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Evaluating the suitability of plant and crustacean microbiotests for assessing soil toxicity caused by ethylbenzene contamination

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Abstract. Plant and crustacean microbiotests were assessed for their suitability in evaluating soil and groundwater contamination with ethylbenzene (EB) following a railway accident. Bioassays using *Lepidium sativum*, *Sinapis alba*, *Sorghum saccharatum*, *Heterocypris incongruens*, and *Thamnocephalus platyurus* were conducted to measure acute toxicity in both naturally and artificially contaminated podzolic soils. Results of direct contact tests showed significant correlations between toxicity endpoints and EB concentrations. In naturally contaminated soils (EB: 67–2865 mg·kg^{-1}), seed germination decreased by 17–52%, and root growth by 55–70%. *L. sativum* and *H. incongruens* exhibited the highest sensitivity. *T. platyurus* also responded to EB in soil pore water and groundwater, although only temporary narcotic effects were observed at lower concentrations (≤76 mg·dm^{-3}). In contrast, artificially spiked soils did not affect seed germination but inhibited root elongation and crustacean growth. These findings highlight the influence of environmental factors, such as contamination duration and soil moisture, on EB toxicity and support the application of microbiotests in evaluating contaminated soils.

Introduction

Ethylbenzene (EB) is a volatile organic compound which occurs naturally in crude oil and coal tar and forms during forest fires. It has also been detected in certain food components, such as orange peels and parsley leaves. However, these natural sources are considered minor compared to anthropogenic emissions. Industrially, EB is synthesised primarily through the alkylation of benzene with ethylene. The pure compound is used almost exclusively in the production of styrene monomers and synthetic polymers in the plastic and rubber industries. In addition, it has several minor applications, including use as a petrol additive to improve the octane rating, as a solvent in paints, varnishes, and other surface coatings, and as a constituent of asphalt (IARC 2000). Due to human activities and demand, EB is emitted from a wide range of consumer products and has been widely detected at low concentrations in both indoor and outdoor environments (Hazrati et al., 2016, Bergoni et al., 2024). Consequently, the potential for human exposure to this chemical is considerable.

Though EB concentrations in uncontaminated areas are expected to be low, they may become highly elevated, as accidents and leakages of petroleum products are unavoidable. EB usually occurs as a constituent in the mixture of volatile organic compounds (VOCs), especially with benzene, toluene and xylene isomers (BTEX), the major air pollutants from vehicle exhaust (Tamrakar et al., 2022). It can also penetrate soil and groundwater due to leakage from underground storage tanks and pipelines, spillage of petroleum products during transport, as well as poor disposal of industrial and household wastes (Durmusoglu et al., 2010, Gross et al., 2013, Rodrigo-Ilarri et al.. 2023). While most documented incidents involve EB as part of BTEX, this study focuses on a rare case involving the release of pure EB during a railway accident. EB's properties and environmental fate are well recognized and documented (ATSD 2010, Yu et al., 2022). When discharged into the atmosphere, it has a low potential for entering other media, as it is rapidly photo-oxidised, although this breakdown may contribute to photochemical smog formation (Huang et al., 2010). In the soil matrix, however, due to its limited water solubility (152 mg·dm^{-3} at 20°C) and significant vapor pressure (1.24 kPa at 20°C), the key processes determining EB's fate are volatilization from the soil surface and biodegradation by soil bacteria (Weelink et al., 2010).

Over the last few decades, researchers have increasingly used bioassays to assess soil contamination and support

bioremediation efforts (Hubálek et al., 2007, Oleszczuk et al., 2012, Wieczorek and Baran, 2022). Considering the leachability of contaminants and the associated threat to groundwater, the toxic effects of the soil solid phase or soil extracts have been demonstrated on both terrestrial and aquatic species (Loureiro et al. 2005, Hentati et al., 2013, Szopka et al., 2021). Unlike chemical analyses, which quantify specific compounds, bioassays measure the combined biological effects of all substances present, accounting for factors such as bioavailability and toxic interactions (Blaise and Gagné, 2009, Łaszczyca et al., 2023). To reflect the complexity of ecological systems, bioassays often include species from different trophic levels, asthe diverse organisms forming complex food webs are simultaneously exposed to natural environments, and their responses may vary (Baker et al., 2022).

In the present study, plant and crustacean bioassays were applied to evaluate the effects of ethylbenzene under both natural and controlled conditions by examining two types of soil contamination. "Field-contaminated" soils were collected from a site affected by an accidental spill of pure ethylbenzene during a railway incident. These samples represent real-world environmental conditions, including the effects of contaminant ageing, soil moisture, redox status, and potential co-contaminants. In contrast, "laboratory-spiked" soils were prepared by amending uncontaminated podzolic soil with defined concentrations of ethylbenzene under controlled conditions. This approach allowed for the assessment of ethylbenzene-specific toxicity with reduced interference from soil heterogeneity or site-specific history.

