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RESEARCH ARTICLE

Beyond medicine: A proof of concept for synergy analysis in ecotoxicology

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Abstract

1. Interactive effects among chemicals, such as synergism and antagonism, are increasingly studied in ecotoxicology and environmental research. However, these interactions are often assessed using a biased, point-wise approach that overlooks dose-dependent effects.
2. In medicinal drug research, the *synergyfinder* method has been widely adopted to characterise dose-dependent drug interactions, yet its application in other scientific fields remains unexplored.
3. Here, as a proof of concept, we demonstrate the suitability of this method for ecotoxicology and environmental research using a model pollinator, the buff-tailed bumblebee and two major environmental pollutants, namely copper and cadmium. As the *synergyfinder* method lacks built-in significance testing, we complemented it with a well-established statistical approach to formally assess survival outcomes within the framework.
4. By exposing bumblebees to increasing concentrations of these metals, both individually and in combination, we found that synergistic effects on survival emerged exclusively at low doses. This study is the first to extend the *synergyfinder* approach beyond medicinal applications and to supplement it with robust statistical hypothesis testing, providing a more rigorous framework for analysing chemical interactions in the face of a global pollution crisis.

KEYWORDS
additivity, antagonistic, bumblebee, chemical, interaction, pollutant, synergistic, synergyfinder

1 | INTRODUCTION

The Earth is experiencing an unprecedented biodiversity crisis, with 28% of all assessed species currently threatened with extinction (IUCN, 2024). This alarming trend spans both vertebrates (Ceballos et al., 2017) and invertebrates, including insects, which have exhibited significant population declines over recent decades (Hallmann et al., 2017). This erosion of biodiversity has profound consequences for human societies, primarily through the disruption of essential ecosystem services like pollination, pest control and nutrient cycling (Cardoso et al., 2020). Anthropogenic pressures such as habitat destruction and climate change are widely recognised as primary

drivers of biodiversity loss (Díaz et al., 2019). Research increasingly highlights the importance of interactions among these stressors, often reporting synergistic effects (Brook et al., 2008; Northrup et al., 2019).

Chemical pollution is another major environmental stressor, with an estimated 220 billion tons of chemicals released annually, leading to widespread contamination across ecosystems and across human populations (Naidu et al., 2021). Among these pollutants, pesticides are well-documented drivers of biodiversity loss. For instance, an 18-year study in the UK demonstrated that seed-treated crops significantly increased local extinction rates in wild bee populations (Woodcock et al., 2016). Beyond individual contaminants, ecotoxicological research has increasingly focused on the interactive effects of multiple pollutants. In this way, synergistic and antagonistic interactions have been reported for pesticides in aquatic communities (Relyea, 2009) and in bees (Siviter et al., 2021; Tosi et al., 2022), as well as for the combined effects of microplastics and trace metals in fish (Wen et al., 2018).

However, the definition of interactive effects among chemicals in ecotoxicology is often biased (Gekière, Ghisbain, Gérard, & Michez, 2024). Laboratory studies typically employ a point-wise design, where organisms are exposed to a single dose of each chemical alone or in combination. While this approach provides insight into potential interactions at specific dose combinations, it overlooks the possibility that interaction effects may shift across a continuum of concentrations (e.g. synergism at low doses but antagonism at high doses) (Jonker et al., 2005). Furthermore, interaction effects are often classified as synergistic when the combined effect exceeds the sum of individual effects (e.g. Favaro et al., 2023; Rossi et al., 2024). However, this definition is conceptually flawed because it fails to account for the shape of each chemical's dose–response curve—it should be noted, however, that obtaining complete and precise dose–response curves can be experimentally challenging. As a result, omitting dose–response curves may conflate genuinely synergistic interactions with super-additive but non-synergistic effects (Twarog et al., 2021). To rigorously assess chemical interactions, a multi-dose factorial approach is recommended (Altenburger et al., 2003; Makariadou et al., 2024). By testing a range of doses for each chemical alone, dose–response curves can be constructed and incorporated into predictive null models. These models estimate expected effects under purely additive interactions, providing a baseline against which deviations—whether synergistic or antagonistic—can be detected (Makariadou et al., 2024; Roell et al., 2017; Vlot et al., 2019). Crucially, this method allows for the identification of dose-dependent interactions, for instance where synergy emerges only at high concentrations (Jonker et al., 2005; Rodea-Palomares et al., 2015).

