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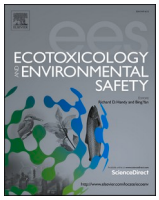

Sorption to mulch film decreases bioavailability of two model pesticides for earthworms in soil

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ABSTRACT

Microplastic pollution and its environmental consequences have been a research topic for decades. Alongside sewage sludge and compost, mulch films are one of the most important sources of organic matter in agricultural soils. As microplastics accumulate in these environments, they increasingly interact with other agrochemical contaminants, such as pesticides. The consequences of the combined presence of mulch film microplastic and pesticides, especially on bioavailability and toxicity, still need to be understood. We investigated the interaction of pesticides with polyethylene mulch films by choosing the neonicotinoid Thiacloprid and the fungicide Tebuconazole in their commercial formulations as plant protection products, serving as representative hydrophobic and hydrophilic substances. The impact of PE microplastic (150 mg/kg) on both pesticides' extractability and earthworm mortality was determined. The hypothesis is that microplastics reduce pesticide bioavailability in soil. Hence, reduced earthworm toxicity might be observed. Acute toxicity testing with *Eisenia fetida* showed a slight decrease in toxicity while MPP was present, shifting the LC50 from 110.3 to 120.6 mg/kg (Tebuconazole) and 20.3–22.6 mg/kg (Thiacloprid). The chemical analysis after two-time extraction of test soil with $CaCl_2$ and then solvents confirmed the reduced availability of pesticide in the presence of MPP. Our research shows that microplastic at application-relevant concentrations can retain pesticides and reduce pesticide toxicity. These findings impact pesticide efficiency in soils that accumulate microplastic, potentially requiring the adaptation of agricultural practices. This study underscores the need for further research and mitigation strategies as our understanding of the interactions between pesticides and microplastics in soil evolves.

1. Introduction

In recent years, the global scientific community has become increasingly concerned about the potential effects of microplastic pollution, with a growing interest in terrestrial ecosystems. This concern is underscored by the fact that the amount of plastic released into terrestrial systems is estimated to be 4–23 times higher than in oceans (Horton et al., 2017a). The topic of microplastics has been intensively discussed in scientific and public media. Historically, microplastic is defined as plastic particles ranging between 1 μm and 5 mm. It occurs in the environment as primary microplastic, which is produced and discharged directly at the microscale, or secondary microplastic, which results from degradation and further downsizing of larger plastic materials through various processes (Thompson et al., 2004). Microplastic particles have emerged as a ubiquitous environmental contaminant, representing an intricate and multifaceted challenge to environmental scientists, policymakers, and society.

One crucial soil-based final receptor of (micro)plastic is agricultural land, where materials such as sewage sludge, gardening foils, and mulch films have been utilized effectively to enhance plant growth and boost

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yields (Iqbal et al., 2020). However, these materials also contribute to the accumulation of embedded and worn microplastic particles (MPP), with levels reaching as high as 67.5 g/kg soil (Fuller and Gautam, 2016). Microplastics in soil can subsequently affect the soil ecosystem by, for example, altering the bioavailability of nutrients through changes in soil structure and microbial activity (Cheng et al., 2024). Furthermore, plastics can release and sorb other soil contaminants and chemicals, though the consequences and mechanisms of these interactions remain not fully understood.

The sorption of pesticides to MPP has several environmental implications. Most notably, it changes the distribution and bioavailability of pesticides in terrestrial target sites (Peña et al., 2023). Pesticides are essential in modern agriculture, providing effective crop protection and increasing productivity (Cooper and Dobson, 2007). However, their widespread use raises serious environmental concerns due to their persistence, mobility, and unintended toxicity to non-target organisms. Understanding how pesticides interact with MPP in soil is crucial, as these interactions may influence the behavior and degradation of both pesticides and MPP. The potential of MPP to act as a vector for transporting and accumulating pesticides in soil and agricultural ecosystems is particularly worrisome.

Since the topic of contaminant sorption began to grow in the scientific community in the early 2000s, Tourinho et al. (2023) found that most studies focus on freshwater and marine samples (97) compared to soil studies (28). Previously, Tourinho et al. (2019) reported greater partitioning of chemical contaminants to MPP in soil than in aquatic ecosystems, highlighting a significant knowledge gap. The authors also pointed out a lack of research involving soil invertebrates, a gap that we aim to address here.

Microplastic particles have been shown to affect the ecotoxicity and accumulation of pesticides in different ways. Many studies have documented the role of microplastic particles as vectors and carriers, revealing that organic pesticides are transmitted more efficiently into exposed earthworms (Sun et al., 2021; Cheng et al., 2020). As Wang et al. (2020a) pointed out, polyethylene (PE) mulch film MPP act as carriers of pesticides in agricultural environments, mainly through physical and chemical interactions and correlating with octanol-water coefficients (log Kow). The effects of pesticides adsorbing onto plastics include increased stability and changes in how these chemicals are available in soil. Interestingly, MPP can also help reduce pollutant effects by adsorbing contaminants, thus decreasing their availability to earthworms, as discussed by Zhang et al. (2022) and Tourinho et al. (2023).

Pesticides are mainly sorbed by microplastic particles through hydrophobic partitioning, which includes surface adsorption and pore filling, thus being more pronounced on aged MPP or those with biofilm presence. This process is supported by hydrogen bonding, π–π interactions, and, for ionizable pesticides or aged plastics, electrostatic interactions. Van der Waals forces also contribute to overall adsorption but are generally less significant (Peña et al., 2023).

Overall, the effects of these interactions heavily depend on the type of (micro)plastic and pesticide, as well as the test conditions, especially the soil chosen. Many studies so far have mainly looked at pristine, lab-made microplastics or single active ingredients (Sun et al., 2021; Gautam et al., 2024; Shi et al., 2022), which might not fully represent the complexity of real-world situations.

This highlights the complex interactions of pollutants in soil ecosystems, requiring detailed research and possible environmental safety measures.

This study aims to assess the impact of mulch film microplastic on pesticide toxicity in soil. While the combination of pesticide active ingredient and microplastic has been assessed a few times already (Cheng et al., 2020; Shi et al., 2022; Baihetiyaer et al., 2023), we combine realistic exposure of pesticide formulations with realistic mulch film fragments in a real soil.

