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Environmental risks of a commonly used pyrethroid: Insights from temporary pond species of the Lake Manyara Basin, Tanzania

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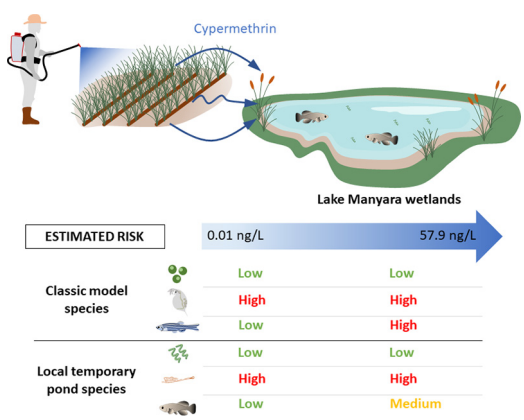
HIGHLIGHTS

- Chemical risk assessments (ERA) rely on toxicity data of classic model species.
- We conducted an ERA for cypermethrin with classic vs. local temporary-pond species.
- Both approaches yielded the similar results for primary producers and consumers.
- ERAs with species from unusual living environments could complement classic ERAs.

GRAPHICAL ABSTRACT



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ABSTRACT

Environmental risks posed by widespread pesticide application have attracted global attention. Currently, chemical risk assessments in aquatic environments rely on extrapolation of toxicity data from classic model species. However, similar assessments based on local species could be complementary, particularly for unusual living environments such as temporary ponds. Here, we carried out an environmental risk assessment (ERA) of a pyrethroid model compound, cypermethrin, based on local temporary pond species. First, we measured cypermethrin residue concentrations in rivers, irrigation canals and temporary ponds in the Lake Manyara Basin (LMB). Then, we estimated the environmental risks of cypermethrin by combining these data with acute toxicity data of three resident species across three trophic levels: primary producers (*Arthrospira platensis*), invertebrate grazers (*Streptocephalus lamellifer*) and fish (*Nothobranchius neumanni*). Furthermore, we compared the derived ERA to that obtained using toxicity data from literature of classic model species. Cypermethrin residue concentrations in contaminated systems of the LMB ranged from 0.01 to 57.9 ng/L. For temporary pond species, *S. lamellifer* was the most sensitive one with a 96 h-LC_{50} of 0.14 ng/L. Regardless of the assumed exposure concentration (0.01 and 57.9 ng/L), the estimated risks were low

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for primary producers and high for invertebrate grazers, both for local species as well as for classic model species. The highest detected cypermethrin concentration resulted in a moderate risk estimation for local fish species, while the estimated risk was high when considering classic fish models. Our results confirm that, at least for pyrethroids, ERAs with classic model species are useful to estimate chemical risks in temporary pond ecosystems, and suggest that complementary ERAs based on local species could help to fine-tune environmental regulations to specific local conditions and conservation targets.

1. Introduction

Agriculture is rapidly intensifying to stay on par with the increasing demand for food, particularly in developing regions of the global South (Carlson, 2018; Kaini, 2020). However, a multitude of diseases and pests compromises agricultural productivity and necessitates farmers to use pesticides to limit their losses (Abhilash and Singh, 2009). These pesticides may accidentally enter the surrounding environment and pollute adjacent freshwater systems which may lead to unwanted changes in the community structure and the functioning of ecosystems (Loch, 2005; Merga and Van den Brink, 2021; Shefali et al., 2021).

To predict and mitigate the impact of pesticides on freshwater ecosystems, pesticides are typically subjected to environmental risk assessments (ERA) that feed into environmental policies and regulations. As part of the first steps of conducting such an ERA, the acute toxicity of a pesticide is determined by means of single-species exposure tests with model organisms that represent three different trophic levels: primary producers (algae), primary consumers (invertebrate grazers) and secondary consumers (fish) (Maund et al., 1998; Vryzas et al., 2011; Köck-schulmeyer et al., 2012; Silva and Cerejeira, 2015; Kapsi et al., 2019). In Europe, typical model organisms that are used for these tests are *Acutodesmus obliquus* (green algae) and *Anabaena* sp. (cyanobacteria) as primary producers, *Daphnia magna* (water fleas) as primary consumers, and *Danio rerio* (zebrafish) as secondary consumers. In the United States, the Environmental Protection Agency (EPA) requires toxicity data (acute and chronic) from aquatic algae and plants (as primary producers), invertebrates (primary consumers), amphibians and fish (secondary consumers from both marine and freshwater systems) (Moore et al., 2021). To assess the acute toxicity of a pesticide, organisms are exposed for 24–96 h to different doses of the pesticide to generate dose-response curves from which typical toxicity parameters (e.g. LC_{50}) can be calculated (Busquet et al., 2014). These toxicity parameters are then extrapolated to other species and the estimated risks are generalized across taxa and habitats (Buckler et al., 2005; van den Berg et al., 2021). The European Food Safety Agency (EFSA) requires the LC_{50}-values to be divided by a specific safety factor to calculate the so-called Predicted No-effect Concentration (PNEC), which is the assumed safe concentration at which no effects are expected to occur. Which safety factors are used typically depends on which toxicity data are available: commonly used factors are 10 or 100 when an adequate number of acute or long-term toxicity data are available (from at least eight invertebrate and five fish species), and 1000 when only acute toxicity data on one representative species are available (EFSA, 2005). Next, a risk quotient (RQ) is calculated as the ratio of measured environmental concentration, also called predicted environmental concentration (PEC), to PNEC. The environmental risk is considered low: when RQ is <0.1, medium: when RQ is 0.1–0.9, and high: when RQ >1 (Bai et al., 2018). A somewhat different approach is adopted in the United States, in which RQ's are calculated as a ratio of Estimated Environmental Concentration (EEC) to acute and chronic toxicity data (EPA, 2016). Calculated RQ's are then compared to the EPA's established levels of concern (LOC) to characterize the risks of a particular chemical (EPA, 2016). Risks are regarded as "Acute", "Acute Restricted Use" or "Acute Endangering Species" when EEC/LC_{50} or EC_{50} is 0.5, 0.1, or 0.05, respectively. When chronic data are used, chronic risks are concluded when the EEC equals the No Observed Adverse Effect Concentration (NOAEC), i.e. when the ratio of EEC to NOAEC is 1 (EPA, 2016).