In medicine, the multi-dose factorial approach is a well-established standard (Calzetta et al., 2024), providing a robust framework that could be more widely adopted in ecotoxicology and environmental research. Recent oncology studies exemplify this approach, such as a paper demonstrating that poly(ADP-ribose) polymerase (PARP) and ataxia telangiectasia and Rad3-related (ATR) kinase inhibitors synergistically kill APOBEC3A (A3A)-expressing cancer cells in a dose-dependent manner, highlighting a promising therapeutic strategy for cancers with A3A activity (Kawale et al., 2024). These studies leverage the *synergyfinder* method, originally developed as a web-based tool for researchers to pre-process, analyse and visualise pairwise drug interactions (Ianevski et al., 2017, 2020, 2022). This method employs four reference models to quantify theoretical additive interactions: (i) the *highest single agent* (HSA) model, which considers the maximum effect of any single compound, (ii) the *Bliss independence* model, which assumes chemicals act independently, (iii) the *Loewe additivity* model, which treats the combination as if the individual chemicals were identical and (iv) the *zero interaction potency* (ZIP) model, which assumes that the chemicals do not alter each other's potency (Yadav et al., 2015).

As a proof of concept, we demonstrate the applicability of the *synergyfinder* method in ecotoxicology and environmental research using a model pollinator, the buff-tailed bumblebee (*Bombus terrestris*) and two major environmental pollutants, the essential metal copper and the non-essential metal cadmium. Following a standardised risk assessment protocol, we exposed bumblebees to increasing doses of these metals, both individually and in combination, and observed dose-dependent interactive effects on survival. However, since the *synergyfinder* method does not include significance testing, we provide an R script to generate reference-based theoretical survival curves and statistically assess differences between observed and expected outcomes. This approach enhances the robustness of interaction analyses in ecotoxicology, enabling a more precise evaluation of synergistic or antagonistic effects in environmental risk assessments.

2 | MATERIALS AND METHODS

2.1 | Bumblebee breeding

In a first experiment in August 2024, 10 standard colonies of *B. terrestris* L. (~50 workers per colony upon receival) were obtained from the commercial supplier *Biobest* (Westerlo, Belgium) and fed with Biogluc® sugar solution (Wäckers et al., 2017). In November 2024, we conducted a second experiment with 10 more colonies to increase the number of replicates in our study. In both experiments, bee workers were retrieved from their colonies, housed in individual Nicot® cages and provided overnight with ad libitum 50% sucrose syrup (sucrose:water 1:1 w/w) through a tip-less 2-mL syringe for acclimatisation before being exposed to their respective treatment. Bees that died within this period were discarded (<3% of the bees). Throughout the experiment, cages were placed in a dark room at constant temperature (27 ± 1°C) and relative humidity (60 ± 10%).

2.2 | Treatments

To investigate the interactive effects of copper and cadmium on the bumblebee *B. terrestris*, we followed a standardised protocol commonly used in risk assessments to evaluate the acute oral toxicity of pollutants

in this model species (OECD, 2017). Bees were exposed to increasing concentrations of copper, increasing concentrations of cadmium or combinations of both metals. Six concentrations of copper ($CuCl_2$; Sigma-Aldrich, CAS 7447-39-4) and six concentrations of cadmium ($CdCl_2$; Sigma-Aldrich, CAS 10108-64-2) were prepared by diluting the metals in a 50% sucrose solution (Table 1). These concentrations were selected to facilitate the calculation of lethal dose–response curves, which are essential baselines for determining chemical interactions on lethal effects (Gekière, Ghisbain, Gérard, & Michez, 2024). Additionally, combinations of both metals were prepared using a full-factorial design, with all possible concentration pairings included. This approach ensures comprehensive detection of interactive effects (Gekière, Ghisbain, Gérard, & Michez, 2024). In total, 36 treatments were tested (6 copper concentrations × 6 cadmium concentrations; Appendix S1).