The comparison of toxicity responses between these two exposure scenarios enabled the evaluation of how environmental factors influence the bioavailability and biological effects of ethylbenzene. This dual approach provided a mechanistically grounded and ecologically relevant interpretation of the results. Accordingly, the aims of the study were: (1) to assess the applicability of microbiotests for evaluating ethylbenzene-contaminated soils, and (2) to determine the extent to which environmental conditions modulate ethylbenzene toxicity.

While microbiotests are well established in ecotoxicological assessments, their application to real-world scenarios involving pure ethylbenzene contamination is rare. In addition, comparative studies evaluating both field-contaminated and laboratory-spiked soils remain limited, particularly for volatile organic compounds. By integrating these two approaches, the present study addresses a notable gap in current knowledge and offers new insights into how environmental conditions influence the bioavailability and toxicity of ethylbenzene in soil systems.

Materials and methods

Field sampling of contaminated soil

The study site was a 400 m^2 forest plot where 47 tons of pure liquid ethylbenzene were spilt due to a railway accident (50°19'13"N, 18°13'50"E). Sampling was conducted in 2021, over a year after the spill and subsequent land remediation. Despite natural attenuation processes such as volatilization and microbial biodegradation, the soil remained EB-saturated (100% of maximum water-holding capacity), appeared dark, and emitted a strong petroleum odor, indicating persistent contamination under anoxic conditions.

Three sampling sites were selected within the plot. At each site, 10 soil subsamples were randomly collected from the soil profile at different depth intervals: 0–25 cm and 25–50 cm. Subsamples from each depth were combined into one composite sample. Soil samples were taken using a Humax stainless steel core sampler and stored in airtight 500 ml glass jars with no headspace. At each site, soil pore water was extracted using a MacroRhizon tension sampler, while groundwater samples were obtained from three piezometers installed at a depth of 1.2 m using a stainless steel bailer. Groundwater was poured into sealed 200 ml glass bottles. All samples were immediately chilled in a portable freezer and transported to the laboratory, where they were stored at 4°C until further analysis (within 1 day).

Although one of the major problems with getting representative VOC samples is the significant loss that can occur throughout the entire procedure, from sample collection and transport to laboratory preparation, none of the solvents recommended in the literature for preservation were added to the EB-contaminated samples, in order to avoid exposing the test organisms to additional substances. Moreover, direct-contact bioassays were performed on the "fresh" soil matrix, i.e., without pretreatment.

Laboratory preparation of EB-spiked soil

Uncontaminated podzolic soil from a nearby forest area, similar to that at the spill site, was collected from a depth of 0–25 cm and used as a control matrix. After sieving through a 2 mm stainless steel mesh and homogenization, the soil was spiked with pure EB (98.5%) at two concentrations: 800 $mg \cdot kg^{-1}$ and 2800 $mg \cdot kg^{-1}$, corresponding to the maximum field concentrations found in the two analyzed layers. For each dose, 500 g of soil was placed in sealed glass jars, shaken on an orbital agitator for 48 hours, and then stored at 4°C for 7 days to ensure even distribution. Soil moisture was maintained at ~70% of water-holding capacity.

Chemical analysis

Standard methods were used to determine soil granulometry (aerometric method), pH (1:2.5 soil-water suspension), electrical conductivity (1:5 soil-water suspension), and organic carbon content (Multi N/C 3100 with HT 1300 furnace, Analytik Jena GmbH).

Ethylbenzene (EB) concentrations in soil samples were determined using a VARIAN CP-3800 gas chromatograph equipped with a flame ionization detector (GC-FID). EB was extracted from the samples using a 9:1 mixture of dichloromethane and hexane in a fexIKA® extractor. The extracts were then concentrated to 0.5 cm^3 and analyzed by gas chromatography coupled with a mass spectrometer (Varian 3900 GC-MS/MS). GC-MS analysis was performed in electron ionization (EI) mode at 70 eV. The ion source temperature was set to 250 °C, and the emission current to 300 µA. The gas chromatograph was fitted with a VF-5MS capillary column (30 m length, 0.25 mm internal diameter, 0.25 µm film thickness). The oven temperature program was as follows: initial temperature of 40 °C (held for 2 minutes), increased at a rate of 5 °C/min up to 100 °C, and held for an additional 3 minutes.

Ecotoxicity tests

Three standardized commercial tests were used:

- **Phytotoxkit** (plant bioassay): Measures seed germination and root elongation in *Lepidium sativum*, *Sinapis alba*, and *Sorghum saccharatum*. Seeds were incubated for 72 hours at 25°C in the dark in test containers filled with soil (10 seeds of each species per plate). Root lengths were measured using Image Tool 3.0. Toxicity was assessed based on percent inhibition relative to controls (Phytotoxkit 2004).
- **Ostracodtoxkit F** (soil contact bioassay): Uses *Heterocypris incongruens* neonates in direct soil contact. Plates (multiwells with 10 ostracods in each well) were incubated for 6 days at 25°C in the dark. Mortality and growth inhibition were recorded. Growth inhibition was calculated only for samples with <30% mortality (Ostracodtoxkit F 2001). The calculation was done according to the formula:
  % growth inhibition = 100 – [(growth in test soil/growth in reference soil) * 100]
- **Thamnotoxkit F** (liquid bioassay): Measures 24-hour mortality of *Thamnocephalus platyurus* exposed to soil pore water and groundwater samples after incubation at 25^{0}C in darkness, using multiwell test plates. Immobilization was recorded as the endpoint (Thamnotoxkit F 1991).