The earthworm *Eisenia fetida* was chosen as a representative soil organism for an acute toxicity test and was exposed to two pesticides. The study investigated the typical triazole fungicide Tebuconazole (Teb) and the neonicotinoid Thiacloprid (Thia) in their commercial formulations, Folicur® and Biscaya®, respectively. The active ingredients have log Kow values of 3.7 and 1.26 (National Center for Biotechnology Information, 2025a, 2025b). Commercial formulations were selected to better reflect real-world conditions. Many cited studies use MPP concentrations that far exceed realistic levels, with effects due to MPP itself, which this study aimed to avoid. Instead, a concentration of 150 mg/kg was set as a realistic hotspot level. To date, no toxic effects have been reported at this concentration (Weltmeyer and Roß-Nickoll, 2024; Rodríguez-Seijo et al., 2017, 2018a,b). The study also included chemical analysis of pesticide concentrations in the soil. Pesticide availability heavily depends on their sorption behavior in soil. Therefore, we hypothesized that: a) the presence of microplastic significantly reduces the extractability of the selected pesticides; b) Tebuconazole, being more hydrophobic, would be retained in the soil and on microplastic particles to a greater extent than Thiacloprid; and c) the strength of binding could be understood through different extraction methods used to remove the pesticide from the soil.

The results are discussed considering the increasing use of mulch films in agriculture and the different factors necessary for safe and sustainable application.

2. Material and methods

2.1. Microplastic production

FKuR Kunststoff GmbH (Willich, Germany) provided polyethylene-based mulch films. Microplastic was created by cryo milling (Thiacloprid experiments) or milling (Tebuconazole experiments) the mulch film. Differences in microplastics arose from the time intervals between pesticide investigations and the availability of the cryo milling facility.

For the experiments with Tebuconazole, the microplastic was manually pulverized with sodium chloride in a ceramic mortar and then rinsed with deionized water. The resulting fractions of MPP were 0.3 mass of 50 μm - 100 μm, 28.5 mass of 100 μm - 500 μm, and 71.2 mass of 500 μm - 1000 μm. All particles used were sieved to a size range of 50 μm to 1 mm with metal sieves and stored in the dark until use.

For the experiments with Thiacloprid, the size range of the resulting polyethylene particles was measured using a confocal Raman microscope alpha300 R (WITec GmbH, Germany) at 5x magnification (Zeiss EC Epiplan-5x/0.13), as described in Wenzel et al. (2022). The image size was 35.8 × 26.3 mm, with 30 × 22 images stitched together, resulting in 660 images, of which 8 were stacked in 500 μm on the z-axis for a single picture. Image analysis was performed with the WITec ParticleScout software (version 5.3.18.110), which automatically set a brightness threshold to mask all bright particles against the dark background. All particles were masked to allow for size and number determination. Particle size was characterized by the maximum Feret diameter, measuring the greatest distance between two parallel tangents at any angle around the particle.

403 particles were identified through contrast analysis, and 270 overlying particles were excluded to minimize adulteration of results caused by electrostatic interaction.

The Appendix (Table A2) provides a detailed characterization of the particles and their size distribution.

2.2. Biological analysis of acute toxicity

Adult *Eisenia fetida* were exposed to the respective pesticide (Tebuconazole (PPP: Folicur) and Thiacloprid (PPP: Biscaya)) exclusively and in co-exposure with microplastics for 14 days according to the acute toxicity test of OECD guideline 207 (1984) and as detailed in, e.g., Lackmann et al. (2023). Due to increased environmental relevance, the pesticides were tested here in their formulation as commercial plant

protection products. For all scenarios, three technical replicates and three biological replicates were examined. Each test day included a negative control with pure wet soil and an additional control with 150 mg/kg mulch film microplastic particles on test days with MPP co-exposure. Each replicate contained 500 g_{ww} soil. The pesticide formulations were dissolved in deionized water to achieve test concentrations of 0, 50, 75, 100, 150, and 300 mg/kg dry soil for Tebuconazole and 0, 0.5, 1, 5, 25, 50, 150, 250, and 500 mg/kg for Thiacloprid. To prevent clustering, the microplastic particles were thoroughly mixed with the soil before slowly pouring the pesticide formulations into the constantly stirred soil.

At the start of the test, 10 adult earthworms were weighed and placed on the soil surface in each vessel. After 14 days of exposure (end of test), worms were gently touched at the mouth to assess mortality by observing the lack of response. Surviving worms were counted and weighed again. The weight change was calculated based on the average weight of the worms in each vessel, excluding dead animals, compared to the control.

Pesticide concentrations were chosen based on previous range-finding experiments. Deionized water was used to eliminate the effects of a co-solvent. For Thiacloprid, not every replicate included all concentrations due to the different sensitivities of the selected group of worms, but there was always a minimum of five concentrations.

The tests used microplastic from PE mulch film measuring 50–1000 μm (see Table A2 for details). The exposure level was set at 150 mg/kg, a realistic concentration for hot spots, but no toxic effects have been reported (this study; Rodríguez-Seijo et al., 2017, 2018).

RefeSol 01-A, a natural reference soil, was provided by the Fraunhofer Institute for Molecular Biology and Applied Ecology IME (Schmallenberg, GER). The soil was collected from the top 25 cm of an agricultural field in Schmallenberg, Germany (51°09′N, 8°18′E), which was previously cultivated with winter wheat and managed extensively without pesticide use for at least three years. After sampling, it was sieved to ≤ 2 mm and stored at 4 °C under field moisture conditions. It is a Dystric Cambisol with slightly loamy sand, medium acidity, and very slight humic content, with physicochemical properties listed in Table A1.

2.3. Chemical analysis of pesticide extractability

To investigate the extractability of the pesticides in co-exposure with microplastics, three soil samples of approximately 1 g wet weight each were taken immediately after mixing the pesticide into the soil from a randomized position within the jar and then dried in air for 24 h. The dry soil was then shaken horizontally in 10 mL of $CaCl_2$ per gram of dry soil for 24 h at 100 rpm, followed by centrifugation at 4000 rpm for 30 min. From each extraction, 500 μL of the supernatant was transferred into a 1 mL pre-cleaned HPLC vial, and the remaining supernatant was discarded.