2.3 | Exposure

After the overnight acclimation period, bees were fasted for 3 h before being provided with 40 µL of their respective treatment via the tip of a 2 mL syringe. Bees were given 6 h to consume the solution, which is a slight deviation from the standardised protocol (typically a 4-h consumption period) (OECD, 2017). This adjustment was made to account for the deterrent properties of high copper concentrations in *B. terrestris* (Gekière, Breuer, Dorio, Evrard, et al., 2024), as extending the duration increased the proportion of bees that consumed their solution. Bees that failed to consume the treatment were excluded from the experiments (non-feeders: 319/1304; 24%). Following exposure, bees were supplied ad libitum with 50% sucrose syrup for 96 h, and mortality was recorded at 24-h intervals (binomial response: bees were either alive ['0'] or dead ['1']). All bees were weighed either upon death or at the end of the experiment if they survived. Overall, 1304 bees entered the experiments, and 985 consumed their treatment.

2.4 | Statistical analyses

To first ensure that the concentrations we used covered the full range of lethal effects (i.e. mortality dose–response curves) in the pure solutions (i.e. copper alone or cadmium alone), we designed median lethal doses (LD_{50}) using three-parameter log-logistic functions via the drm() and LL.3() functions in the *drc* package (Ritz et al., 2015) for each metal. Since LD_{50} was significant for each metal ($p < 0.001$), we were confident in testing interactive effects across the full range of combinations (Appendix S2).

Then, to explore combinations with potential synergistic or antagonistic effects, we relied on the *synergyfinder* method (Ianevski et al., 2017, 2020, 2022). Basically, based on the dose–response curve of each pure chemical, this method computes the theoretical values that should be observed for all the respective concentrations in the combination. This method relies on four reference models to calculate these theoretical values, namely HSA, Bliss, Loewe and ZIP models. Then, for a given reference model and combination, a score is computed to quantify the deviation (in percentage) of observed values from the expected values. A positive score indicates a response exceeding the expected outcome, while a negative score reflects a response below expectation. For example, a score of 20 signifies a response that is 20% greater than the expected value. Although no universally established thresholds define the significance of these scores, the guidelines proposed by the package developers underline that a score below −10 suggests a significant antagonistic interaction, a score between −10 and 10 indicates no significant interaction (i.e. an additive effect), and a score above 10 suggests a significant synergistic interaction (Ianevski et al., 2017, 2020, 2022) (https://synergyfinder.aittokallio.group/synfin_docs/).

We calculated synergy scores for all drug combinations using the ReshapeData() and CalculateSynergy() functions from the *synergyfinder* package (Zheng et al., 2022). For the ZIP reference model, we generated contour plots with the Plot2DrugContour() function to visually highlight potential antagonistic or synergistic interactions. The ZIP model was chosen for graphical representation due to its ability to address several limitations inherent to other reference models (e.g. the Loewe model assumes that drugs act exactly the same way; Yadav et al., 2015). To validate combinations yielding notable synergy or antagonism scores (i.e. <−10 or >10), we compared the observed survival curves with the theoretical curves generated by all four reference models. This comprehensive comparison ensured that significant interactive effects on bumblebee

TABLE 1 Concentrations and doses of copper and cadmium used in this study.

