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EDITED BY

Melida Gutierrez,
Missouri State University, United States

REVIEWED BY

Md Muzammel Hossain,
Jiangsu University, China
MariaTeresa Alarcon-Herrera,
Centro de Investigación de Materiales
Avanzados (CIMA), Mexico

*CORRESPONDENCE

Lihong Li
✉ lihong413413@163.com

RECEIVED 18 September 2025

ACCEPTED 03 November 2025

PUBLISHED 14 November 2025

CITATION

Qi H, Liu Z, Wang H, Li X and Li L (2025)
Toxicity identification evaluation of riverine
water using seed germination: lead toxicity in
the Fen River's Taiyuan section of China.
Front. Water 7:1707872.
doi: 10.3389/frwa.2025.1707872

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Toxicity identification evaluation of riverine water using seed germination: lead toxicity in the Fen River's Taiyuan section of China

Hongxue Qi, Zhenchao Liu, Hemei Wang, Xiaodong Li and
Lihong Li*

Department of Chemistry and Chemical Engineering, Jinzhong University, Jinzhong, Shanxi, China

A phytotoxicity-based toxicity identification evaluation (TIE) approach was employed to identify toxic substances in river water, focusing on the Taiyuan section of the Fen River in China as a case study. Four vegetable seed species—amaranth, oilseed rape, cabbage, and lettuce—were selected for toxicity screening. An analysis of variance was performed on the germination index (GI%) of these seeds exposed to nine initial water samples from the Fen River. This analysis revealed that the sampling site with the lowest GI% was S6. Consequently, samples from the S6 site were used for subsequent TIE treatments, which included aeration, EDTA chelation, reduction with oxidizing agents, filtration, solid-phase extraction using C18, and pH adjustment. As a result, EDTA and its related treatment significantly improved seed viability across nearly all species, underscoring the role of cationic metals in contributing to toxicity. Correspondingly, 10 metals in the initial water samples were analyzed using inductively coupled plasma mass spectrometry. Linear correlations were observed between the concentrations of metals (cobalt, chromium, nickel, and lead) and the phytotoxicity observed in the four vegetable species, with correlation coefficients exceeding 0.5 ($p < 0.01$). Finally, Canonical correlation analysis suggested that exposure to lead in riverine water was most likely associated with the phytotoxic effects observed in oilseed rape and cabbage, contributing to overall toxicity. In conclusion, lead was confirmed as the primary toxicant responsible for the observed phytotoxic effects in oilseed rape and cabbage in the Fen River. Although metal concentrations remained below the Environmental Quality Standards for Surface Water thresholds, the identified ecological risks underscore the need for enhanced monitoring and management. The phytotoxicity-based TIE approach proved effective in identifying specific toxicants, providing valuable insights for water quality management and ecological risk assessment.

KEYWORDS

toxicity identification evaluation, metals, water, phytotoxicity, river

1 Introduction

River contamination represented a significant global environmental concern, threatening the health of aquatic ecosystems. The principal sources of this pollution included agricultural runoff, wastewater treatment facility discharges, and industrial wastewater. River water functioned as a complex system containing a variety of pollutants, including pesticides (Ramage et al., 2025), pharmaceuticals (Rodrigues et al., 2024), heavy metals (Wang et al.,

2025), personal care products (Hawash et al., 2023), microplastics (Priya et al., 2025), halogenated flame retardants (Olaniyan et al., 2023), estrogens (Waniek et al., 2025), per- and polyfluoroalkyl substances (Kali et al., 2025), and other emerging contaminants (Picinini-Zambelli et al., 2025). These pollutants had the potential to disrupt species abundance (Muhie et al., 2025), alter biome composition (Malla et al., 2025), and compromise the ecological functionality of aquatic ecosystems (Jiang et al., 2025). The environmental risk posed by these pollutants in water bodies may be underestimated by conventional chemical monitoring and biological toxicity testing alone (Yan et al., 2025).

The toxicity identification evaluation (TIE) technique, which integrated organism toxicity testing with chemical manipulations, proved effective in identifying causative toxicants in complex environmental samples (USEPA, 1991, 1993a, 1993b). It successfully characterized toxicity in various contexts, including sediments (Ho and Burgess, 2013; Ke et al., 2015), seafood wastewater (Tsipa et al., 2023), shale gas wastewater (Zhou et al., 2024), pharmaceutical wastewater (Chen et al., 2025), and excess sludge (Nobili et al., 2024). Within the TIE framework, various test organisms were employed, with *Daphnia magna*, fish, and plants being the most commonly used. Furthermore, terrestrial plant seeds demonstrated their ability to assess phytotoxicity in environmental media, offering advantages such as sensitivity, simplicity, rapidity, low cost, and minimal equipment requirements. However, seed germination was infrequently utilized in TIE procedures for riverine water, despite its application in identifying toxicity in landfill leachate (Budi et al., 2016) and toxic industrial effluents (Ra et al., 2016).