Subsequently, a more rigorous extraction was performed using the same samples to retrieve more firmly bound pesticide molecules. 25 mL of a methanol and dichloromethane mixture (1:1, v:v) was added to 1 g of centrifuged soil and shaken thoroughly until the soil was evenly dispersed in the solution. The tubes were then placed in a cooled ultrasound bath (Bandelin Sonorex, 50/60 Hz) for 30 min, followed by centrifugation at 4000 g for 5 min. From each sample, 500 μl of the supernatant was taken for analysis.

The aqueous phase, the 500 μL supernatant, was analyzed for pesticides using high-performance liquid chromatography (1200 Series, Agilent Technologies, USA) combined with mass spectrometry and an LTQ XL linear trap (Thermo Fisher, Waltham, USA). Ionization was performed with heated electrospray ionization (HESI). Tebuconazole and Thiacloprid were separated on a Phenomenex Synergi Hydro-RP column (250 ×2 mm; particle size: 4 μm, pore size: 80 Å) using mobile phases of pure water with 0.1 % formic acid and methanol with 0.1 % formic acid. Using a flow rate of 0.6 mL/min, retentions times resulted in 6.98 min (Tebuconazole) and 6.5 min (Thiacloprid).

Xcalibur and Quan/Qual Browser version 2.0.7 (Thermo Fisher) were used for data analysis. Data were calculated based on the respective negative controls, which were set to 0 mL/mg as the total absence of pesticides. For quantification, a standard curve with 8–12 concentrations was created for interpolation.

2.4. Statistical analysis

The statistical analysis for toxicity testing was performed using the GLP-compliant software ToxRat (ToxRat Solutions GmbH) and the template "Earthworm, Acute Toxicity Test (OECD, 1984) Soil." Probit analysis and the Step-down Rao-Scott-Cochran-Armitage test procedure were employed to determine the lethal and effect concentrations of 50 % (LC50, EC50) for the acute toxicity tests. Weight differences were assessed with Shapiro-Wilk's test for normality and Levene's test for homoscedasticity (using residuals) before applying the Williams Multiple Sequential *t*-test procedure. Differences between exposure scenarios were evaluated with paired *t*-tests at a significance level of $p = 0.05$.

Analytical results were compared (co-exposed/non-co-exposed) using a Welch ANOVA with the Brown-Forsythe test and post-hoc Dunnett's multiple comparisons tests. Data from the negative controls were used to normalize the data.

3. Results and discussion

3.1. Chemical analysis

3.1.1. Bioavailability of active pesticide ingredients in soil

The chemical analysis of soil extracted with CaCl2 showed no significant differences in Tebuconazole amounts concerning MPP, except for the middle concentration of 75 mg/kg (Dunnett's test, $p = 0.01$). In contrast, MeOH/DCM extraction revealed significant reductions ($p < 0.02$) in extractable amounts with MPP co-exposure for all concentrations except 150 mg/kg ($p = 0.06$). Data are presented in Fig. 1 and Tables A4 and A5.

The detected Tebuconazole concentrations after solvent extraction were significantly higher at each concentration compared to $CaCl_2$ extraction for both treatments.

Significant differences between treatments were observed for CaCl2-extracted Thiacloprid, especially at the three highest comparable concentrations (Dunnett's test, $p < 0.0001$). For solvent-extracted Thiacloprid, only the highest concentration of 250 mg/kg revealed significantly higher levels in the treatment without MPP ($p = 0.0002$).

Further, the amount extracted with calcium chloride compared to the organic solvents was higher for Thiacloprid, whereas solvent extraction was much more effective for Tebuconazole.

Trend lines derived from the linear regression for Thiacloprid indicate that the slope of the fitted curves is significantly different from the slope with co-exposure in both pesticide and extraction scenarios ($p < 0.0001$). For Tebuconazole, the slopes for MeOH/DCM extraction also differ significantly ($p < 0.0001$), but not for CaCl2 extraction ($p = 0.3$) (Fig. 1).

Our results do not support the hypothesis that polyethylene microplastic particles in soil retain Tebuconazole more effectively than Thiacloprid. Tebuconazole was completely extracted with and without MPP; however, the presence of MPP slightly increased the $CaCl_2$ extractable fraction while decreasing the MeOH/DCM extractable amounts. Specifically, $CaCl_2$ extracted 0.67–1.05 (Ø 0.8) times less Tebuconazole without MPP. In solvent extraction, the amount extracted was 1.19–1.71 (Ø 1.4) times higher without MPP (Table A4). Thiacloprid availability was significantly greater without MPP, with extraction amounts being 10–28 (Ø 15.6) and 4–19 (Ø 7.8) times higher with $CaCl_2$ and MeOH/DCM, respectively.

The greatest impact of MPP was observed at lower concentrations for both extraction methods. The desorption of pesticide molecules depends