	Copper				Cadmium			
	[$CuCl_2$] (mg L^{-1})	[Cu] (mg L^{-1})	M (mM)	Cu (µg)	[$CdCl_2$] (mg L^{-1})	[Cd] (mg L^{-1})	M (mM)	Cd (µg)
Conc 1	0	0	0	0	0	0	0	0
Conc 2	1000	472.64	7.43	18.9	45	27.59	0.25	1.1
Conc 3	1300	614.43	9.67	24.58	55	33.73	0.3	1.35
Conc 4	1700	803.48	12.64	32.14	65	39.86	0.35	1.59
Conc 5	2200	1039.8	16.36	41.59	80	49.06	0.44	1.96
Conc 6	3300	1559.7	24.54	62.39	110	67.45	0.6	2.7

Note: Metals were also used in combinations in a full-factorial design, resulting in 36 treatments.
Abbreviation: Conc, concentration.

mortality were consistently supported across multiple models and not solely by the ZIP model. Since no function exists to directly extract survival curves from a *synergyfinder* object, we manually retrieved the data (R code in Supporting Information) and analysed survival curves using the *survival* (Therneau, 2021) and *survminer* (Kassambara et al., 2021) packages, including model as a fixed effect. When a given interaction was statistically significant ($p < 0.05$), we conducted custom post hoc contrasts with false discovery rate correction to compare the observed survival curves against those predicted by the four reference models using the *emmeans* package (Lenth, 2022). In this study, no global correction for multiple testing was applied across all statistical analyses, as the number of notable synergy or antagonism scores to be tested was limited. However, implementing such corrections may be advisable in future studies involving a larger number of tests, to more rigorously control the false discovery rate (Jafari & Ansari-Pour, 2019).

3 | RESULTS

By comparing observed mortality rates with theoretical rates from the reference ZIP model, the contour plot revealed combinations suggestive of potential synergistic interactions (i.e. Cu 7.43/Cd 0.25, Cu 7.43/Cd 0.30, Cu 9.67/Cd 0.25 and Cu 9.67/Cd 0.30 mM) and one indicative of a potential antagonistic interaction (i.e. Cu 16.36/Cd 0.60 mM) (Figure 1).

To validate these interactions, we compared the observed survival curves for these combinations with their corresponding theoretical curves derived from four reference models (i.e. HSA, Loewe, Bliss and ZIP). The analyses revealed that observed mortality was significantly higher than the predictions from three of the four models (i.e. HSA, Bliss and ZIP) for the Cu 7.43/Cd 0.25 mM ($\chi^2 = 12.4$, df = 4, $p = 0.015$; Figure 2a) and Cu 7.43/Cd 0.30 mM ($\chi^2 = 18.6$, df = 4, $p < 0.001$; Figure 2b) combinations. These findings indicate significant synergistic effects on bumblebee mortality for these combinations. In contrast, survival curve analyses for other combinations suggestive of potential interactions did not reveal significant synergistic or antagonistic effects (Appendix S3).

4 | DISCUSSION

Using copper and cadmium, two environmentally prevalent metals and the buff-tailed bumblebee, our study is the first to rigorously demonstrate dose-dependent synergistic interactions between chemical pollutants in a model organism through the *synergyfinder* method. We found that these metals exhibited a dose-dependent synergistic effect by significantly increasing mortality in buff-tailed bumblebee workers, but exclusively at low, yet field-unrealistic, doses. Typically, studies investigating chemical interactions in living organisms expose their models to a single concentration of each compound, either alone or in combination (e.g. Favaro et al., 2023; Rossi et al., 2024), failing to capture the variability of interactive effects across a broad range of concentrations. A recent review on bee-pesticide interactions highlights this gap: only 3% ($n = 2$) of the studies employed a near-factorial multi-dose approach to assess interactive effects (Misiewicz et al., 2024; Robinson et al., 2017), and none implemented a full-factorial design as we did in this study (Gekière, Ghisbain, Gérard, & Michez, 2024).