While typically classic study species are used to determine the toxicity of compounds, conducting an ERA based on toxicity data from local, non-standard species (in addition to classic model species) could be complementary and may help to further fine-tune ERAs to local conditions or specific conservation targets (Wepener and Chapman, 2012). For example, organisms from temporary ponds often have non-generic life histories or other unique biological attributes that facilitate life in extreme environments but may lead to differences in pesticide sensitivity compared to that of established model organisms (Lahr, 1998; Kafula et al., 2022). Typical inhabitants of temporary ponds, including fairy shrimps and annual killifish, survive dry periods by producing drought-resistant dormant eggs that are buried in the pond sediments (Pinceel et al., 2017; Grégoir et al., 2018; Rogers et al., 2021; Brendonck et al., 2022). As soon as the pond is inundated again, part of these eggs hatch and organisms rapidly grow and complete their life cycle before ponds dry out again (Nhiwatiwa et al., 2014; Pinceel et al., 2021). In Tanzania, for instance, temporary ponds typically only hold water for about two months before drying, during which resident organisms have to complete their life cycles. Furthermore, temporary pond systems are often poorly buffered against ambient changes and typically experience large daily fluctuations in abiotic conditions (e.g. temperature, oxygen and conductivity) as well as over the course of their hydroperiod (i.e. the time during which ponds contain water). Even though the sensitivity of some temporary pond species to chemical exposure has been shown to be largely in range of that of classic model species (Philippe et al., 2017; Thoré et al., 2021b), it is sometimes hypothesized that temporary pond organisms may be more sensitive to chemicals than their counterparts from permanent freshwater ecosystems (Lahr, 1998; Kafula et al., 2022). This higher sensitivity would result from a trade-off between their ability to survive in highly variable and ephemeral environments – which demands extra energy for survival, fast growth and reproduction rates – and their resistance to pesticide exposure (Thoré et al., 2021a; Kafula et al., 2022).

Temporary ponds offer a wide range of ecosystem services, such as clean water for irrigation or domestic purposes (Van den Broeck et al., 2015; Zongo et al., 2017), drinking places for cattle and wildlife (Van den Broeck et al., 2019) as well as wallowing spots for buffaloes and elephants (Vanschoenwinkel et al., 2011). Temporary ponds are also feeding and nesting places for birds (Moreno-Opo et al., 2011). However, the integrity of temporary pond ecosystems, like other freshwater ecosystems, is threatened by ongoing global change, including not only habitat alteration, introduction of alien species and various effects of climate change, but also pollution with various agrochemicals (Brain and Prosser, 2022).

Because temporary pond organisms are adapted to live under extreme conditions, carrying out chemical environmental risk assessment using local species could help to fine-tune classic ERAs and tailor them to the relatively unusual conditions of temporary pond ecosystems. Our study focuses on temporary ponds in the LMB of northern Tanzania as a case-study to perform an ERA with local species and compare it with an ERA based on classic models. Northern Tanzania is a suitable study area not only because it has several lakes, rivers and numerous temporary pond systems that are valuable in terms of biodiversity and service to the local communities, but also because these systems are increasingly threatened by growing agricultural activities in the region (Ngana et al., 2004; Bachofer et al., 2015; Maerker et al., 2015; Manyilizu and Mdegela, 2015). Cypermethrin, a synthetic pyrethroid, is by far the most commonly used insecticide in Tanzania (Manyilizu and Mdegela, 2015). This pesticide has a broad activity spectrum, is highly toxic to insects, and is used in agriculture

as well as for veterinary applications and mosquito control programs (Li et al., 2005; Afolabi et al., 2019).

