman. Toxicity of the interaction of glyphosate and cadmium to wheat Ecology and Environment. 2004;13(2):158-160..
David, R., Ebbels, T., Gooderham, N., 2016. Synergistic and Antagonistic Mutation Responses of Human MCL-5 Cells to Mixtures of Benzo[a]pyrene and 2-Amino-1-Methyl-6-Phenylimidazo[4,5-b]pyridine: Dose-Related Variation in the Joint Effects of Common Dietary Carcinogens. Environ. Health Perspect. 124 (1), 88–96 (Jan).
Glaab, W.E., Kort, K.L., Skopek, T.R., 2000. Specificity of mutations induced by the food-associated heterocyclic amine 2-amino-1-methyl-6-phenylimidazo-[4,5-b]-pyridine in colon cancer cell lines defective in mismatch repair. Cancer Res. 60, 4921–4925.
Bukowska, B., Mokra, K., Michałowicz, J., 2022. Benzo[*a*]pyrene-environmental occurrence, human exposure, and mechanisms of toxicity. Int J. Mol. Sci. 23 (11), 6348. Jun 6.
Meynard, A., Espinoza-González, C., Núñez, A., et al., 2021. Synergistic, antagonistic, and additive effects of heavy metals (copper and cadmium) and polycyclic aromatic hydrocarbons (PAHs) under binary and tertiary combinations in key habitat-forming kelp species of Chile. Environ. Sci. Pollut. Res 28, 18300–18307.
Babu, M.Y., Palanikumar, L., Nagarani, N., Devi, V.J., Kumar, S.R., Ramakritinan, C.M., Kumaraguru, A.K., 2014. Cadmium and copper toxicity in three marine macroalgae: evaluation of the biochemical responses and DNA damage. Environ. Sci. Pollut. Res 21, 9604–9616.
Kumar, M., Kumari, P., Gupta, V., Anisha, P.A., Reddy, C.R.K., Jha, B., 2010. Differential responses to cadmium induced oxidative stress in marine macroalga Ulva lactuca (Ulvales, Chlorophyta). Biometals 23, 315–325.