All tests were run in triplicate following manufacturer protocols (MicroBioTests Inc., Belgium).

Data analysis

All data were tested for normality using the Shapiro–Wilk test and for homogeneity of variance using Kolmogorov–Smirnov test. Due to violations of these assumptions, nonparametric methods were applied. Spearman's rank correlation was used to assess relationships between EB concentrations and bioassay outcomes. Differences among plant species were tested using Kruskal–Wallis test, while comparisons between field- and laboratory-contaminated soils were performed using the Mann–Whitney U test. All analyses were conducted in STATISTICA 13.3 with $\alpha = 0.05$.

Results

Bioassays on field-contaminated soils with ethylbenzene (EB)

Soil characteristics

Granulometric analysis of the investigated soils indicated that the soils had a coarse-texture with limited fine particles and minor variations in finer fractions. The contaminated soil was classified as loamy sand, containing 89% sand, 6% clay, and 5% silt. The control soil, collected from a nearby uncontaminated area and also used in the laboratory experiment, showed a similar granulometric profile (87% sand, 7% clay, and 6% silt. Basic chemical parameters and their ranges within the soil profile are summarized in Table 1. Previous liming increased soil pH, which ranged from 6.3 to 7.6. The upper soil layer (0-25 cm) was generally neutral to slightly alkaline, whereas the deeper layer (25-50 cm) was slightly acidic. Electrical conductivity (EC) and organic carbon (C_{org}) content decreased with the profile depth. In contrast, the EB content varied substantially between individual sampling sites and horizons within the contaminated area ranging from 67 to 800 mg·kg^{-1} in the upper layer and from 870 to 2865 mg·kg^{-1} in the deeper layer, with the highest contamination consistently found at greater depths.

The EB concentration in soil pore water was also excessively high, ranging from 369 to 1314 mg·dm^{-3}) across the studied sites. In groundwater, concentrations were more than tenfold lower (from 25 to 76 mg·dm^{-3}) but still exceeded regulatory thresholds.

Plant bioassays

Field-contaminated soil adversely affected the development of the plants used in the acute toxicity test. All plant species showed reduced seed germination, which was negatively correlated with EB content (Spearman's R = – 0.83 for *S. saccharatum*, – 0.82 for *L. sativum*, – 0.65 for *S. alba*). Although EB caused visible inhibition of seed germination, especially in *L. sativum* and *S. saccharatum*, in all soil samples (Fig. 1A), statistically significant differences compared to their controls occurred only in samples collected from the deeper soil layer , and in *L. sativum* also in samples from the upper layer (Kruskal–Wallis test, $p < 0.05$). After 3 days of exposure, average reductions in seed germination were: *S. alba* – 17%, *L. sativum* – 43%, and *S. saccharatum* – 52%.

The results of root growth inhibition were consistent with those for seed germination (Fig. 1B). Root elongation was negatively correlated with EB content in soil, with correlation coefficients of –0.59 (*S. saccharatum*), – 0.48 (*L. sativum*), and – 0.46 (*S. alba*). In the control soil, median root lengths ranged from 94.7 mm in *L. sativum* to 69.6 mm in *S. saccharatum*. No significant differences in root elongation were observed between control and upper-layer soil samples, due to the considerable variability in *L. sativum* and *S. alba* responses, which ranged from growth inhibition to stimulation. Comparable results were obtained for *S. saccharatum*. In contrast, root elongation in plants grown in soil from a depth of 25–50 cm differed significantly from both the control and the upper layer (Kruskal–Wallis test, $p < 0.05$).Median lengths

Table. 1. Selected chemical properties of EB-polluted soil (min–max. range)

Soil	Depth (cm)	pHH_2O	EC (µS·cm^{-1})	C_{org} (%)	EB (mg·kg^{-1})
Contaminated	0–25	7.0–7.6	79–113	0.55–0.72	67–800
	25–50	6.3–7.4	27–40	0.32–0.55	870–2865
Uncontaminated (Control)	0–25	5.3–5.6	76–92	1.36–1.61	0.048–0.057
	25–50	4.9–5.2	30–46	0.42–0.51	0.029–0.032

decreased to ~14 mm in *L. sativum* and *S. saccharatum*, and to 21.3 mm in *S. alba*. The greatest inhibition (70%) occurred in *L. sativum* and *S. saccharatum.*

Crustacean bioassays

For the ostracod species *H. incongruens*, mortality strongly correlated with soil EB content (R = 0.82) and it also differed significantly between the two layers of the soil profile. At the end of the 6-day exposure, mortality reached 100% in all deep-layer samples and ranged from 50% to 100% in upper-layer soils, depending on EB concentrations. *T. platyurus* showed 40 - 100% mortality in soil pore water, with strong correlation with EB levels (R = 0.94). In groundwater, no mortality was observed, though narcotic symptoms (immobilization) occurred at higher EB concentrations. These effects were temporary and subsided within 24 hours.