In this study, we did the first steps of an ERA to characterize environmental risks of cypermethrin on temporary pond ecosystems in the LMB in northern Tanzania. Specifically, we first determined the current environmental concentrations of cypermethrin in rivers, irrigation canals and temporary ponds. Water samples were collected during the rainy season which coincides with peak pesticide application in the region. Moreover, as the majority of pesticide pollution occurs mid-stream, samples in rivers and irrigation canals were collected downstream of agricultural hotspots, while for temporary ponds an integrated sample (from several micro-habitats per pond) was taken to increase the chances of detection. Next, we estimated the predicted no-effect concentrations (PNEC) for a blue-green alga (*Arthrospira platensis*), invertebrate grazer (the fairy shrimp *Streptocephalus lamellifer*) and fish (the killifish *Nothobranchius neumanni*) based on acute toxicity data of these three species. These three species were selected as they are native to the temporary pond ecosystems of the LMB. Toxicity data for algae and fish were derived from literature, while toxicity data for fairy shrimps were obtained by conducting an acute toxicity test. Finally, we calculated the risk quotients (RQ) of the three native species to estimate the environmental risks of cypermethrin on the temporary pond ecosystems of LMB. We then compared the derived ERA to that obtained using literature toxicity data of classic model species in ecotoxicology, specifically the green alga (*A. obliquus*), an invertebrate (*D. magna*) and five fish models: common carp (*Cyprinus carpio*), zebrafish (*D. rerio*), rainbow trout (*Oncorhynchus mykiss*), Nile tilapia (*Oreochromis niloticus*) and japanese medaka (*Oryzias latipes*). Moreover, we compared the sensitivity of classic models and the focal temporary pond species by means of a species sensitivity distribution (SSD).

Temporary ponds in the LMB are either rain-fed or flooded by rivers flowing in adjacent areas (Bachofer et al., 2014). Connectivity of rivers and temporary ponds in floodplains can facilitate contamination of temporary ponds with pesticides. Because temporary ponds are not well buffered against daily temperature fluctuations and typically have high amounts of organic matter that may promote the removal of dissolved pesticides, we expect the residue concentrations of cypermethrin to be lower in temporary ponds and higher in rivers and irrigations canals. Moreover, we hypothesise that there will be high risks of cypermethrin on representative temporary pond species (invertebrate and fish) due to possible trade-offs between their environmental robustness and resistance to pesticide exposure, making them more sensitive to cypermethrin.

2. Materials and methods

2.1. Measuring environmental concentrations of cypermethrin

Duplicate water samples (500 mL) from 12 rivers, 23 temporary ponds and three irrigation canals (Fig. 1) were collected in April–May 2021 (during the long rainy season) in amber glass bottles (preserved with 10 % NaCl) and transported to the Chemistry laboratory at the University of Dar es Salaam under cooled conditions (4 °C) until pesticide extraction.

2.1.1. Chemicals used for analysis

The certified pesticide reference standard used for the identification and quantification of cypermethrin was obtained from Sigma-Aldrich (Germany) with purity of 99 % (CAS number: 52315-07-8) and stored in the freezer to minimize degradation. Pesticide analytical grade acetonitrile (CAS number: 75-05-8) and dichloromethane (CAS number: 75-09-2) were purchased from Sigma-Aldrich (Germany). In addition, all other reagents and solvents used were of analytical grade purchased from Sigma-Aldrich (Germany) including anhydrous sodium sulphate (CAS number: 7757-82-6). Cypermethrin stock solution (100 μg/mL) standards were prepared by pipetting the appropriate aliquot of the synthetic pyrethroid pesticides into 25-mL volumetric flasks, and then dissolving and diluting to the marks with ethyl acetate with the aid of a vortex mixer (Thermolyne MaxiMix-Plus). All solutions prepared for gas chromatography (GC) were filtered through a 0.45-μm nylon filter.

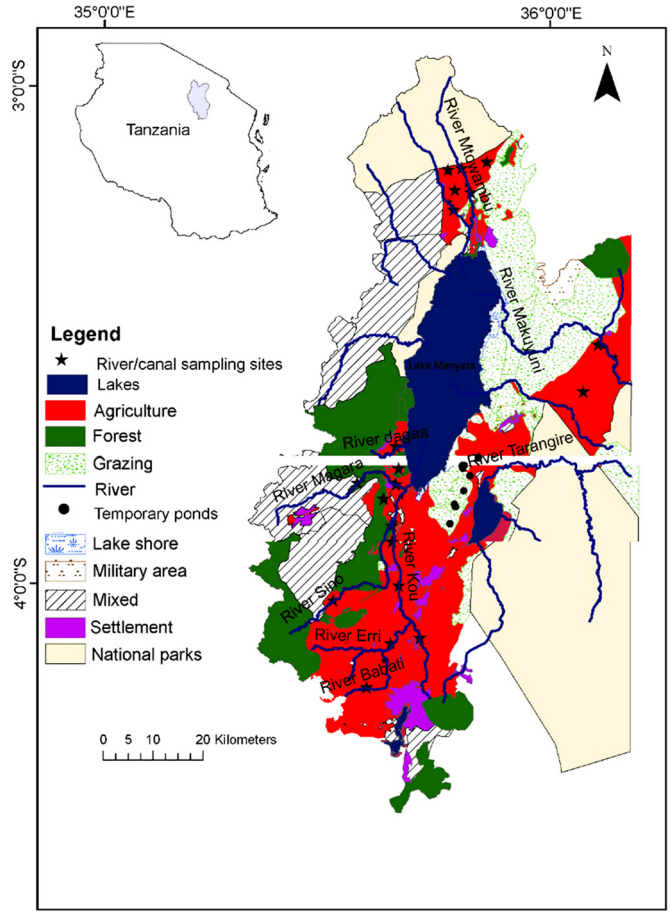

Fig. 1. Map of the Lake Manyara Basin showing river- and temporary pond sampling sites.