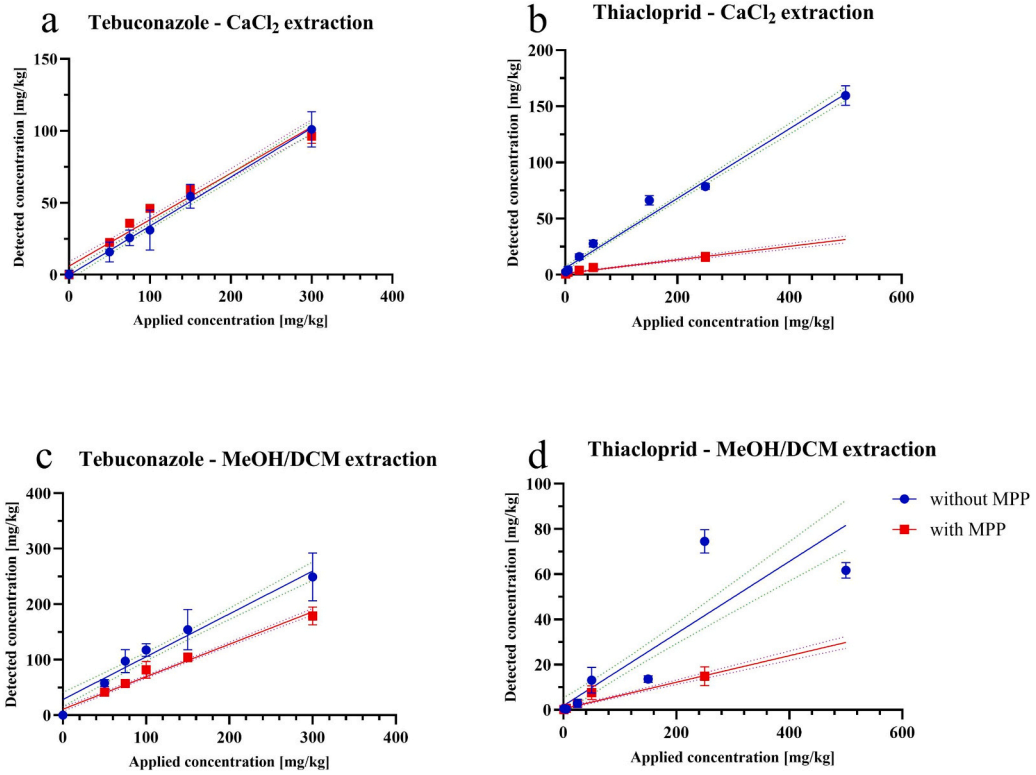

Fig. 1. Pesticide LC-MS analysis. Determined bioavailable pesticide concentrations after $CaCl_2$ (a/b) and solvent (c/d) extractions and LC-MS analysis of the active ingredient Tebuconazole (a/c, n = 9) and Thiacloprid (b/d, n = 3–9) with (red) and without (blue) microplastic co-exposure. Linear regression is applied for all scenarios with a 95 % confidence interval (dotted). R^2 of the linear regression with/without MPP are 0.96/0.94 (a), 0.88/0.98 (b), 0.95/0.87 (c), 0.88/0.77 (d).

on the strength of the solvent gradient. When molecule concentrations are higher, the gradient becomes saturated, decreasing its ability to release tightly bound molecules from MPP. Therefore, the significant differences even at higher concentrations indicate a strong tendency of Thiacloprid to be retained by microplastic particles. In contrast, this trend was not observed with Tebuconazole.

Combined with the toxicity data, both investigations support the finding of a slight decrease in readily bioavailable Tebuconazole in soil. For Thiacloprid, we expected toxicity to be higher when no microplastic was present, since $CaCl_2$ extraction was significantly more efficient—on average 15.6 times—without MPP across all concentrations (see Table A5). Overall, the extractable amount of pesticide with and without MPP is much lower for Thiacloprid, which was unexpected because toxicity differences in OECD 207 were similar for both pesticides. Therefore, the chosen extraction method may be more suitable for Tebuconazole with total extraction (Table A4) than for Thiacloprid, which showed 69.5 % extraction with MeOH (excluding the outlier at 1 g/kg) and 43 % with $CaCl_2$.

The analytical results confirmed that microplastic retains pesticides. The larger differences in extractability between the substances did not impact the organisms, as the MPP co-exposure effects for both pesticides were similar (Thia LC50: +11.3 %, Tebu LC50: +9.3 %, see Table 1). The median lethal and effect concentrations, LC50 and EC50, are the statistically calculated levels of a substance expected to cause death or effects in 50 % of the exposed animals during the study period. For Thiacloprid, this suggests that worms primarily took up the pesticide through soil ingestion or dermal absorption rather than microplastic. Wang et al. (2019b) showed how different uptake pathways contribute at co-exposure to polychlorinated biphenyls and PE or PS microplastic. As microplastic concentrations increased, the main uptake shifted from dermal absorption to soil ingestion—about 30 % in control, 45 % at 0.1 % PE MPP, and 75 % at 1 % PE MPP—reducing dermal uptake and increasing plastic ingestion. Plastic ingestion was only observed at 1 % PE, accounting for 1.03 % of total uptake. Therefore, the main uptake mechanisms are mostly dermal, with some contribution from MPP and soil particles.

Table 1
Overview of the earthworm LC50 and EC50 (probit analysis, 95 % confidence limit) and their upper and lower limits for the pesticides with and without co-exposure to 150 mg/kg PE microplastic. EC50 was calculated from weight change data.

Exposure scenario (*E. fetida*)	Tebuconazole LC50 / EC50	Thiacloprid LC50 / EC50
Without MPP	110.3 (106.7–114.3) mg/kg / 172.5 (67.7–418.4) mg/kg	20.3 (8.5–43.7) mg/kg / 2.5 (0.4–17.3) mg/kg
With MPP	120.6 (116.6–124.8) mg/kg / 130 (84.9–194.8) mg/kg	22.6 (10.7–51.0) mg/kg / 3.3 (1.3–8.6) mg/kg

3.1.2. *Influence of MPP presence and extraction setup*

The presence of MPP negatively affected the extractability of Tebuconazole more with MeOH/DCM than with $CaCl_2$. The ratio of the amount extracted without and with MPP was consistently higher in the MeOH/DCM scenario compared to $CaCl_2$. This shows that MPP's negative effect on Tebuconazole extraction was more significant during MeOH/DCM extraction.

In the case of Thiacloprid, the extraction ratio with CaCl2 versus MeOH/DCM was higher at every concentration except at 25 mg/kg when there was no co-exposure to MPP, unlike the measurements from co-exposed soil. The amounts of solvent-extracted Thiacloprid were generally less affected by MPP, except at the 25 mg/kg concentration. This contrasts with the extraction amounts observed for Tebuconazole.

This is probably because of the polarity differences of both substances, which, as discussed later, influence their binding strength to the applied microplastic.

$CaCl_2$ is an aqueous solution used as a pore water simulant. It does not extract harshly but washes out substances that are not firmly bound. In studies with similar investigation targets, aqueous solutions are well-established and still commonly used (Baskaran et al., 1996; Siedt et al., 2023). Our study suggests that Thiacloprid is less available for this type of extraction. A factor may have been the initial soil pH value, which was slightly lower in Thiacloprid testing (average pH=5.6 ± 0.2) compared to Tebuconazole testing (average pH=6.3 ± 0.1). Hüffner et al. (2019) observed higher pesticide sorption to PE MPP at lower pH. They also found that Ca+ ions can decrease the distribution coefficient between soil and MPP, as they are more sorbed to MPP at lower pH.

Furthermore, only a small amount of Thiacloprid was extracted with MeOH/DCM, while most of the Tebuconazole was nearly completely extracted with the harsher method. Additional reasons for a lower extractable amount include pesticide loss within the soil due to formation of biogenic non-extractable residues, degradation, and sorption to the vial or soil.