The Fen River, recognized as the largest river in Shanxi Province and the second-largest tributary of the Yellow River in China, experienced notable improvements in water quality in recent years. However, prior research revealed concerning levels of pollutants, including heavy metals (Li et al., 2023), estrogens (Liu et al., 2017), nitrates (Xiao et al., 2022), and polyfluoroalkyl substances (Zhou et al., 2019). Despite improvements in chemical quality, the ecological factors contributing to phytotoxicity in the Fen River remained unclear, as conventional chemical analysis failed to account for plant-relevant exposure pathways. Identifying the primary causes of biotoxicity was essential for establishing a scientific foundation for effective water management in the Fen River. Based on the technical route outlined in Figure 1, it was hypothesized that the observed phytotoxicity during seed germination resulted from volatile pollutants, cationic metals, oxidants, particulate matter, or nonpolar organic compounds. This study aimed to identify key toxicants in riverine water using a phytotoxicity-based TIE approach, following an assessment of initial water toxicity on seed germination, with the Taiyuan section of the Fen River serving as a case study.

2 Materials and methods

2.1 Sample collection

In September 2024, nine water samples were collected from the upper water column at a depth of 0.5 meters in the Taiyuan section of the Fen River in China, as illustrated in Figure 2. Seed toxicity tests were performed within 1 day of sample collection. For heavy metal analysis, the samples were acidified, stored at 4°C, shielded from light, and tested within 3 days.

2.2 Chemical analysis

The concentrations of 10 metals—cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb), vanadium (V), and zinc (Zn)—were analyzed using an inductively coupled plasma mass spectrometer (ICP-MS, Agilent 7,700) with internal calibration, as presented in Supplementary Table S1. Additional details regarding the metal analysis are available in the Supplementary material.

2.3 Toxicity test for seeds

The seeds used in this study included amaranth (*Amaranth tricolor* L.), oilseed rape (*Brassica napus* L.), cabbage (*Brassica campestris* L.), and lettuce (*Lactuca sativa* L.), all obtained from Shanxi Dafeng Seed Industry Co., Ltd. in China. These species were chosen for their rapid germination rates, sensitivity to pollutants, and significance as common vegetable crops in the region. Phytotoxicity tests were conducted in accordance with the agricultural seed testing guidelines established in China (GB/T3543.4–2025) (CSAMR, 2025). In brief, 50 seeds from each species were germinated on filter paper within a clean 10 cm-diameter petri dish, each containing 10 mL of treatment water. The Petri dishes were incubated in the dark at 25°C. After 5 days of exposure, seed germination was evaluated by measuring primary root length. Only seeds with a primary root length of at least 3 mm were considered successfully germinated. All tests were independently replicated three times. Following the recommendation from the literature (Zucconi et al., 1985), the germination index (GI) was calculated using the following formula:

$$GI(\%) = (G/G_c) \times (RL/RL_c) \times 100$$

Where G represents the number of seeds germinated in the test, G_c denotes the number of seeds germinated in the control, RL indicates the mean root length in the test, and RL_c signifies the mean root length in the control.

2.4 TIE procedure

The TIE procedure was conducted with the recommendations of the United States Environmental Protection Agency (USEPA, 1991). The evaluation included several treatments: aeration, ethylenediaminetetraacetic acid (EDTA) chelation, oxidant reduction, filtration, solid phase extraction with C18, and pH adjustment. These methods allowed for the tentative classification of toxic substances into the following categories: volatile pollutants, cationic metals, oxidants, particulate matter, non-polar organics, and pH-sensitive chemicals. (1) The water used for toxicity testing was lightly aerated at a controlled rate of 4 L/min, ensuring optimal conditions with an air pump. (2) EDTA was added through dilution, achieving a final concentration of 30 mg/L for the four seeds. (3) In the oxidant reduction test, sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$) was carefully introduced into the solution at a concentration of 0.1 g/L for four seeds, effectively reducing oxidants. (4) Samples were filtered using a 0.45 μm Whatman GF/C filter membrane to eliminate harmful particles. (5) Solid phase