1.2. Bioassays on laboratory-spiked soils

Soil characteristics

The chemical properties of the soil used in the experiment are shown in Table 1. The control soil was acidic (mean pH 5.4) with typical podzolic organic carbon content (1.52%).

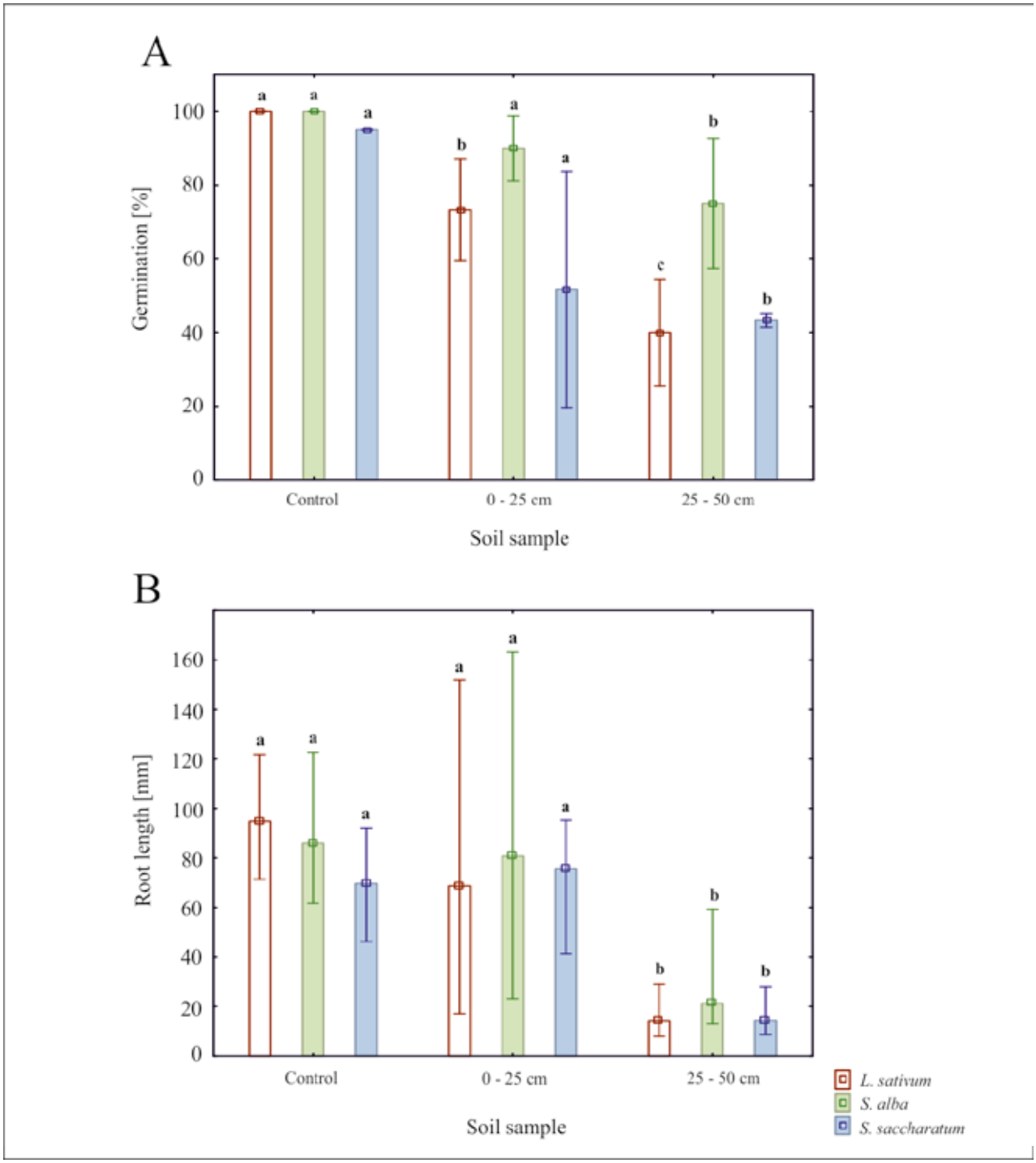

Figure 1. Seed germination (A) and seedling growth (B) of tested plants in control and individual layers of EB-contaminated soils. Bars indicate the median, whiskers the 25th and 75th percentiles, respectively. Significant differences from controls (p < 0.05) are marked with letters.