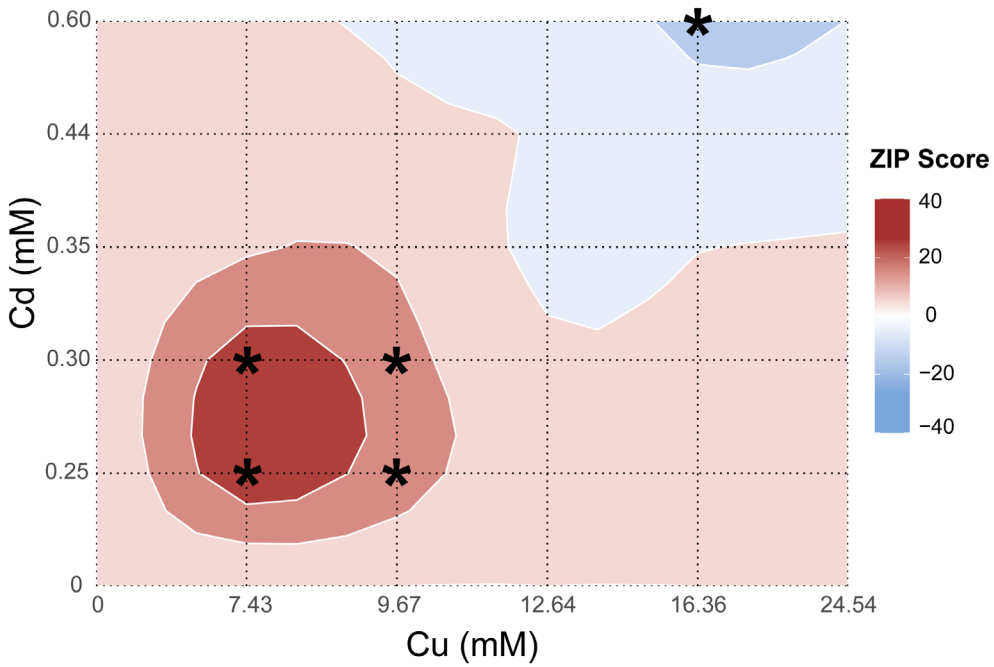

FIGURE 1 Contour plot highlighting zero interaction potency (ZIP) scoring of the effects of copper and cadmium combinations on bumble bee mortality. Asterisks highlight combinations with potential antagonistic (i.e. score <−10) or synergistic (i.e. score >10) effects.

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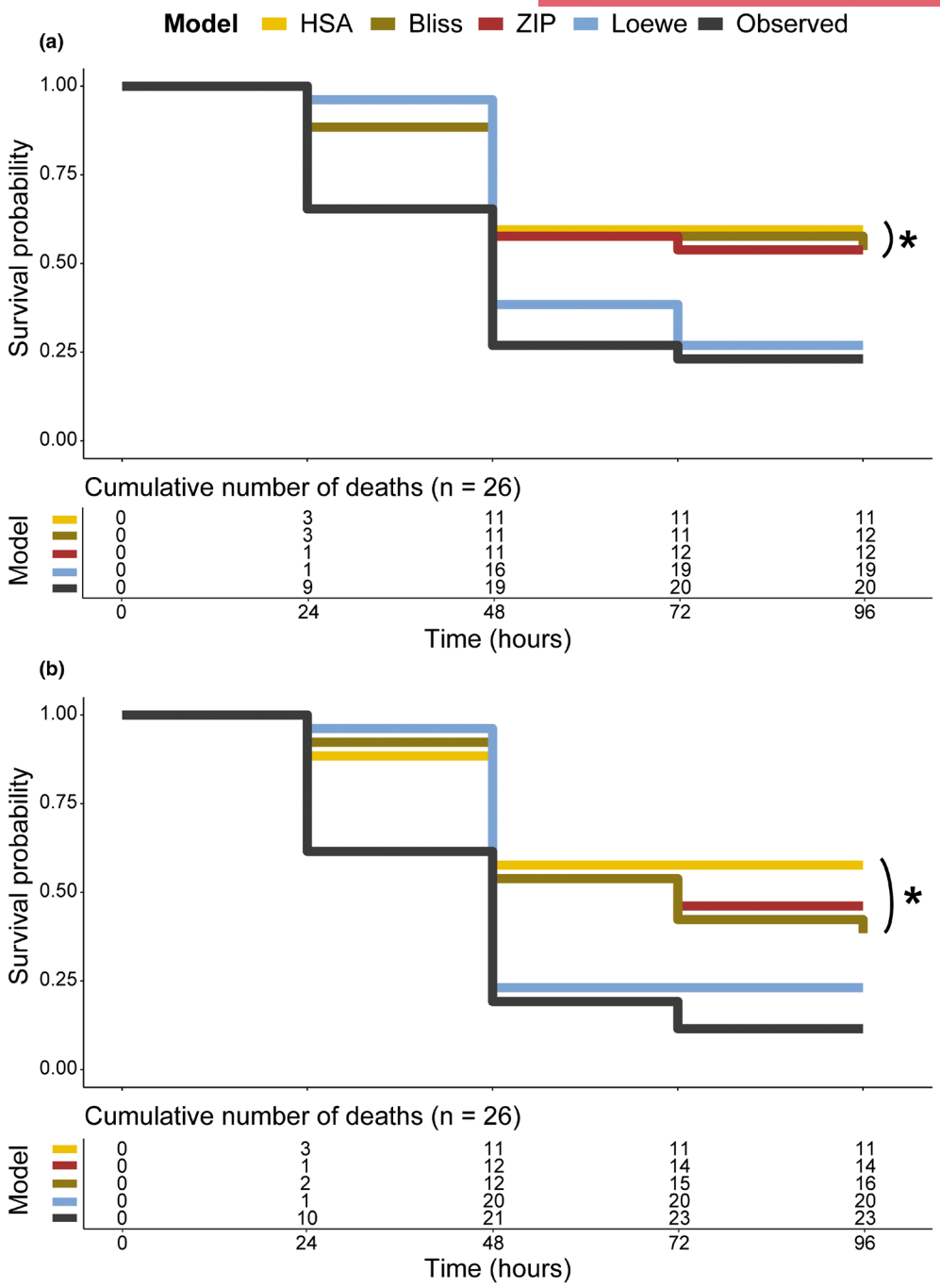