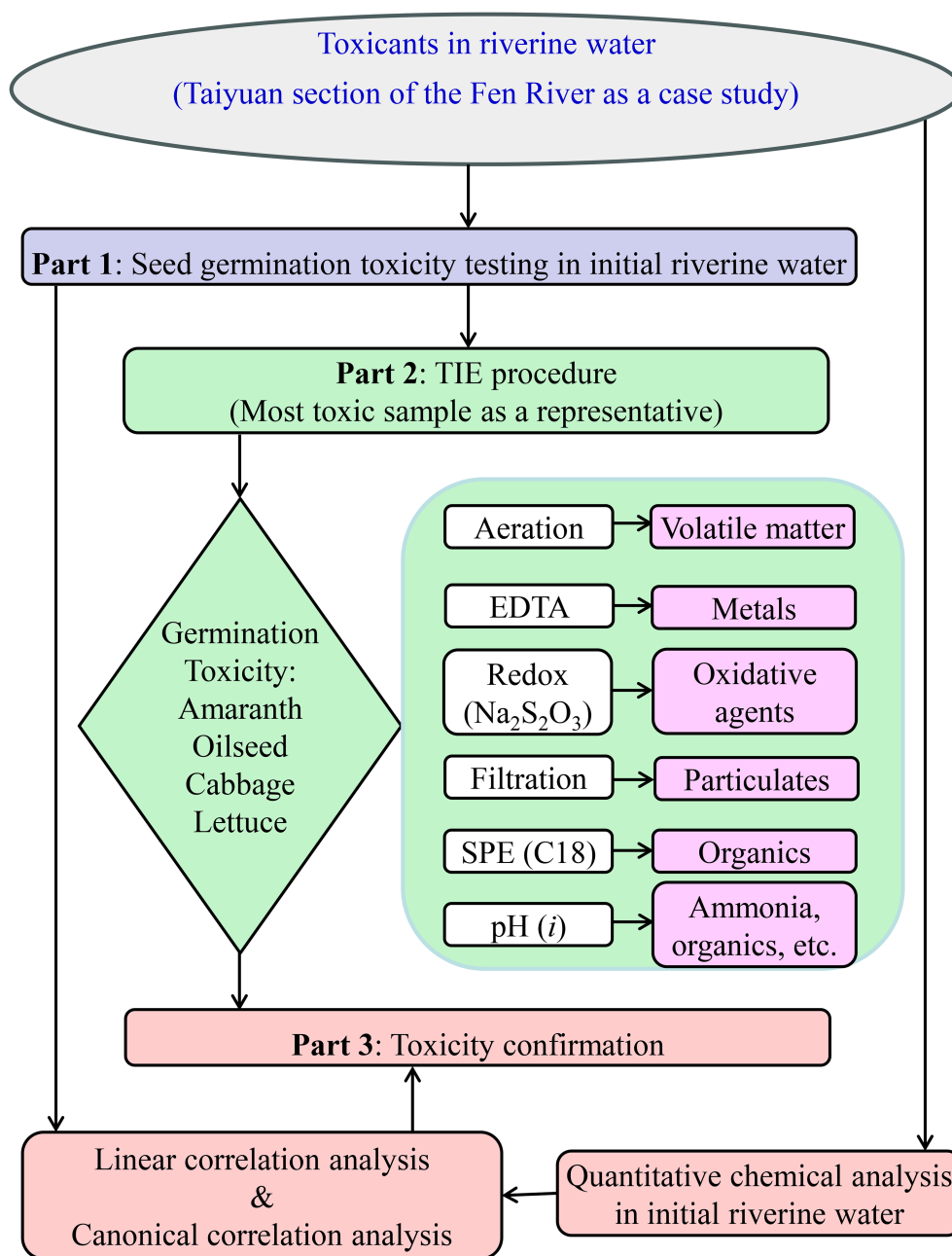


FIGURE 1

Scheme of toxicity identification evaluation based on seed germination toxicity in riverine water.

extraction (SPE) was carried out with a C18 column (2000 mg/12 mL, Shanghai Titan Scientific Co., Ltd.). (6) During the pH adjustment tests, the initial pH of the water was accurately modified to pH 3 with hydrochloric acid (HCl) or adjusted to pH 11 with sodium hydroxide (NaOH), facilitating thorough assessments of pH-sensitive chemicals.