2.1.2. Extraction of cypermethrin from water samples

Unfiltered water samples were extracted by liquid–liquid extraction (LLE) as described by Kishimba et al. (2004). Briefly, each sample (0.5 L) was transferred to a 1-L separating funnel and the bottle rinsed with dichloromethane (30 mL), and combined with the sample in the separating funnel. The samples were mixed by inverting the flask four times. 50 mL of extraction solvent (dichloromethane) was added and samples were vigorously shaken manually for three minutes, while occasionally releasing the pressure. The phases were allowed to separate for five minutes and the dichloromethane extracts (organic layers) were separated from the aqueous layers. The extraction for each water sample was repeated twice with 30 mL of dichloromethane and the organic layers put together and dried by anhydrous sodium sulphate. The combined extracts were concentrated in vacuo at 30 °C, and the final extract was made up in 2 mL cyclohexane: acetone (9:1 *v*/v), ready for GC analysis. Absence of sediments, colour and debris ensured that all samples were sufficiently clean, which were therefore not subjected to further clean-up procedures.

2.1.3. GC–MS analysis of cypermethrin concentration

GC–MS analysis was performed in a GCMS-QP 2010Ultra (Shimadzu instrument) operating in Electron Ionization (EI) mode (MS) at 70ev, and Flame Ionization Detector (FID) for GC. A Restek-5MS column (30 m × 0.25 mm × 0.25 μm) was used. The oven temperature program was 90 °C for two minutes and increased to 280 °C for two minutes at the rate of 25 °C per minute. The injection temperature was 250 °C with split injection mode. The flow rate of carrier gas helium was 1.21 mL min^{-1}. The ion

source temperature and interface temperature in MS were 230 °C and 300 °C, respectively. The identification of compounds in the sample was done by the scanning method which involved the use of Mass Spectral Library and Search Software (NIST). Quantification of compounds in the extract was done using the Peak Integration method whereby ion allowance was 20 %. Target ion and other five quantification ions were used on quantitative analyses. For injection, 1 μL of the sample in dichloromethane was injected in GC–MS.

2.1.4. Quality assurance and quality control

All glassware used for analysis was rigorously washed with detergent, rinsed with distilled water and thoroughly rinsed with analytical grade acetone before drying overnight in an oven at 150 °C. The glassware was then allowed to cool down and stored in dust-free cabinets. The quality of cypermethrin residues was assured through the analysis of solvent blanks and duplicate samples. All reagents used during the analysis were exposed to same extraction procedures and subsequently ran to check for interfering substances. In the blank for each extraction procedure, no pesticide was detected. Samples of each series were analyzed in duplicates. Recalibration curves were run with each batch of samples to ensure that the correlation coefficient was always >0.98. The efficiency of the analytical methods was determined by recoveries of an internal standard. The recoveries of internal standards ranged between 80 and 100 %. The measured concentrations of the internal standard were in range with the detected cypermethrin concentrations. Recovery values show that the method used was reliable and reproducible. The minimum limit of cypermethrin detection was 0.001 ng/L.

2.2. Determination of algae and fish toxicity

Toxicity data for algae were obtained from the ECOTOX database (US Environmental Protection Agency), Google Scholar, Web of Science and PubMed. Keywords included 'cypermethrin', 'algae toxicity', '*Acutodesmus obliquus*', 'pesticide-induced algae growth inhibition', 'toxicity', and 'LC_{50}' to find acute toxicity data of algae to be used for derivation of PNEC values. Only toxicity data published in peer-reviewed journals were considered. Cypermethrin acute toxicity values (LC_{50}) for *Nothobranchius neumanni*, which is the only fish species inhabiting temporary ponds of the LMB, were obtained from Kafula et al. (2022).

2.3. Streptocephalus lamellifer acute toxicity assessment

2.3.1. Fairy shrimp hatching and maintenance

The fairy shrimp *Streptocephalus lamellifer* is the most common anostracan in the study region (Kafula et al., in review). An integrated dry sediment sample with *S. lamellifer* dormant eggs was collected from 14 temporary ponds in the LMB as described by Boven et al. (2008). Briefly, before taking sediments for dormant eggs, deeper areas of the pond were pinpointed, from which additional samples were taken. Then, sediments were sampled from four established transects that radiate from the center. Along the transect lines, 5–20 sediment samples were taken from each pond from different locations. Sediment (~1 kg) was collected from the upper 3 cm as most viable dormant eggs are found at the upper 2 cm of the pond bottom (Boven et al., 2008). Sediments with dormant eggs were air-dried, homogenized, wrapped in aluminum foil and stored in the dark at room temperature before being inundated. Sediment samples were screened by means of sugar floatation to select samples with high abundance of *S. lamellifer* eggs (Pinceel et al., 2017). Then, hatching and maintenance of fairy shrimps was done (with slight modifications) as described by Thoré et al. (2021a). Briefly, 3 kg of mixed sediment sample were inundated in each of the 15 plastic containers with 140 L dechlorinated tap water at 26 °C under a 14:10 h light:dark regime. Seven days after inundation, hatchlings were transferred individually to 500-mL glass jars with reconstituted water at 27 ± 1 °C and a 14:10 h light:dark regime. Glass jars were placed in heat-regulated water tubs to maintain a constant temperature. Reconstituted water was made by adding standardized Instant Ocean Sea Salt (Instant Ocean-Aquarium Systems, Fiji) to dechlorinated tap water till a conductivity of 450 μS/cm. Individuals were fed ad libitum with live *Acutodesmus obliquus* (CCAP 276/3A).