FIGURE 2 Survival curves comparing the observed and theoretical mortality of *Bombus terrestris* workers exposed to a combination of (a) Cu 7.43/Cd 0.25 mM or (b) Cu 7.43/Cd 0.30 mM. Asterisks indicate reference models that significantly differ from the observed mortality. Colour legend is in the first panel.

While our study demonstrates the utility of the *synergyfinder* framework for assessing interactive effects among environmental pollutants, it also underscores the critical need to complement this tool with robust statistical hypothesis testing. Notably, *synergyfinder* calculates synergy or antagonism scores based solely on the proportion of dead individuals, without accounting for the underlying sample size. For instance, this method treats two deaths out of four individuals (i.e. 50% dead) equivalently to 50 deaths out of 100 (i.e. 50% dead), despite the latter result providing a more reliable estimate. This insensitivity to sample size renders the method vulnerable to false positives and false negatives when applied to small datasets, and it does not include any built-in correction for this limitation. To address this issue, we supplemented the *synergyfinder* analysis with a comparison of observed survival curves to theoretical curves predicted by reference models. We then applied a well-established statistical approach (i.e. the Cox proportional-hazards model) to test whether observed deviations were statistically significant. This additional step proved essential since among the five

notable interaction scores identified by *synergyfinder*, only two were supported by statistically significant differences in survival outcomes. These findings highlight the importance of integrating formal survival statistical testing with *synergyfinder* outputs to mitigate the risk of erroneous conclusions.

Intriguingly, for the two combinations exhibiting synergistic effects, we observed differences between the predicted and observed survival curves when using the HSA, Bliss and ZIP reference models. In contrast, the theoretical predictions generated by the Loewe model closely matched the observed data. These divergences among models stem from their differing underlying assumptions. For instance, the Loewe model assumes that both compounds act on the same biological target and can be considered as dilutions of one another, whereas the Bliss model assumes the independent action of each compound (Cedergreen, 2014). Consequently, reference models often yield inconsistent interpretations of interactions, as their outputs are sensitive to the shape of the individual dose–response curves, including Hill slopes and maximal efficacy (Kashif et al., 2017; Twarog et al., 2021; Vlot et al., 2019). The ZIP model, which integrates features from both Loewe and Bliss models, aims to provide a more robust framework. However, no standardised guidelines currently exist for selecting the most appropriate reference model (Yadav et al., 2015). Ultimately, a reliable identification of synergistic or antagonistic interactions requires elucidation of the underlying biological mechanisms. Yet, studies that simultaneously report interaction outcomes and investigate their mechanistic basis remain notably scarce.