Table 2. Endpoints of bioassay with ostracod *H. incongruens* on soil treated with EB.

Soil	Mortality (%)		Body length (µm)	
	range	mean ± SD	range	mean ± SD
Control	0	0	500–600	545.4±32.5
S1	0–20	10±8.9	300–450	333.3±48.0
S2	90–100	96±5.2	—*	—*

* No body measurement due to high mortality

Plant bioassays

The effect of artificially contaminated soil on plant germination differed from the results obtained with soil collected from the accident area. Seed germination remained above 80%, indicating no significant inhibition at EB concentrations of 800 and 2800 mg·kg^{-1}. In contrast, root growth was adversely affected by contaminated soil (Spearman's R = -0.84 for *L. sativum*, -0.71 for *S. alba,* -0.48 for *S. saccharatum*), species-specific responses differed (Fig. 2). *L. sativum* showed the strongest response, with median root length differing significantly among all treatment groups (Kruskal–Wallis test, $p < 0.05$) and inhibition of approximately 28% and 45% at 800 mg·kg^{-1} (S1) and 2800 mg·kg^{-1} (S2), respectively. *S. alba* showed comparable average inhibition (33% and 45%) but with greater variability. *S. saccharatum* had the lowest root elongation rate overall, even in the control soil (median 52.4 mm), and showed inhibition of approximately 26% and 32% in soils S1 and S2, respectively.

Crustacean bioassays

At 800 mg·kg^{-1} EB (S1), mortality of the ostracod species *H. incongruens* was low (<20%), but growth was inhibited by 30 - 40%. At 2800 mg·kg^{-1} EB (S2), mortality exceeded 90%, preventing growth assessment (Tab. 2).

Field vs. laboratory comparison

The results of the toxicity analysis using direct contact tests differed markedly. Toxicity was significantly higher in field-contaminated soils than in laboratory-spiked soils with similar EB concentrations (Mann–Whitney U test, $p < 0.05$). Figure 3 illustrates the phytotoxicity of soils containing approximately 800 mg·kg^{-1} of EB. Field soils had reduced endpoint variability and greater phytotoxicity, likely due to differences in soil moisture, structure, and ageing effects. A similar data distribution was obtained at higher EB concentrations.