In Álvarez-Martín et al. (2016), Tebuconazole showed 10–15 % of the non-extractable amount, which was extracted with methanol on day 0 of application, more than in our study, where it was almost fully extracted with MeOH/DCM.

Because there is only a limited amount of time remaining in the soil, degradation likely plays a minor role since pesticide soil half-lives range from 9.3 to 24 days for Thiacloprid (Chen et al., 2021) and several months or years for Tebuconazole (Siek and Paszko, 2019). Regarding the degradation of Thiacloprid, reduction, hydrolysis, and oxidation are the main reactions (Chen et al., 2021), although hydrolysis seems unlikely since the test system pH was only slightly acidic with an average pH of 5.6. In conclusion, degradation remains a factor in the loss of Thiacloprid; however, it is unlikely to be a significant factor for Tebuconazole.

The differences between MPP and soil extractable amounts are pronounced for both pesticides, indicating competition for sorption sites. Similar conclusions were drawn by Wu et al. (2022), who demonstrated that Imidacloprid and Flumioxazin sorption to soil slowed down when LDPE and PBAT mulch film MPP were present. Wu et al. (2022) also observed an increased adsorption strength of the soil system when MPP was present. In contrast, Šunta et al. (2020) found that MPP presence decreased pesticide retention and increased the mobility of pesticides bound to MPP, which was the focus of this study.

3.1.3. *Factors influencing pesticide availability*

Our extraction experiments showed that co-exposure with microplastic decreased the extractable amount in most cases; however, this was not statistically significant for the CaCl2 extraction of Tebuconazole.

Sorption between polymers and chemicals can be attributed to various factors such as polymer properties (e.g., type, size, surface structure), properties of the adsorbate (e.g., log Kow, polarity), and environmental or experimental conditions, mainly time, pH, salinity, organic matter content, ionic strength, and temperature.

Sorption properties vary among polymer types, depending on factors such as their sorption type, strength, and sorbate affinity. Aqueous sorption of non-polar organic compounds to PE is generally assumed to occur through mostly linear absorption into the bulk polymer, in contrast to PS, PA, or PVC, where adsorption mainly occurs on the polymer surface (Hüffner and Hofmann, 2016). Differences between polymer types were also observed in desorption, with pollutant desorption from PE being much faster than from other polymers (Bakir et al., 2014; Rodríguez-Seijo et al., 2018 b). Polymers differ in their monomeric composition and, consequently, their molecular interactions. PE engages in non-specific van der Waals interactions but cannot perform π-π interactions like PS. Since we did not compare different polymer types, no conclusions can be drawn from our data, and generalizing the observed effects to other polymers should be approached cautiously.

Besides polymer type, age also significantly influences sorption behavior (Peña et al., 2023).

In this study, pristine MPP represents an environmentally favorable sorption scenario because their surface area and sorption capabilities increase with aging (Lan et al., 2021), leading to higher substance loads per particle (Antunes et al., 2013; Cheng et al., 2020). The increased sorption mechanism is related to improved H-bonding between N and –OH groups at the functional groups (O) induced by aging and at microcracks, as summarized in Peña et al. (2023).

Antunes et al. (2013) also observed higher concentrations of all studied contaminants in darker and aged particles, making this particularly important for leftover black mulch film.

The bond between pesticides and particles also heavily depends on their interaction. Therefore, the octanol-water partition coefficient, log Kow, of the studied substance is believed to play a key role in adsorption to PE MPP (Wang et al., 2019a; Lan et al., 2021; Peña et al., 2023), as also assumed in this study. For Thiacloprid, which has a lower log Kow, bonding to PE MPP is primarily driven by van der Waals forces and hydrogen bonds (Pan et al., 2023), whereas Tebuconazole's bonding is mainly influenced by hydrophobic interactions (Wang et al., 2020a). Hydrophobic interactions involve the clustering of non-polar, hydrophobic molecules such as Tebuconazole and MPP, which can be disrupted by adding organic solvents.

Thus, MPP are expected to retain a higher amount of Tebuconazole since $CaCl_2$ will not disrupt hydrophobic interactions, as confirmed by our data (Tables A4, A5). The results suggest that, with organic solvent extraction, differences compared to co-exposure are more noticeable: We hypothesize that the fraction extracted with $CaCl_2$ is the part that has not bound to MPP but was readily available in the soil, since all binding sites of the comparatively low concentration of MPP were occupied (Wang et al., 2020b).

For Thiacloprid, both extractions showed significant effects of co-exposure. This is surprising, especially compared to Tebuconazole, and may stem from the differently produced microplastic particles. Even though they were both ground, variations in particle shapes and size distributions due to different methods could have affected their interactions with the pesticides (Wang et al., 2019c). Tebuconazole's MPP was ground with the addition of salt. However, they were thoroughly washed afterward, making an influence of it unlikely.

Compared to other studies, Xu et al. (2020) found that pure Thiacloprid was unaffected by the presence of MPP, showing similar bioavailability both with and without MPP. They used higher amounts of MPP than in this study and extracted with acetonitrile. Their test soil contained more silt and had three times the organic carbon content. Organic matter significantly influences the adsorption process because dissolved organic matter has a high affinity for pesticides, leading to minimal interactions with MPP (Mo et al., 2021). Factors such as lower organic carbon levels, sandy soil type, and different application methods in our study favor different sorption outcomes, indicating that more evidence of adsorption behavior is necessary.

Adjacent to the sorption to microplastic, the potential impact of microplastic particles on preventing degradation is an intriguing area for future research. Wu et al. (2022) explored this by examining how different particle types (new and aged LDPE MPP, biodegradable MPP) affect the adsorption and degradation of two pesticides (imidacloprid and flumioxazin). Their results showed that fresh LDPE MPP slightly reduce half-lives at low MPP concentrations (0.2 %), but the half-lives of pesticides increase when co-exposed with aged LDPE MPP. However, comparisons with other mulch film products should be made cautiously due to their varying and unknown compositions.