Interactive effects among chemical pollutants can be attributed to six key mechanistic processes, in which chemicals influence each other's bioavailability, uptake, transportation, metabolisation, binding or excretion (Cedergreen, 2014). However, empirical evidence supporting some of these mechanisms remains scarce (Table 2). For instance, nanoparticles have been shown to enhance the toxicity of co-occurring contaminants through the 'Trojan horse' effect, where nanoparticle-contaminant complexes facilitate the uptake of adsorbed toxicants (Forest, 2021). The mechanistic basis underlying the synergistic effects of copper and cadmium remains unclear. Since these metals do not form direct complexes in solution, we can rule out the possibility that they influence each other's bioavailability. However, interactions may occur at the level of uptake, transport, binding, metabolism and excretion. Non-essential cadmium ions are known to compete with essential copper ions for transporters and metal-binding proteins, potentially disrupting metal homeostasis and the regulation of shared metal-responsive elements (Luo et al., 2020; Slobodian et al., 2021; Yiwen et al., 2022). Additionally, both metals induce cellular damage by targeting DNA and proteins, impairing critical repair mechanisms such as DNA repair and protein refolding (Kanellis & dos Remedios, 2018; Oliveira et al., 2022). This disruption may exacerbate toxicity, as the presence of one metal could compromise the cellular response required to mitigate damage caused by the other, thereby contributing to synergistic effects.

As our understanding of the mechanisms driving general chemical interactions is already limited, our knowledge regarding dose-dependent interactions is even more constrained. For instance, in the case of dose-dependent antagonistic effects, hormetic phenomena provide a compelling explanation. Hormesis describes a biphasic dose–response, in which low doses of a chemical stimulate beneficial biological processes, such as detoxification or immune function, while high doses lead to detrimental effects (Cutler et al., 2022). Consequently, exposure to a low dose of a chemical may enhance an organism's resistance to subsequent stressors. This has been demonstrated in mites, where exposure to a hormetic concentration of imidacloprid led to a twofold increase in tolerance to the insecticide diazinon (Alimirzaee et al., 2023). In contrast, the mechanisms

TABLE 2 Mechanisms underlying interactives effects among chemical pollutants (inspired from Cedergreen, 2014).

Mechanism	Description	Example (reference)
Bioavailability	A chemical influences the bioavailability of the other chemical in the substrate or food	In toy shrimps, the combination of zinc pyrithione (PT) and copper ions leads to the formation of the more toxic copper PT complex, resulting in a mixture that is more toxic than predicted from the toxicities of zinc PT and copper alone (Mochida et al., 2006)
Uptake	A chemical influences the efficacy of the other chemical to penetrate tissues	The insecticide 1,8-cineole increases the cuticular penetration of the insecticide camphor in cabbage loopers (Tak & Isman, 2015)
Transportation	A chemical influences the ability of the other chemical to reach its target site	*Knowledge gap*
Metabolization	A chemical influences the detoxification process of the other chemical	The fungicide prochloraz inhibits cytochrome P450-mediated detoxification, necessary for the detoxification of the insecticide λ-cyhalothrin, and thus increased effects are found in honey bees when these pesticides are mixed (Pilling et al., 1995)
Binding	A chemical changes the conformation of the other chemical, or changes the conformation of the target site, increasing the binding affinity to the target site	*Knowledge gap*
Excretion	A chemical influences the active excretion of the other chemical by the organism	In humans, at low doses, selenium decreases arsenic toxicity via the formation of selenium-arsenic compounds that are readily excreted (Sun et al., 2014)