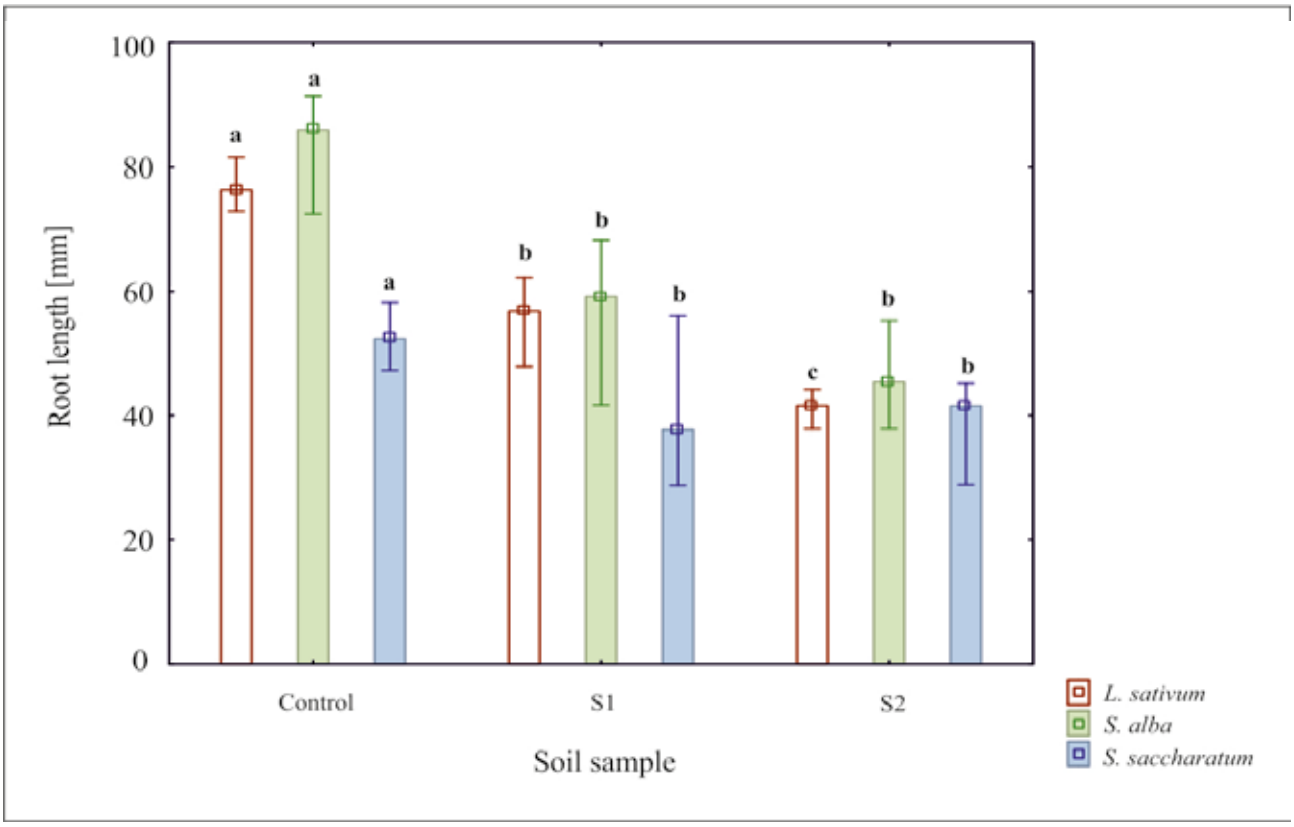

Figure 2. Root growth of tested plants in control and artificially EB-contaminated soils. Bars indicate the median, whiskers the 25th and 75th percentiles, respectively. Significant differences from controls ($p < 0.05$) are marked with letters.

Equally significant differences were observed in seed germination. In contrast to laboratory-spiked soils, where inhibition ranged from 0 to 20%), seed germination was limited in field-contaminated soil samples under anaerobic conditions, with greater variability among plant species. Overall, inhibition was 15% (*S. alba*), 52% (*L. sativum*) and 65% (*S. saccharatum*).

Discussion

This study confirmed the usefulness of plant and crustacean microbiotests for assessing ethylbenzene (EB) toxicity in both soil and aqueous environments. The use of multiple bioassays spanning different trophic levels allowed for a comprehensive understanding of EB's ecological impact, particularly in a real-world contamination scenario following a chemical spill.

It is recognized that field-contaminated and laboratory-spiked soils differ substantially in terms of environmental complexity, contaminant history, and exposure conditions. However, rather than serving as directly comparable systems, these two soil types provide complementary perspectives on ethylbenzene toxicity. The field-contaminated soils reflect long-term environmental exposure, including the effects of aging, soil saturation, and potential co-contaminants. In contrast, the laboratory-spiked soils offer a controlled context in which ethylbenzene is the sole variable. By examining toxicity patterns across both settings, this study highlights how environmental factors modulate the expression of chemical toxicity and supports a more ecologically informed interpretation of bioassay results.

The natural rate of EB attenuation in soil is influenced by soil temperature, rainfall and soil depth. In the area of accidental EB spill, its content increased with the depth of the soil profile because in deeper layers, dispersion prevails over volatilization, and the dispersal process depends not only on the EB volatility but also on the EB-soil matrix interaction (Serrano et al. 2006). Acute toxicity was fully confirmed in direct contact tests on the soil matrix from the spill site by plants and the ostracod *H. incongruens*. The highest toxicity was observed in the deeper soil layers, where EB concentrations were markedly elevated. Both root growth inhibition in plants and ostracod mortality increased with EB content. Numerous studies have shown that germination rate and root elongation are valuable outcome parameters in assessing soil phytotoxicity (e.g., He et al., 2023, Pusz et al., 2024). Consistent with previous findings, root elongation proved to be a more sensitive phytotoxicity endpoint than seed germination for assessing petroleum hydrocarbon effects (Tang et al., 2011). Interestingly, *H. incongruens* exhibited 100% mortality in all deep-layer soil samples, highlighting its sensitivity to EB exposure in saturated, anaerobic conditions. Pore water samples also exhibited high toxicity to *T. platyurus*, whereas groundwater samples did not cause mortality.

These outcomes emphasize how test matrix and chemical behavior (e.g., volatility, solubility) influence toxicity results. They also align with previous research demonstrating that bioassays on whole soil often yield stronger toxicity signals than tests on soil extracts. For example, Domínguez-Rodríguez et al. (2020) showed 100% mortality of the earthworm *Eisenia fetida* after direct exposure to herbicide-contaminated soil matrix, whereas no mortality was observed in soil extracts. When assessing the phytotoxicity of soils enriched with sewage sludge, Oleszczuk et al. (2012) also pointed out the higher toxicity of the solid phase of soil than its extracts.