2.3.2. Acute exposure test experimental setup

Prior to the experiment, range-finding tests were carried out to determine the appropriate concentration range to be used in the acute toxicity test (results of the last range finding experiment are presented in Supplementary Figure 1). The acute toxicity test ran for 96 h as described in OECD TG 202 (*Daphnia* sp. Acute Immobilisation Test) and Kim et al. (2008). One hundred and twenty (120) *S. lamellifer* juveniles (96 h old) were divided into eight experimental groups at different concentrations of cypermethrin, each group of which was replicated 15 times ($n = 15$). After the initial 48 h of exposure, test organisms were fed ad libitum with live *Acutodesmus obliquus* algae twice throughout the 96 h experimental duration and test media renewed. Basic water-chemistry parameters were measured before and after water renewal (electric conductivity: 490 ± 25.4 μS/cm, pH: 6.8 ± 0.4, temperature: 27 ± 1 °C). Immobility was scored once daily as a proxy for mortality, and was defined as an individual being motionless for 15 s after gentle agitation of the test vessel, based on the OECD TG 202. Nominal concentrations used were 0 (control), 0.0329, 0.0987, 0.2963, 0.889, 2.67, 8 and 24 ng/L. Achieved concentrations were measured using GC–MS (Model QP 2010, Shimadzu corporation, Japan; $n = 3$ per concentration) and ranged from 75.98 to 109 % of the nominal concentrations used. Achieved concentrations were 0.03 ± 0.05, 0.095 ± 0.0004, 0.301 ± 0.008, 0.78 ± 0.09, 2.48 ± 0.45, 7.8 ± 0.14 and 25 ± 0.8 ng/L.

2.4. Ecological risk assessment

The ecological risk of cypermethrin on algae, invertebrate grazers and fish was assessed using the EFSA risk quotient (RQ) method, as described by Bai et al. (2018). Briefly, RQ's were calculated for each of the tested species using the formula $RQ = C/PNEC$ where C is the measured environmental concentration and PNEC is the predicted no-effect concentrations for cypermethrin. PNEC data were obtained by dividing the 96 h-LC_{50} by a safety factor of 1000 (Chapman et al., 1998). The choice of 1000 as a safety factor is conservative, but is based on absence of cypermethrin chronic toxicity data for selected temporary pond species which could have justified the use of other safety factors (10 or 100). Inference of ecological risks were made after Bai et al. (2018). Environmental risks were classified into three levels based on RQ values: 0.01–0.1 represents relatively low risk, 0.1–1 a medium risk, and values above 1 indicate high risk. Moreover, we used the 96 h-LC_{50} data of both classic models and temporary pond species to generate SSD curves in R (version 4.2.2, ssdtools package).

3. Results

Cypermethrin residues were detected in rivers and irrigation canals within the LMB. Detected concentrations in contaminated systems ranged from 0.01 to 57.9 ng/L (Table 1). Cypermethrin was not detected in 8 of the 15 rivers (53 %) and in 1 of the 3 (33 %) irrigation canals (detection limit: 0.0001 ng/L). Moreover, Cypermethrin was not detected in any of the studied temporary ponds. Results further indicate that the rivers Babati and Erri (9.29 and 57.9 ng/L, respectively) and irrigation canals in Kissangeji (5.32 and 17.36, respectively ng/L) had higher levels of cypermethrin residues compared to rivers and canals from other areas within the LMB (i.e rivers Dagaa, Magara and Mto wa Mbu which had 0.01, 0.489 and 2.18 ng/L, respectively) (Table 1). Additionally, cypermethrin was not detected in any of the rivers in the Lake Manyara national park, even the ones originating outside the park (such as river Iyambi, Endala and Msasa). Despite originating in agricultural active areas (Mbulu district), rivers Dagaa and Magara had lower levels of cypermethrin, ranging from 0.01 to 0.489 ng/L, compared to the ones in Babati (cypermethrin residues ranging from 9.29 to 57.9 ng/L) (Table 1).

Table 1
Levels of cypermethrin in temporary ponds, irrigation canals and rivers of the Lake Manyara Basin.

Temporary pond ID	Geographical coordinates (UTM)		Cypermethrin residue (ng/L)	River/irrigation canal	Geographical coordinates (UTM)		Cypermethrin residue (ng/L)
	Eastings	Northings			Eastings	Northings	
MG1	810,424	9,560,639	BDL	River Iyambi	803,677	9,594,405	BDL
MG2	810,185	9,560,310	BDL	River Endabashi	805,999	9,605,387	BDL
MG3	810,124	9,559,705	BDL	River Endala	809,347	9,612,631	BDL
MG4	810,075	9,559,680	BDL	River Msasa	811,730	9,619,303	BDL
MG5	810,046	9,559,674	BDL	River Mto wa mbu	816,951	9,624,005	2.18
MG6	809,864	9,559,441	BDL	Kissangeji canal 1	802,913	9,570,902	5.32
DR1	817,159	9,578,751	BDL	River Oltukai	176,703	9,606,276	BDL
DR2	817,212	9,578,492	BDL	River Babati	806,203	9,536,517	9.29
DR3	815,744	9,580,569	BDL	River Erri	798,236	9,544,061	BDL
DR4	813,142	9,569,313	BDL	River Rohu	798,436	9,544,661	BDL
DR5	807,855	9,554,566	BDL	River Dagaa	798,104	9,583,207	0.01
SA1	825,949	9,620,995	BDL	River Magara	800,308	9,578,884	0.489
SA2	825,416	9,621,428	BDL	River Erri-Babati	803,265	9,554,063	57.9
SA3	821,689	9,623,855	BDL	Kissangeji canal 2	803,461	9,570,185	17.36
SA4	821,215	9,622,375	BDL	Mto wa mbu canal	808,932	9,557,931	BDL
SA5	821,675	9,623,421	BDL				
SA6	821,855	9,623,529	BDL				
PR1	803,486	9,590,362	BDL				
PR2	803,987	9,592,115	BDL				
PR3	804,099	9,593,542	BDL				
PR4	804,316	9,595,857	BDL				
PR5	803,035	9,604,154	BDL				
PR6	809,296	9,615,281	BDL				