2.5 Statistical analysis

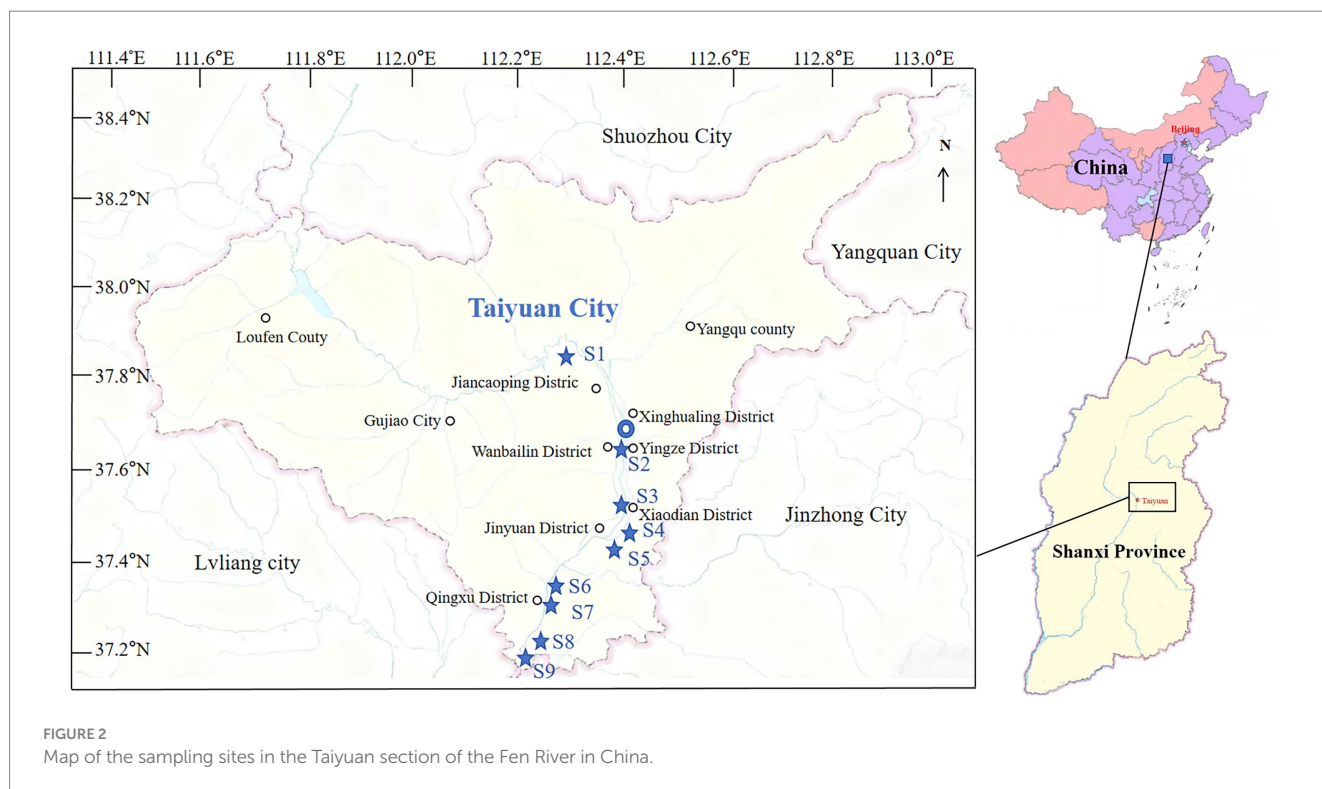
Data on biological effects were reported as the mean $\pm$ standard deviation from three independent experiments. A one-way analysis of variance (ANOVA) was employed to identify statistically significant

differences, and the Tukey Test was utilized for multiple comparisons. Canonical correlation analysis was performed using SPSS 26.0 software.

3 Results and discussion

3.1 Toxicity screening

Four vegetable seeds were chosen for toxicity tests of the initial water due to the relative simplicity and sensitivity of seed toxicity screening. The GI% was analyzed using one-way ANOVA for multiple



comparisons. As shown in [Supplementary Table S2](#) and [Table 1](#), no statistically significant differences were found among items labelled with the same letter within the same column. Conversely, statistically significant differences were observed between items labelled with different letters, suggesting that they could be classified into distinct categories. Among the evaluated categories, seed viability consistently ranked lowest for amaranth (C), oilseed rape (E), cabbage (C), and lettuce (D) at site S6. Consequently, a sample from site S6, located downstream along the Fen River, was selected for further TIE studies.

3.2 Toxicity characterization in TIE

To statistically differentiate among the various TIE treatments, a one-way ANOVA was performed on the mean GI% across treatments. As shown in [Supplementary Table S3](#) and [Figure 3](#), significant differences were primarily observed in the EDTA and redox treatments compared with raw water, except for amaranth, which showed statistically significant differences not only in the EDTA and Redox treatments but also in the pH adjustment.

Analysis of variance was used to identify statistically significant differences, and the Tukey Test was employed for conducting multiple comparisons. Notably, items marked with the same letter within a column exhibited no statistically significant differences ($p < 0.05$).

Regarding EDTA, the treatments included EDTA chelation, filtration combined with EDTA treatment, and SPE combined with EDTA treatment. Firstly, seed viability significantly improved ($n = 3$, $p < 0.01$) after EDTA treatment in oilseed rape, cabbage, and lettuce compared to the initial water treatment. Amaranth also showed a significant difference under EDTA chelation ($n = 3$, $p < 0.05$). Secondly, for filtration combined with EDTA treatment, seed viability significantly improved ($n = 3$, $p < 0.01$) across all four plant species compared to the initial water treatment. Thirdly, for

TABLE 1 The analysis of variance and multiple comparisons for the (GI%) in the Taiyuan section of the Fen River in China (mean $\pm$ SD, $n = 3$).

Site	Amaranth	Oilseed rape	Cabbage	Lettuce
S1	43 $\pm$ 3 (C)	27 $\pm$ 3 (DE)	27 $\pm$ 3 (BC)	53 $\pm$ 4 (BC)
S2	53 $\pm$ 5 (BC)	23 $\pm$ 3 (E)	23 $\pm$ 4 (BC)	70 $\pm$ 5 (A)
S3	53 $\pm$ 4 (BC)	36 $\pm$ 3 (D)	29 $\pm$ 3 (BC)	32 $\pm$ 3 (C)
S4	74 $\pm$ 2 (A)	88 $\pm$ 3 (A)	77 $\pm$ 3 (A)	63 $\pm$ 3 (A)
S5	62 $\pm$ 2 (B)	87 $\pm$ 4 (A)	86 $\pm$ 5 (A)	20 $\pm$ 3 (D)
S6	50 $\pm$ 3 (C)	15 $\pm$ 3 (E)	16 $\pm$ 2 (C)	19 $\pm$ 3 (D)
S7	57 $\pm$ 2 (BC)	69 $\pm$ 3 (B)	14 $\pm$ 2 (C)	53 $\pm$ 4 (BC)
S8	40 $\pm$ 3 (C)	54 $\pm$ 3 (C)	30 $\pm$ 3 (B)	55 $\pm$ 4 (B)
S9	41 $\pm$ 3 (C)	24 $\pm$ 3 (E)	19 $\pm$ 2 (C)	43 $\pm$ 3 (C)