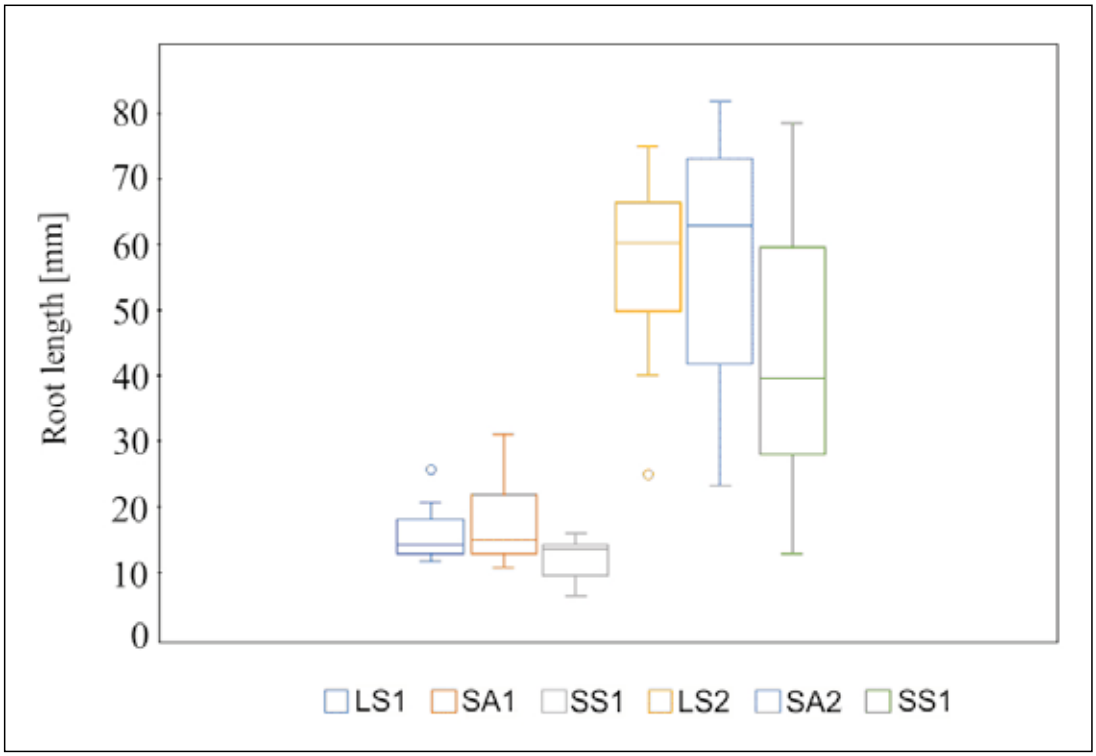

Figure 3. Root elongation of tested plants in EB-contaminated soils (1 in field, 2 in laboratory). The solid line, box, and whiskers indicate the median, interquartile range, and min–max range without outliers. LS – *Lepidium sativum*; SA – *Sinapis alba*; SS – *Sorghum saccharatum*.

These differences may result from many factors, including differences in the physicochemical properties of the tested matrix (Balseiro-Romero et al., 2016), the sensitivity of individual species (Di Marzio and Saenz, 2006, Wu et al., 2021) and the bioavailability of contaminants, which may lead to discrepancies between the level of contamination determined chemically and the observed toxic effects (Loureiro et al., 2005, Czerniawska-Kusza and Kusza, 2011). The described pattern, however, is attributable to direct exposure routes and enhanced bioavailability of hydrophobic compounds in solid matrices.

The test procedure is also crucial when it comes to VOCs, since static methods are generally subject to the problem of VOCs' stability. EB's high volatility and poor water solubility, which favor its rapid loss from solution, may cause differences in toxicity depending on the system used: flow-through, static renewal or static (Masten et al., 1994). For example, the mean 48-hour LC_{50} values for *D. magna* exposed to ethylbenzene were 75 $mg \cdot kg^{-1}$ in the static test (LeBlanc, 1980) and 20 $mg \cdot kg^{-1}$ in the flow-through one (Bobra et al., 1983). This is probably why acute toxicity was not confirmed in groundwater samples. In the static system, the test plate wells' capacity of 1 ml did not ensure the longer maintenance of the toxic concentration of the volatile compound in solution when its content in the sample at the beginning of the procedure was no more than 76 $mg \cdot kg^{-1}$. In groundwater samples, *T. platyurus* experienced only temporary narcotic symptoms. Above a certain level within tissue lipids, the mode of EB toxicity is narcotic (Di Marzio and Saenz, 2006). Zheng and Zhou (2017) also did not observe mortality after exposing the freshwater snail *Bellamya aeruginosa* to EB concentrations ranging from 1 to 100 $mg \cdot kg^{-1}$, but they did not observe a distress neurotoxic effect (96-hour EC_{50} was 13.3 $mg \cdot kg^{-1}$ in the renewed test solution system). Thus, for volatile compounds like EB, static test systems can lead to concentration losses, underestimating toxicity in aqueous media.

While ethylbenzene concentration was clearly associated with increased toxicity across all bioassays, the interpretation of these effects must consider differences in soil physicochemical properties, particularly between the field-contaminated and reference soils. Although the control soil was collected from an adjacent, uncontaminated area, it differed in both pH and organic carbon content. Specifically, the field-contaminated soils had a higher pH due to prior liming and lower organic carbon content, whereas the reference soil was more acidic and richer in organic matter. Higher organic matter typically enhances the sorption of VOCs, reducing their availability to organisms, whereas lower organic content and higher pH may facilitate desorption and increase toxicant exposure (Albergaria et al. 2010, Insam and Seewald, 2010). These differences could partly explain the stronger toxic responses observed in the field soils, even when EB concentrations were comparable to those in laboratory-spiked samples.