underlying the synergistic effects of copper and cadmium at low doses remain unclear. Both metals exhibit non-linear, threshold dose-mortality relationships in bumblebees (Gekière, Breuer, Dorio, Vanderplanck, & Michez, 2024), suggesting that once a critical threshold is exceeded, physiological responses—such as the upregulation of metal-sequestering proteins—may mitigate interactive effects, thereby preventing synergy at higher doses. The absence of synergistic effects at higher doses may also reflect a limitation of the *synergyfinder* method, as mortality rates approach a ceiling effect, leaving little range for detecting further increases in mortality rates indicative of synergy. Future research should explore dose-dependent interactions in greater detail, with a particular focus on elucidating the molecular and physiological mechanisms driving these dose-dependent effects.

5 | CONCLUSIONS AND PERSPECTIVES

Our study is the first to establish the suitability of the *synergyfinder* method, widely used in medicinal drug research, for investigating dose-dependent interactive effects among environmental pollutants. As a proof of concept, we demonstrated that copper and cadmium—two widespread contaminants—exhibit synergistic toxicity in the buff-tailed bumble bee, but only at low doses. To address the absence of built-in significance testing in the *synergyfinder* method, we also complemented its use with robust statistical hypothesis testing, thereby strengthening the reliability of the interaction outcomes.

We encourage future risk assessments exploring chemical interactions in model organisms to adopt this framework, as it provides a robust and quantitative approach to assessing interactions. When chemicals are likely to co-occur in natural habitats, risk assessments should first establish dose–response curves for each individual compound and then apply the *synergyfinder* framework to identify potential detrimental, dose-dependent interactive effects on wildlife. Furthermore, most research on interactive effects focuses primarily on outcomes while largely overlooking the underlying mechanisms driving these interactions. To bridge this gap, we advocate for future studies to unravel the molecular and physiological processes that govern these interactions, as this knowledge is crucial for understanding dose-dependent outcomes.

A current limitation of the *synergyfinder* method is its reliance on relative response variables (e.g. percentage of viability), whereas ecotoxicology and environmental research often require absolute measurements (e.g. body mass). Developing graphical and statistical approaches to characterise dose-dependent interactive effects on such variables would therefore be highly relevant (e.g. assessing whether metals exhibit synergistic effects on body mass loss at low concentrations). Additionally, mounting evidence suggests that interactions between chemical and non-chemical stressors are key drivers of wildlife decline (e.g. agriculture and climate change synergistically impact insect populations; Outhwaite et al., 2022). However, unlike the framework we propose for chemical–chemical interactions, no standardised methodology currently exists to rigorously assess cross-category stressor interactions. Given the accelerating global biodiversity crisis, developing such integrative frameworks sounds both timely and essential.

AUTHOR CONTRIBUTIONS

Antoine Gekière: Conceptualisation; methodology; software; validation; formal analysis; investigation; visualisation; supervision; writing—original draft; writing—review and editing. **Maïlys Paulet:** Investigation; writing—review and editing. **Lauralyne Paulet:** Investigation; writing—review and editing. **Manon Fievet:** Investigation; writing—review and editing. **Dimitri Evrard:** Investigation; writing—review and editing. **Maxence Gérard:** Supervision; investigation; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

Data available via https://doi.org/10.5281/zenodo.15881356 (Gekière, 2025a) and R script available via https://doi.org/10.5281/zenodo.15988748 (Gekière, 2025b).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1. Number of replicates per treatment.

Appendix S2. Dose–response curves of *Bombus terrestris* workers exposed to (a) copper (LD_{50} = 40.3 μg/bee) or (b) cadmium (LD_{50} = 2.8 μg/bee). Bees were either alive ('0') or dead ('1') 96 h after exposure (acute oral).

Appendix S3. Survival curves comparing the observed and theorical mortality of *Bombus terrestris* workers exposed to a combination of (a) Cu 9.67/Cd 0.25 mM, (b) Cu 9.67/Cd 0.30 mM or (c) Cu 16.36/Cd 0.60 mM. Colour legend is in the first panel.

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