Finally, the time needed for complete pesticide sorption is an important factor, heavily influenced by polymer and soil characteristics, especially organic matter. In Wu et al. (2022), the sorption of two

pesticides to mulch film MPP in soil reached equilibrium after 30 h, with the first 10 h showing particularly rapid adsorption. For Tebuconazole and Thiacloprid, equilibrium was achieved within 2–3 days (Bošković et al., 2020; Xu et al., 2020). Therefore, the incubation period in this study can be considered sufficient for sorption to the available MPP. Preliminary tests also suggest there is no difference in solvent-extractable concentrations of Tebuconazole after 0, 7, and 14 days (see Table A3).

3.2. Acute toxicity testing

The acute lethal effects of the two pesticides' active ingredients on *E. fetida* were determined and are shown in Fig. 2 in blue, along with the respective effects of co-exposure to microplastics shown in red. By testing different pesticide concentrations in the acute toxicity test with *E. fetida*, we observed a shift in the LC50 value from 110 mg/kg to 121 mg/kg for Tebuconazole and from 20 to 23 mg/kg for Thiacloprid when co-exposed to 150 mg/kg polyethylene MPP (Table 1). Exposure to only 150 mg/kg microplastic particles (control) resulted in a mean mortality of 0 % for Tebuconazole and 3.3 ± 3.3 % for Thiacloprid.

Differences between treatments were not statistically significant, with p-values of 0.3957 (paired *t*-test, Thiacloprid) and 0.3893 (paired *t*-test, Tebuconazole); therefore, we cannot confidently confirm our hypothesis. However, for Tebuconazole, the trend is clearly visible and aligns with our expectations. Since we tested three technical replicates with three biological replicates each, this suggests a reduced toxic effectiveness of the tested model pesticides when mulch film particles are present.

Lackmann et al. (2023) found that the LC50 in E. andrei for Thiacloprid, formulated as Calypso, was 102.035 mg/kg in LUFA 2.2 soil, which is significantly higher than our measured LC50 of 20.28 mg/kg. However, they did not achieve 100 % mortality but extrapolated from 53 %. In artificial soil, Wang et al. (2015) determined an EC50 of 2.68 mg/kg for Thiacloprid in Calypso. Similar to our study, Renaud et al. (2018) and Wang et al. (2012) calculated EC50 values of greater than 12.9 mg/kg and 10.96 mg/kg, respectively, for Thiacloprid in artificial OECD soil. While testing acute toxicity within the same system, results can vary substantially due to differences between pure active ingredient and formulation applications, which presents a major challenge for open and transparent assessment (Hollert and Backhaus, 2019). Additionally, toxicity data from different soils may vary because of differences in pH levels, soil moisture, organic matter content, and other factors (Natal-da Luz et al., 2008). These factors can also contribute to wide ranges in EC50 and LC50 values, as shown in Table 1.

Previous studies have observed a shifted effect strength between single- and co-exposed test organisms; however, this mainly indicates an aggravating impact of MPP on toxicity.

Similar to this study, Cheng et al. (2020) investigated PE mulch film MPP at 2.5 g/kg with co-exposure to atrazine and found increased biomarker responses. Aggravated effects on earthworm health were also observed for the co-exposure of different microplastic particles with pesticides or cadmium (Baihetiyaer et al., 2023; Sun et al., 2021; Zhou et al., 2020).

Baihetiyaer et al. (2023) found the combination of PLA MPP and imidacloprid to severely damage earthworm growth and survival at 10 g/kg amendment rates after 14 d. Sun et al. (2021) observed several effects (bioaccumulation, oxidative damage) regarding co-exposure of MPP (40–50 μm) with Dufulin in exposure with 3000 mg/kg MPP but not 300 mg/kg. The particles themselves did not cause oxidative damage to the investigated earthworm but aggravated the toxicity of Dufulin. Zhou et al. (2020) observed enhanced oxidative damage and accumulation when cadmium was co-exposed with PP MPP. However, they worked with higher MPP concentrations (300 – 9000 mg/kg), where microplastic particles caused effects regardless of the presence of other contaminants.

In this study, we focus on using a high, but currently reasonable, concentration of pristine MPP in German soil, which has no measurable effect on earthworms. Higher microplastic concentration would generally increase the adsorption capacity until binding sites are saturated, as observed previously (Hu et al., 2023).

Interestingly, Shi et al. (2022) observed that co-exposure to concentrations of 125 mg/kg MPs (PE, PVC, PS, size: approx. 150–220 μm) added to the toxicity of fluoranthene, shifted the LC5014d from 131 to 98–124 mg/kg and also increased growth inhibition rates in *E. fetida*. They also observed a polymer-specific effect; PE MPP only reduced the LC50 after 14 days to 118.4 mg/kg, whereas PS reduced it to 98.1 mg/kg. However, as we see it, only one assay with three replicates was conducted, which might limit the informative value. Additionally, commercially obtained MPP can contain readily available additives compared to final products such as mulch film from this study and a study by Cheng et al. (2020).

Most studies observed increased effects when MPP was present. However, few found results similar to this study. Wang et al., (2019a) noted lower arsenic accumulation in earthworms when co-exposed to concentrations of 10 g PE MPP per kg dw soil, but no changes occurred at 1 g/kg dw.

In addition to non-detestable and incomparable factors such as additive content, different particle size ranges, soils used, and pesticides, we wanted to explore potential pathways that could lead to toxicity limitation due to MPP presence.

3.2.1. Why was toxicity reduced by microplastics?

We discuss three possible reasons why toxicity is reduced in co-exposure: a) earthworms avoided loaded MPP and were thus exposed to lower concentrations in soil, b) loaded MPP were not avoided, but the availability of the pesticides was limited due to binding to the PE particles, or c) digestion of microplastics led to adsorption of pesticides within the worm's gastrointestinal tract, making the particles act as a

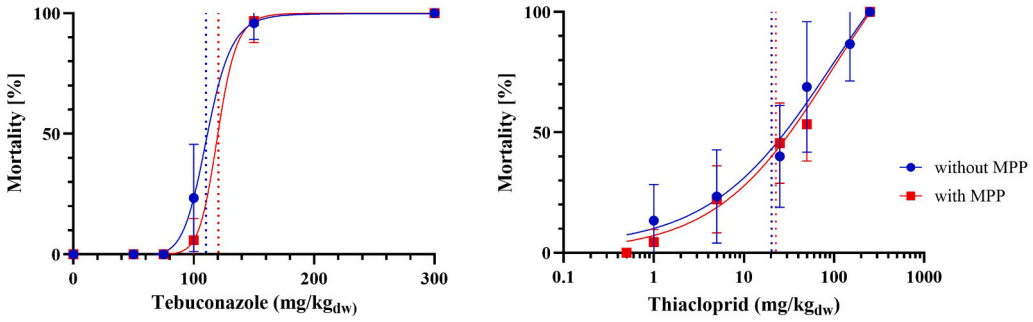

Fig. 2. Dose-response relationship between the concentration of applied pesticide (left: Tebuconazole, right: Thiacloprid) and mortality of worms with (red) and without (blue) co-exposure to 150 mg/kg PE microplastic particles and indicated standard deviation. The dashed lines indicate the calculated LC50 values. Note the logarithmic scaling of the right X-axis.