Analysis of variance was used to identify statistically significant differences, and the Tukey Test was employed for conducting multiple comparisons. Notably, items marked with the same letter within a column exhibited no statistically significant differences ($p < 0.05$).

the SPE combined with EDTA treatment, significant differences were observed in amaranth, oilseed rape, and lettuce ($n = 3$, $p < 0.01$). However, cabbage did not show a statistically significant difference ($n = 3$, $p > 0.05$). These reductions in toxicity suggested that the presence of certain metals in the water was consistent with previous studies linking heavy metals to reduced phytotoxicity ([Sperdouli, 2022](#)). EDTA is a potent chelator that forms stable complexes with cations such as aluminium, barium, cadmium, cobalt, copper, iron, lead, manganese, nickel, strontium, and zinc. This process reduces metal bioavailability and alleviates phytotoxicity. Conversely, arsenic and mercury exhibit relatively weak chelation, while the formation of complexes such as selenides, chromates, and hydrochromates proved more challenging ([USEPA, 1991](#)).

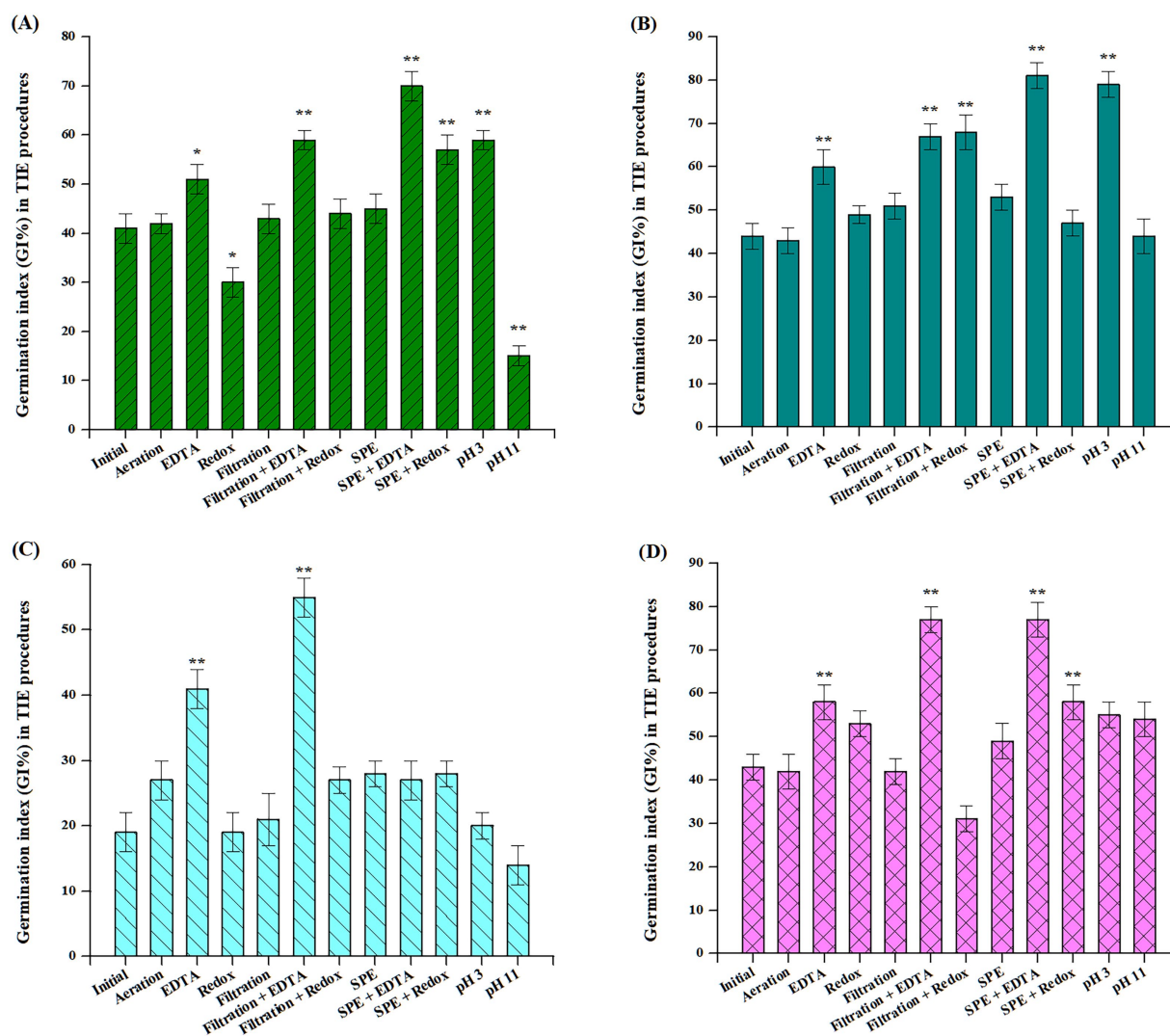


FIGURE 3

Analysis of variance for the germination index (GI%) among TIE treatment groups. (A): amaranth, (B): oilseed rape, (C): cabbage, (D): lettuce. Significant differences between the test and initial water were determined by a one-way analysis of variance and indicated by * $p < 0.05$ and ** $p < 0.01$, respectively. Error bars represent standard deviation ($n = 3$).

Regarding the redox treatments, the options included redox treatment alone, filtration combined with redox treatment, and SPE combined with redox treatment. Firstly, only amaranth showed a significant difference ($n = 3$, $p < 0.05$) with the redox treatment, while oilseed rape exhibited a highly significant difference with the filtration combined with redox treatment ($n = 3$, $p < 0.01$). Additionally, the SPE combined with redox treatments demonstrated the substantial differences ($n = 3$, $p < 0.01$) for amaranth, cabbage, and lettuce, with oilseed rape being the exception ($n = 3$, $p > 0.05$). The reduction in toxicity associated with redox treatments indicated the presence of toxic oxidizers, such as chlorine, certain electrophilic organics, or specific cationic metals (USEPA, 1991). Chlorine, functioning as an oxidant and biocide, oxidizes sodium thiosulfate to form sodium chloride and sodium tetrathionate, both of which are non-toxic to the samples. Furthermore, electrophilic organic compounds were neutralized by sodium thiosulfate, thereby reducing their toxicity. The observed reduction in toxicity may also be attributed to the formation of metal complexes with thiosulfate anions, similar to the EDTA

chelation process, which is designed to decrease toxicity (Hockett and Mount, 1996).