Comparisons between field and laboratory experiments revealed that EB toxicity is influenced not only by concentration, but also by soil moisture and ageing. Szopka et al. (2021) reported that ostracod mortality and growth inhibition were greater under waterlogged,anoxic conditions compared to 70% soil moisture conditions, which is consistent with our results. The concept of ageing - time-dependent changes in contaminant binding and bioavailability – further explains why artificially spiked soils showed lower toxicity despite matching EB concentrations. In freshly contaminated soils, EB may not have sufficient time to form strong sorptive interactions with the soil matrix, resulting in higher organismal exposure (Bahar et al., 2024).

Moreover, other environmental factors such as redox conditions, microbial activity, and the presence of co-contaminants likely influenced toxicity outcomes. Although only EB was measured in this study, the site's proximity to a railway suggests that additional toxicants may have been present, potentially acting additively or synergistically. Such interactions are common in contaminated environments and could contribute to the variability observed in plant responses, especially in *L. sativum* and *S. alba*, which showed both inhibition and stimulation in upper-layer soil samples under varying conditions. On the one hand, liming might suppress toxic effects and increase soil pH up to values where nutrient availability is higher (Paradelo et al., 2015), especially in soils with the lowest EB content. On the other, the co-occurrence of various contaminants may enhance their toxicity through mutual interactions (Aivalioti et al., 2010).

Nonetheless, the strong and consistent correlations between EB levels and toxicity endpoints (e.g., Spearman's $R = -0.83$ for seed germination, $R = 0.82$ for ostracod mortality) indicate that ethylbenzene was the primary driver of the observed effects. However, the role of soil matrix characteristics cannot be excluded, as these properties may either attenuate or amplify toxic responses. Such interactions should be considered when interpreting ecotoxicological data.

Conclusions

This study demonstrates the utility of microbiotests using plants and crustaceans for assessing the ecotoxicological impact of ethylbenzene (EB) in soil and water. Acute toxicity was observed in all bioassays using field-contaminated soil, with strong correlations between EB concentration and biological effects. *Lepidium sativum* and *Heterocypris incongruens* were particularly sensitive, showing significant reductions in root growth of up to 70% and high mortality rates of 50-100%, respectively.

Differences between field-contaminated and laboratory-spiked soils underscore the importance of environmental factors, such as contamination ageing, soil moisture, pH, and organic matter, in shaping contaminant bioavailability and toxicity. Although EB was the principal toxicant, variations in soil properties likely influenced organism responses and should be accounted for in future assessments.

Combining direct-contact and aqueous-phase tests provided complementary insights into the effects of EB across environmental compartments. While pore water showed acute toxicity to aquatic crustaceans (40-100%), groundwater effects were limited to reversible narcotic symptoms, highlighting the limitations of static testing systems for volatile compounds.

Overall, this study supports the inclusion of standardized microbiotests in soil contamination monitoring, particularly for volatile organic pollutants. These tools offer rapid, cost-effective, and ecologically relevant data that can guide environmental risk assessments and remediation strategies.

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Oszacowanie przydatności mikrobiotestów z roślinami i skorupiakami w ocenie toksyczności gleby spowodowanej zanieczyszczeniem etylobenzenem

Streszczenie. Oceniono przydatność mikrobiotestów roślinnych i ze skorupiakami w ocenie skażenia gleb i wód gruntowych etylobenzenem (EB) po katastrofie kolejowej. Przeprowadzono analizy z użyciem biotestów zawierających Lepidium sativum, Sinapis alba, Sorghum saccharatum, Heterocypris incongruens i Thamnocephalus platyurus w celu określenia toksyczności ostrej w glebach bielicowych zanieczyszczonych w środowisku naturalnym oraz w warunkach laboratoryjnych. Wyniki testów bezpośredniego kontaktu wykazały istotne korelacje pomiędzy efektami końcowymi a stężeniami EB. W glebach zanieczyszczonych (EB 67–2865 mg.kg-1) odnotowano spadek kiełkowania nasion o 17-52% i zahamowanie przyrostu korzeni o 55-70%. L. sativum i H. incongruens wykazały najwyższą wrażliwość. T. platyurus również zareagował negatywnie na EB w wodzie porowej gleby i wodach gruntowych, choć przy niższych stężeniach (≤76 mg.dm-3) obserwowano jedynie tym czasowe efekty narkotyczne. Natomiast gleby zanieczyszczone EB w warunkach laboratoryjnych nie wpłynęły na kiełkowanie nasion, ale hamowały wzrost korzeni oraz rozwój skorupiaków. Uzyskane wyniki podkreślają wpływ czynników środowiskowych, takich jak, czas trwania skażenia i wilgotność gleby, na toksyczność EB i wskazują na przydatność stosowania mikrobiotestów w ocenie zanieczyszczonych gleb.