"cleaning agent."

Rodríguez-Seijo et al. (2018b) co-exposed *E. fetida* to MPP and chlorpyrifos. They observed that the organisms avoided contact with contaminated MPP in the top layer by hiding in the lower part of the test vessel. However, the MPP was sprayed before being incorporated into the soil, which depicts a scenario different from ours. Shi et al. (2022) observed increased avoidance when MPP were mixed into soil contaminated with fluoranthene. It is unlikely that the avoidance was caused by the microplastic itself, as previous studies did not report significant avoidance of microplastic (Hodson et al., 2017; Rodríguez-Seijo et al., 2018b; Shi et al., 2022). This could indicate that the earthworm specimen used avoided pesticide-loaded particles.

The second possible explanation relates to pesticide interaction with microplastic particles and soil. Adsorption onto plastics is influenced by the pesticide's physico-chemical properties, such as its log Kow, along with other factors like soil pH, organic content, or age (Lan et al., 2021; Mo et al., 2021; Fu et al., 2021).

When we assume loaded MPP were not avoided, either the binding to microplastic particles is stronger than to the soil, or the desorption is less likely. Most studies generally observe decreased contaminant bioaccumulation when co-exposed to MPP, with bioaccumulation mostly occurring in experiments with high MPP concentrations (Tourinho et al., 2023). Therefore, MPP are probably not avoided, but contaminants are bound so strongly that uptake and accumulation in earthworms only happen at high MPP concentrations.

Furthermore, Horton et al. (2017) found that zinc desorbs more easily from soil than from MPP in a $CaCl_2$ solution. In contrast, zinc desorption in the earthworm gut was 10–30 times higher from MPP than from soil particles. However, they concluded that in synthetic earthworm gut extract with equal amounts of soil and MPP material, the microplastic might even reduce the earthworms' exposure to zinc. Similar results were obtained by Pan et al. (2023), who observed that Thiacloprid binds more strongly to MPP than to humic acid in water.

Conversely, some studies have observed MPP acting as a carrier of pollutants into organisms: When exposed to pollutant-adsorbed PVC particles, Browne et al. (2013) found that lugworms take up chemicals mainly through ingestion and that pollutants and additives accumulated in the gut to a larger extent than in the sediment. The concept of MPP as a vector for microplastics is complex and debated in the related literature (Hartmann et al., 2017).

In this study, microplastic retention of the two model pesticides may have resulted in a lower bioavailable concentration for the earthworms.

Finally, the potential role of MPP as a cleaning agent is evaluated. It is important to highlight that earthworm gut surfactants have been shown to aid in desorbing pollutants significantly (Bakir et al., 2014). However, there is limited evidence that microplastic particles can adsorb pollutants inside the organism and then transport them outside. In another experiment using the same MPP as in this study, we observed that earthworms absorbed and excreted MPP, indicating this process is generally possible (Weltmeyer and Roß-Nickoll, 2024). Environmental factors such as pH, ionic strength, organic matter, and overall MPP characteristics influence the adsorption process.

The pH effect on sorption depends on the chemical species, for example, making some species more susceptible to adsorption at pH= 5 when electrostatic repulsion is minimized (Zhang et al., 2018). A lower pH increases the adsorption capacity of microplastics for organic pollutants (Fu et al., 2021). However, pH in soil was generally lower (see Table A1) than in the nearly neutral earthworm gut (Horn et al., 2003).

Unfortunately, there is a lack of information and metadata to assess this phenomenon for our study.

Lastly, it is important to note that although acute exposure showed reduced effects due to microplastic presence, sorption can also potentially cause long-term adverse effects: Slow desorption of pesticides in the animal's gut or gradual release from MPP over time can expose animals for an extended period (Zhang et al., 2022). Chronic exposure to pesticides such as thiacloprid can lead to changes in essential functions, including enzymatic processes, metabolism, and DNA damage, which can have further impacts on higher biological levels (Lackmann et al., 2023; Feng et al., 2015). Future research should therefore also consider examining the effects and sorption behavior over an extended period.

4. Conclusions

Mulch films are a significant source of plastic in agricultural soils and their use is expected to grow with advancing climate change with increasing droughts and heat stress. The accumulation of microplastic particles and other agricultural chemicals, like pesticides, poses a threat to soil health due to limited remediation options and potential unintended interactions.

Our results indicate that short-term co-exposure decreases bioavailability significantly and potentially reduces toxicity in earthworms. The adsorption of pesticides to soil is the most likely explanation for the reduced toxicity in earthworms, rather than MPP acting as a cleaning agent or avoidance behavior from animals.

Unlike previous studies, we prioritized a basic, but more realistic test setup, using commonly used agrochemicals and mulch film in a real agricultural soil. Although, this study is limited by its single-species approach and the use of fresh, not aged, mulch film, our results indicate that that interactions between MPP and pesticides can both affect soil health and pesticide efficiency.

Thus, we contribute valuable information to the heterogeneous study situation of effects on soil organisms from combined exposure, helping to clarify the key factors such as polymer type, size, and age, as well as pesticide and soil properties. Following our results, pesticide effectivity could be influenced by a lower availability of pesticides, decreasing their direct effect to (non)target organisms. To mitigate this effect, farmers should aim to diversify their cropping rotation and use biodegradable mulch film or organic amendments to reduce persistent MPP input and its transport to deeper soil layers. The modification of soil properties by increasing organic matter through, e.g., biochar addition, might also decrease binding to microplastics as a competing sorption target.