In summary, the seeds of the four plant species exhibited varying degrees of restorable toxicity, primarily due to treatments with EDTA and Redox agents. This evidence suggested that cationic metals and certain electrophilic organic compounds significantly contributed to phytotoxicity in the Taiyuan section of the Fen River in China. Consequently, further identification of TIE was conducted, focusing on the metals present in the riverine water. The identification of the electrophilic organics will be carried out in future research.

3.3 Toxicity identification in TIE

To confirm the potent candidates, the levels of metals in the Taiyuan section of the Fen River were measured using ICP-MS. The concentrations of 10 metals were detailed in Supplementary Table S4, with their descriptive statistics summarized in Table 2. The detection

rates for these metals were 100% in the water samples. The mean concentrations of heavy metals were ranked in the following order: $\text{Mo} > \text{Zn} > \text{Ni} > \text{Mn} > \text{Cu} > \text{Pb} > \text{Cr} > \text{V} > \text{Co} > \text{Cd}$. Mo was the most abundant metal, with a mean of $8.94 \pm 5.67 \mu\text{g}\cdot\text{L}^{-1}$, while Cd exhibited the lowest mean concentration at $0.016 \pm 0.01 \mu\text{g}\cdot\text{L}^{-1}$. Furthermore, the maximum concentrations of heavy metals in all samples remained below the Chinese Environmental Quality Standard for Surface Water (GB 3838–2002) (MEPC, 2002).

Water quality benchmarks are essential not only for physicochemical monitoring but also for ecological risk assessment. Until now, only three water quality ecological criteria have been issued in China in 2020 for ammonia nitrogen (CMEE, 2020a), cadmium (CMEE, 2020b), and phenol (CMEE, 2020c). However, the technical guideline for deriving water quality criteria of freshwater organisms (HJ 831–2022) (CMEE, 2022) was issued on March 10, 2022. The release of this guideline is of great significance for establishing a comprehensive water quality criteria system, effectively controlling toxic and harmful pollutants, and protecting aquatic biodiversity and the integrity of water ecosystems.

3.4 Toxicity confirmation in TIE

3.4.1 Linear correlation analysis between phytotoxicity and the concentrations of metals

Phytotoxicity was first confirmed by evaluating nine initial water samples from the Fen River. Subsequently, a linear correlation analysis was performed to investigate the relationship between the GI% of seeds converted to probit values and the logarithmic values of metal concentrations in the corresponding water samples. The correlation coefficients are presented in Table 3. The toxicity of plant seeds exhibited strong correlations with the concentrations of Co, Cr, Ni, and Pb, with correlation coefficients exceeding 0.5 ($p < 0.01$). Consequently, these four metals were selected for a canonical correlation analysis to confirm their toxic effects further.