BDL = Below Detection Limit.

During the acute exposure test, *S. lamellifer* survival was 90 % in the control condition. Cypermethrin LC_{50} values at 24, 48, 72 and 96 h are presented in Table 2. Cypermethrin LC_{50} values decreased with increasing exposure duration. Mortality after 24 h exposure was very low, and the resulting LC_{50} value could not be assessed reliably. The 48-, 72-, and 96 h-LC_{50} values were reliable and ranged from 0.597 ng/L at 24 h to 0.14 ng/L at 96 h. The dose-response curve after 48 h of exposure is shown in Fig. 2.

Because cypermethrin could only be detected in rivers and irrigation canal samples, we used the lowest and highest detected concentration (0.01 and 57.9 ng/L, respectively) to assess the potential environmental risks of the compound to temporary pond species (Table 3) and classic model species should temporary ponds become contaminated with cypermethrin (Table 4). Acute toxicity results show that *S. lamellifer* is more sensitive to cypermethrin exposure compared to *A. platensis* (algae) and *N. neumanni* (fish). Sensitivity of temporary pond species was in line with that of classic model species. Fig. 3 shows that the sensitivity of the focal temporary pond species (*A. platensis*, *S. lamellifer*, *N. neumanni*) does not deviate strongly from that of classic model species.

Arthrospira platensis appears to be the most resistant species among the focal temporary pond species, with a 96 h-LC_{50} of 53.12 mg/L. For both fish and algae from temporary ponds, the calculated risk quotients at the lowest detected concentration (0.01 ng/L) were < 0.001 (1.88×10^{-7} and 4×10^{-5}, respectively) indicating a low risk to both fish and algae (Table 3). However, the calculated risk quotient for *S. lamellifer* was >1 indicating high risk for invertebrate grazers. At the highest recorded environmental concentration (57.9 ng/L), the risk quotient for *N. neumanni* increased from 4×10^{-5} to 0.214 (Table 3), suggesting a moderate risk of cypermethrin to fish at this concentration. For *A. platensis*, however, the estimated risk remained low.

At the lowest detected concentration (0.01 ng/L), results of the ERA based on toxicity data of classic model species (Table 4) were similar to those of temporary pond species. Here, the risk quotient indicated high risk only to invertebrates (*D. magna*) similar to what was observed for invertebrate temporary pond species (*S. lamellifer*). However, at the highest detected concentration (57.9 ng/L), calculated risk quotients indicated high risks to both *D. magna* and classic fish models. The estimated risk for algae (Table 4) was low at both cypermethrin concentrations.

Table 2
LC_{50} values at 24, 48, 72 and 96 h after the start of juvenile *Streptocephalus lamellifer* exposure to cypermethrin, including the corresponding standard errors and *P*-values.

Duration (h)	LC_{50} (ng/L)	Standard error	*P-value*
24	143.15	422.56	0.736
48	0.597	0.09	0.001
72	0.16	0.017	0.001
96	0.14	0.013	0.001

4. Discussion

We assessed environmental residue concentrations of the commonly used pesticide cypermethrin in rivers, temporary ponds, and irrigation canals in the LMB, a region in northern Tanzania with intensifying agriculture activities. Using algae and fish toxicity data obtained from literature, and

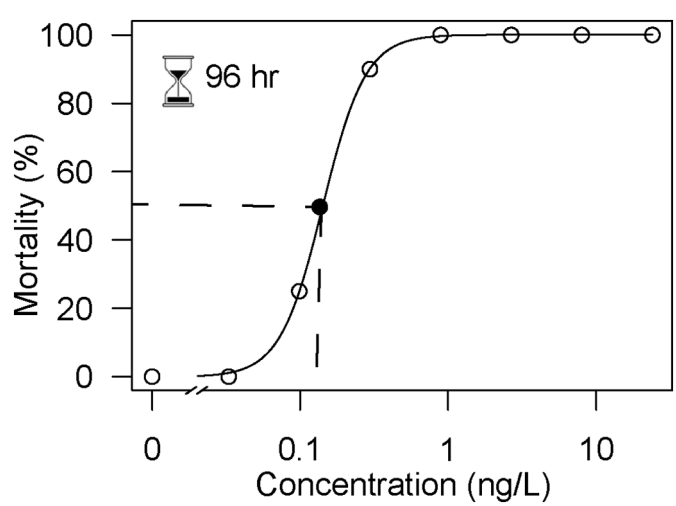

Fig. 2. Concentration-mortality curve after 96 h of exposure of *Streptocephalus lamellifer* juveniles to cypermethrin. The black dot represents the LC_{50} value.