The essential take-home message is that the co-existence of pesticides and (micro)plastic could lead to less effective pesticide use on the target organism. The potential impact of plastic pollution on the soil environment is complex and requires careful examination before taking action, given plastic's vital role in our society. Future research should thus explore how the use of mulch films and pesticides might affect sustainable land use in the future.

List of abbreviations

Acetylcholine esterase - AChE
Analysis of Variance – ANOVA
Catalase – CAT
Dichlormethane – DCM
Dimethyl sulfoxide – DMSO
Glutathione S-transferase – GST
Heated electron spray ionization – HESI
High-performance liquid chromatography – HPLC
Methanol – MeOH
Mean lethal/effect concentration – LC50/EC50
Lowest/No observed effect concentration – LOEC/NOEC
Microplastic (particles) – MP(P)
Optical density – OD
Plant protection product – PPP
(Low density) Polyethylene – (LD)PE
Polyamide - PA
Polybutylene adipate terephthalate - PBAT
Polylactic acid - PLA
Polystyrene - PS
Polyvinylchloride - PVC
Tebuconazole – Teb

Thiacloprid - Thia

CRediT authorship contribution statement

Thomas-Benjamin Seiler: Writing – review & editing, Funding acquisition, Conceptualization. **Isaac Heimbach:** Investigation, Data curation. **Antonia Seidel:** Writing – original draft, Visualization, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Martina Roß-Nickoll:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Lars M. Blank:** Writing – review & editing, Project administration, Funding acquisition. **Henner Hollert:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

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Declaration of Competing Interest

The authors declare that they have no competing interests.

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Appendix

Table A1
Overview of the soil properties from different delivery dates of the RefeSol 01-A from Fraunhofer Institute for Molecular Biology and Applied Ecology IME (Schmallenberg, GER). *according to DIN ISO 11277

Physiochemical properties	02/2020	05/2020	05/2020	05/2020	06/2021	09/2021	Mean ± SD
Sand (%)*	73.1	69.8	73.1	69.8	69.8	69.8	70.9 ± 1.6
Silt (%)*	19.8	24.4	19.8	24.4	24.4	24.4	22.8 ± 2.2
Clay (%)*	7	5.9	7	5.9	5.9	5.9	6.3 ± 0.5
Corg (%)	0.96	0.95	0.96	0.95	0.95	0.95	0.95 ± 0.004
Total N (g/kg)	0.9	0.81	0.9	0.81	0.81	0.81	0.84 ± 0.04
pHCaCl2	5.65	5.72	5.65	5.72	5.72	5.72	5.69 ± 0.03
KAKeff (mmol/kg)	3.5	40	7	40	40	40	28.4 ± 16.4
Water holding capacity (g/kg)	293	287	293	287	287	287	289 ± 2.8
Biomass (mg/kg Cmik)	198	174	198	174	174	174	182 ± 11.3

Table A2
Size distribution of the polyethylene microplastic used. Feret,min describes the minimum size of the MPP in μm. Feret,max describes the maximum size of the MPP in μm. Only particles to a size range of 1000 μm were used in testing

Feret,min [μm]	Number Particles (Feret, min)	Feret,max [μm]	Number Particles (Feret, max)
50–100	3	150–200	2
100–150	7	200–400	12
150–200	4	400–600	15
200–400	26	600–1000	28
400–600	19	1000–1500	28
600–1000	30	1500–2000	13
1000–1500	29	2000–3000	28
1500–2000	13	3000–4000	7
2000–3000	2		

Table A3
Analytical results of preliminary tests with Tebuconazole in soil for 0, 7 and 14 d and subsequent extraction with Methanol and DCM as described in 2.3

Applied concentration (mg/kg)	Mean detected concentration (mg/kg)		
	T0 (0 d)	T1 (7 d)	T2 (14 d)
50	31.6 ± 6.3	34.9 ± 3.3	25.7 ± 3.2
75	47.2 ± 6.5	52.8 ± 5.3	48.8 ± 2.6
100	72.0 ± 15.3	72.9 ± 9.0	86.8 ± 35.2
150	94.2 ± 7.8	99.9 ± 15.7	104.3 ± 19.3
300	168.9 ± 16.0	184.8 ± 11.3	167.7 ± 28.4

Table A4
Mean extracted amount of $CaCl_2$ and MeOH/DCM extraction of Tebuconazole from three replicate day measurements. Proportion between extraction methods and the presence of MPP are calculated. n.d. = no data, - MPP = without MPP, + MPP = with 150 mg/kg MPP present

Tebuconazole	- MPP			+ MPP		
Appl. Conc. (mg/kg)	**CaCl2 (mg/kg)**	**MeOH-DCM (mg/kg)**	**CaCl2/ MeOH-DCM**	**CaCl2 (mg/kg)**	**MeOH-DCM (mg/kg)**	**CaCl2/ MeOH-DCM**
300	101,00	249,23	0,41	96,32	178,85	0,54
150	54,51	154,13	0,35	59,28	104,17	0,57
100	31,04	97,56	0,32	46,17	81,99	0,56
75	25,64	97,56	0,26	35,77	57,13	0,63
50	15,82	57,78	0,27	22,40	41,58	0,54
Mean			0,32			0,58

Table A5
Mean extracted amount of $CaCl_2$ and MeOH-DCM extraction of Thiacloprid from three replicate day measurements. Proportion between extraction methods and the presence of MPP are calculated. n.d. = no data, - MPP = without MPP, + MPP = with 150 mg/kg MPP present

Thiacloprid	- MPP			+ MPP		
Appl. Conc. (mg/kg)	**CaCl2 (mg/kg)**	**MeOH-DCM (mg/kg)**	**CaCl2/ MeOH-DCM**	**CaCl2 (mg/kg)**	**MeOH-DCM (mg/kg)**	**CaCl2/ MeOH-DCM**
500	159,61	61,67	2,59	n.d.	n.d.	n.d.
250	78,55	74,55	1,05	15,98	14,88	1,07
150	66,28	13,65	4,86	n.d.	n.d.	n.d.
50	27,79	13,13	2,12	6,37	7,59	0,84
25	15,89	2,89	5,56	3,94	2,79	1,41
5	2,80	0,36	11,81	2,28	0,69	3,28
1	2,48	0,52	5,04	0,58	0,16	3,52
0,5	n.d.	n.d.	n.d.	0,21	0,09	2,37
Mean			3,69			1,81

Data availability

Data will be made available on request.

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