3.4.2 Canonical correlation analysis between phytotoxicity and candidate metals

A canonical correlation analysis was conducted to confirm the relationship between phytotoxicity and the candidate metal concentration, based on the results of the preceding linear correlation analysis. One set of variables (X_1 – X_4) represented phytotoxicity, which included the GI% of amaranth, oilseed rape, cabbage, and lettuce. The other set of variables (Y_1 – Y_4) consisted of the logarithmic values of the concentrations of Co, Cr, Ni, and Pb in the water. As a result, the analysis yielded four pairs of canonical correlations; however, only the first pair [$\text{Corr}(U_1, V_1)$] exhibited a significant correlation, as indicated in Table 4, with a canonical coefficient (ρ_1) of 0.99 ($p < 0.01$) and an explained variance ratio of 22.9%. The initial variables (X_i and Y_i) with canonical loadings exceeding 0.35 (in absolute value) suggested a meaningful interpretation between U_1 and V_1 . The canonical variables to phytotoxicity effects (X_i) were notably higher for oilseed rape (0.651) and cabbage (0.695). In contrast, the variables representing metal concentrations (Y_i) showed a strong negative correlation with Pb concentration (canonical loading: -0.422), indicating its likely role in the observed phytotoxicity. Contributions from the remaining toxicity responses and toxicants were minimal. Consequently, this analysis identified Pb

TABLE 2 Statistical description of metal concentrations in 2024 from the Taiyuan section of the Fen River in China ($n = 9$, $\mu\text{g}\cdot\text{L}^{-1}$).

		III grade ^a	Min	Median	Max	Mean	SD
1	Cd	5	0.009	0.013	0.04	0.016	0.01
2	Co	1,000	0.1	0.18	0.59	0.32	0.21
3	Cr	50	0.26	0.59	3.49	1.19	1.07
4	Cu	1,000	1.35	2.58	5.61	2.96	1.48
5	Mn	100	1.65	2.93	7.41	3.53	1.83
6	Mo	70	1.22	8.7	19.87	8.94	5.67
7	Ni	20	1.29	3.97	11.74	4.78	3.01
8	Pb	50	0.62	2.38	5.25	2.44	1.52
9	V	50	0.15	0.72	1.95	0.83	0.59
10	Zn	1,000	2.91	6.67	12.45	7.43	3.31

^aEnvironmental Quality Standards for Surface Water of China (GB 3838–2002) (MEPC, 2002).

TABLE 3 The linear correlation coefficients between the GI% converted to probit and the log values of the metal concentrations in water.

	Amaranth	Oilseed rape	Cabbage	Lettuce
Cd	−0.2	−0.49	−0.42	0.47
Co	−0.5	−0.25	−0.53	−0.2
Cr	−0.63	−0.46	−0.39	−0.21
Cu	−0.45	−0.37	−0.34	0.43
Mn	−0.44	−0.16	−0.48	0.23
Mo	0.35	0.45	0.29	−0.43
Ni	−0.4	−0.36	−0.37	−0.59
Pb	−0.51	−0.49	−0.75	0.51
V	−0.42	−0.48	−0.13	−0.4
Zn	0.33	0.03	−0.01	0.28

Note: Absolute values above 0.5 are given in bold.

TABLE 4 Canonical correlation between the two sets of variables [toxicity effects (X_1 – X_4) converted to probit and log values of metal concentrations in water (Y_1 – Y_4)].

Corr (U_1, V_1), $\rho_1 = 0.99$ ($p = 0.002 < 0.01$) ^a					
Variable (X)	a_1^b	Loading ^c	Variable (Y)	b_1^b	Loading ^c
Amaranth	−3.778	0.068	Co	5.711	−0.108
Oilseed rape	0.933	0.651	Cr	3.895	−0.017
Cabbage	1.071	0.695	Ni	−9.376	−0.196
Lettuce	0.142	−0.058	Pb	−5.597	−0.422

^aCorr (U_1, V_1): first pair of canonical correlation ($U_1 = a_1 X_1 + a_2 X_2 + \dots + a_4 X_4$, $V_1 = b_1 Y_1 + b_2 Y_2 + \dots + b_4 Y_4$). ρ_1 : coefficient of the first pair of canonical correlation. ^b a_1 and b_1 : canonical coefficients. ^cLoading: canonical loadings, absolute values above 0.35, are given in bold. Values above 0.35 are believed to provide a meaningful interpretation between the new pair of variables.

as the primary toxic metal influencing the phytotoxicity effects observed in oilseed rape and cabbage in water from the Taiyuan section of the Fen River in China.