Table 3
Measured environmental concentration of cypermethrin (C), LC_{50} values for algae (*Arthrospira platensis*), invertebrates (*Streptocephalus lamellifer*) and fish (*Nothobranchius neumanni*), with their respective PNEC values and risk quotients for the lowest (0.01 ng/L) and highest (57.9 ng/L) measured environmental concentrations. Risks were classified into three levels (Low, Medium, High), after Bai et al. (2018).

	Species	C (mg/L)	96 h-LC_{50} (mg/L)	PNEC (mg/L)	RQ	Risk	Reference
Lowest concentration	*A. platensis*	1×10^{-8}	53.12	0.05312	1.88×10^{-7}	Low	(Tunca et al., 2021)
	S. lamellifer	1×10^{-8}	1.4×10^{-7}	1.4×10^{-10}	71.43	High	*
	N. neumanni	1×10^{-8}	0.25	2.5×10^{-4}	4×10^{-5}	Low	(Kafula et al., 2022)
Highest concentration	*A. platensis*	5.79×10^{-5}	53.12	0.05312	0.001	Low	(Tunca et al., 2021)
	S. lamellifer	5.79×10^{-5}	1.4×10^{-7}	1.4×10^{-10}	4.1×10^{5}	High	*
	N. neumanni	5.79×10^{-5}	0.25	2.5×10^{-4}	0.23	Medium	(Kafula et al., 2022)

* This study.

Table 4
Measured environmental concentration of cypermethrin (C), LC_{50} values for algae (*Acutodesmus obliquus*), invertebrates (*Daphnia magna*) and five classic fish models, with their respective PNEC values and risk quotients for the lowest and highest measured environmental concentrations. Risks were classified into three levels (Low, Medium, High), after Bai et al., (2018).

	Species	C (mg/L)	LC_{50} (mg/L)	Time (h)	PNEC (mg/L)	RQ	Risk	Acute toxicity reference
Lowest concentration	*A. obliquus*	1×10^{-8}	112	96	0.112	8.93×10^{-8}	Low	(Li et al., 2005)
	D. magna	1×10^{-8}	6.1×10^{-7}	96	6.1×10^{-10}	16.39	High	(Kim et al., 2008)
	H. azteca	1×10^{-8}	3.5×10^{-6}	96	3.5×10^{-9}	2.86	High	(Clark et al., 2015)
	C. carpio	1×10^{-8}	0.0026	96	2.7×10^{-6}	3.7×10^{-3}	Low	(Saha and Kaviraj, 2008)
	D. rerio	1×10^{-8}	0.0021	96	2.1×10^{-6}	4.8×10^{-3}	Low	(Uddin et al., 2018)
	O. mykiss	1×10^{-8}	0.0031	96	3.1×10^{-6}	3.2×10^{-3}	Low	(Velisek et al., 2006)
	O. niloticus	1×10^{-8}	0.006	96	6.0×10^{-6}	1.67×10^{-3}	Low	(Sarikaya, 2009)
	O. latipes	1×10^{-8}	0.0385	48	3.85×10^{-5}	2.59×10^{-4}	Low	(Kim et al., 2008)
Highest concentration	*A. obliquus*	5.79×10^{-5}	112	96	0.112	5.2×10^{-4}	Low	(Li et al., 2005)
	D. magna	5.79×10^{-5}	6.1×10^{-7}	96	6.1×10^{-10}	94.92×10^{4}	High	(Kim et al., 2008)
	H. azteca	*5.79 × 10–5*	*3.5 × 10–6*	*96*	*3.5 × 10–9*	*1.65 × 104*	Low	(Clark et al., 2015)
	C. carpio	5.79×10^{-5}	0.0026	96	2.6×10^{-6}	22.23	High	(Saha and Kaviraj, 2008)
	D. rerio	5.79×10^{-5}	0.0021	96	2.1×10^{-6}	27.57	High	(Uddin et al., 2018)
	O. mykiss	5.79×10^{-5}	0.0031	96	3.1×10^{-6}	18.68	High	(Velisek et al., 2006)
	O. niloticus	5.79×10^{-5}	0.006	96	6.0×10^{-6}	9.65	High	(Sarikaya, 2009)
	O. latipes	5.79×10^{-5}	0.0385	48	3.85×10^{-5}	1.50	High	(Kim et al., 2008)