In this study, lead (Pb) was identified as the primary toxicant impairing seed germination in oilseed rape and cabbage in the Taiyuan section of the Fen River. A negative correlation between Pb concentrations and the germination index was also observed, which was consistent with findings reported in previous literature (Collin et al., 2022b; Zulficar et al., 2019). Furthermore, the findings of this study were consistent with those of the entire Fen River (Li et al., 2023), which highlighted Pb and Cr as the main ecological risk factors. Notably, the Pb concentrations observed in this study were mostly lower than those of the entire Fen River (Li et al., 2023). On the one hand, this difference can be partially explained by the study area's site in the upper reach of the Fen River, where pollution levels tend to be lower. On the other hand, it also indicated that ecological and environmental remediation strategies in the Fen River basin have achieved some success. As previously reported, concentrations of heavy metals in the water bodies of the Fen River have declined in recent years (Chai et al., 2021).

Although the mean Pb concentration in the Fen River (mean: $4.78 \mu\text{g}\cdot\text{L}^{-1}$) was below the Class I threshold ($50 \mu\text{g}\cdot\text{L}^{-1}$) established by China's Environmental Quality Standards for Surface Water (GB 3838–2002) (MEPC, 2002), this finding suggested that current water quality standards may not adequately protect aquatic vegetation from Pb toxicity. It highlights a notable gap between regulatory chemical thresholds and their actual ecological effects. Lead, even at relatively low concentrations, can adversely impact plant growth and development by inhibiting seed germination, root elongation, transpiration, and chlorophyll production, ultimately impacting crop yields (Ashraf et al., 2017). The pervasive presence of Pb in freshwater ecosystems is primarily due to human activities such as mining, fossil fuel combustion, and manufacturing (Adnan et al., 2024), which underscores its persistent environmental threat. Furthermore, when Pb-contaminated water is used for irrigation, lead can accumulate in plants (Collin et al., 2022a). Lead can also enter aquatic organisms and the human body in both inorganic and organic forms through ingestion, inhalation, and dermal contact (Collin et al., 2022b). These concerns have prompted increasingly stringent regulatory measures regarding lead pollution, as evidenced by significant reductions in lead levels in various consumer products (Levin et al., 2021) and the U. S. Environmental Protection Agency's establishment of a Maximum Contaminant Level Goal of zero for lead in drinking water, acknowledging its potent neurotoxicity even at low concentrations (Waterhouse, 2024). The International Agency for Research on Cancer has classified lead as a Group 2B carcinogen, indicating its possible carcinogenicity to humans (IARC, 2025). Therefore, despite achieving regulatory compliance, the phytotoxicity observed in this study underscores the need for rigorous monitoring and remediation of lead contamination to mitigate potential ecological and human health risks associated with this persistent toxic metal (Kumar et al., 2022).

3.5 Limitations

Firstly, the TIE method employed had certain limitations, primarily its focus on individual classes of toxicants and their contributions to phytotoxicity, while neglecting potential interactions among various toxicants. When two or more heavy metals coexist, they can produce synergistic effects (Hoang et al., 2021), antagonistic effects

(Arreguin-Rebolledo et al., 2024), or additive effects (Emenike et al., 2021), resulting in significantly more complex toxic outcomes. Moreover, the interactions between metals and organic pollutants, such as PAHs (Yang et al., 2024), pesticides (Ayala Cabana et al., 2024), and microplastics (Ge et al., 2024), have not been thoroughly investigated. This lack of such a comprehensive analysis may substantially influence the overall toxicity and ecological risk evaluation of these contaminants.

Secondly, the scope for toxicological identification during the confirmation phase of the TIE procedure was limited. While this study successfully identified heavy metals as the primary toxicants, a minor portion of the toxic contribution in the Fen River waters also originated from certain electrophilic organic compounds. Future research should specifically focus on identifying these organic pollutants.

Finally, although the study targeted 10 heavy metals, it did not cover all metallic elements present in the Fen River. Other potentially toxic elements, such as arsenic and mercury, were not investigated due to the mercury memory effect and the interference of arsenic with the ArCl method.

4 Conclusion

This study utilized a TIE method based on seed germination to identify toxicants in the Taiyuan section of the Fen River as a case study. Toxicity screening with four vegetable seeds revealed significant phytotoxicity, particularly at site S6. Subsequent TIE treatments involved aeration, EDTA chelation, oxidant reduction, filtration, solid-phase extraction using a C18 column, and pH adjustment. Notably, lead was identified as the primary toxicant impairing seed germination in oilseed rape and cabbage, even though its concentrations were below regulatory thresholds. This finding underscores a latent ecological risk and demonstrates that the phytotoxicity-based TIE approach serves as a valuable tool for transitioning water-quality assessment from traditional chemical monitoring to more ecologically relevant evaluations.

Data availability statement

The original contributions presented in the study are included in the article/[Supplementary material](#), further inquiries can be directed to the corresponding author.

Author contributions

HQ: Writing – original draft. ZL: Methodology, Writing – original draft. HW: Methodology, Writing – original draft. XL: Visualization, Writing – review & editing. LL: Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. This work was supported by the Innovation and Entrepreneurship Training Program for College Students in Shanxi Province (20241194) and the project for optimizing

and standardizing the water supply and drainage system of the chemical laboratory (2025140711000092).

Conflict of interest

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Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/frwa.2025.1707872/full#supplementary-material>

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