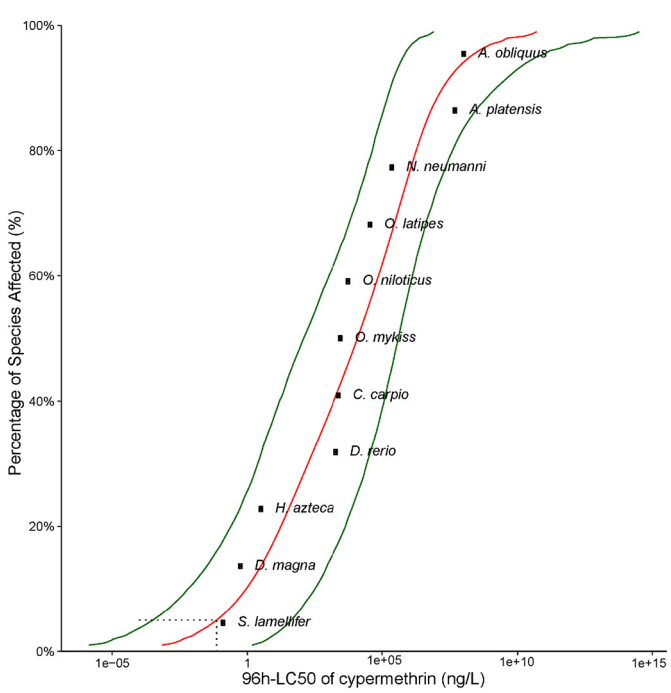

Fig. 3. Species Sensitivity Distribution curve including both classic models and the focal temporary pond species, based on 96 h-LC_{50} data of cypermethrin. The red line is a model-fitted distribution and green lines indicate the 95 % prediction intervals. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

invertebrate toxicity data acquired from an acute exposure test, we determined safe concentrations to be used in environmental risk assessments for temporary ponds. Specifically, we determined the potential environmental risks of cypermethrin using both local inhabitants of temporary ponds in the LMB, as well as based on toxicity data from classic model organisms. Cypermethrin concentrations in contaminated rivers (6 out of 12) and irrigations canals (2 out of 3) ranged from 0.01 to 57.9 ng/L. In temporary ponds, samples did not contain cypermethrin above the detection limit. Even though no cypermethrin was detected in the temporary ponds, we used the detected concentrations in rivers/canals (0.01 and 57.9 ng/L) as likely exposure scenario's – based on the fact that temporary pond often receive water input from neighboring rivers/canals (Zacharias and Zamparas, 2010) – in order to compare the potential environmental risks of cypermethrin exposure to local vs. classic test species as a proof-of-principle. When comparing the three temporary-pond species, the invertebrate *S. lamellifer* was the most sensitive species with a 96 h-LC_{50} of 0.14 ng/L. At 0.01 ng/L cypermethrin, high risks were derived for both *S. lamellifer* and *D. magna*. Additionally, at 0.01 ng/L cypermethrin, low risks were derived for algae and fish (both classic models and temporary pond species). At 57.9 ng/L, cypermethrin posed high risk to both *S. lamellifer* and *D. magna* and all five classic fish models while the risk remained moderate for *N. neumanni*. These findings are regardless of whether a EU- or US-style ERA are used.

As we expected, cypermethrin residues were recorded in rivers and irrigation canals. Our findings were lower than those reported in the surface waters of Southern Malawi (8.12–15.46 mg/L) (Kanyika-Mbewe et al., 2020), but were in line with those reported in South Africa (<0.1 μg/L) (Ansara-Ross et al., 2006, 2012). Residue concentrations were higher in rivers flowing through the Babati and Mbulu districts compared to rivers flowing through the Monduli district. This is not surprising, because agricultural activities, including pesticide use, are higher in Babati and Mbulu

compared to Monduli, so that higher levels of pesticide contamination can be expected (Burn, 2003; Passeport et al., 2014; Carluer et al., 2017). Variation in measured cypermethrin concentrations between Babati and Mbulu may reflect the difference in pesticide application frequency and intensity in the two districts. Intensive agricultural activities in Babati areas suggests that farmers use higher amounts of cypermethrin-based insecticides compared to Mbulu.

Despite being used all year round, irrigation canals in Mto wa Mbu had lower levels of cypermethrin compared to the ones in Kissangeji. Variation of cypermethrin residue between these areas, both of which experience intensive agriculture, could be linked to the type of crops cultivated and associated differences in pesticide use. In Kisangeji, farmers rotate annual and horticultural crops in one area, resulting in a continuous pesticide use during the year (Scherm et al., 2001; Mrema et al., 2017). In contrast, farmers in Mto wa Mbu focus on rice farming with little to no horticultural crops. Rice farming needs less applications of pesticides compared to horticultural crops, as also observed in Cheju Zanzibar (Stadlinger et al., 2011). In contrast to the rivers in developed areas, cypermethrin was not detected in rivers passing through the Lake Manyara National Park (which is devoid of agricultural activities). Still, it would not be unexpected that agricultural activities in upstream areas could result in pesticide residues in the downstream surface waters of the National Park. In support of this, pesticides have been detected in other national parks in previous studies, including in Kruger National Park (South Africa) and Tortuguero Conservation Area (Costa Rica) to levels that pose risks to freshwater species (Castillo et al., 2000; Wolmarans et al., 2021). It is possible that our sampling campaign may have been carried out prior to pesticide application in upstream areas which could explain why cypermethrin was not detected in the National Park.

Cypermethrin was not detected in any of the 23 sampled temporary ponds, even though several of these ponds are in or adjacent to agricultural land and often connect to rivers during floods. These ponds are typically shallow, and have a low capacity to buffer against temperature fluctuations during the day. Ponds may consequently heat up substantially, which accelerates pesticide degradation (Op et al., 2016) and could explain why cypermethrin was below detection limit in temporary ponds. Furthermore, temporary ponds typically have higher amounts of organic matter compared to rivers and canals. This facilitates sorption of lipophilic compounds, such as cypermethrin (log Kow of 6.60), to the organic matter and decreases the levels of dissolved pesticide.

It is important to note that the current sampling campaign only captures a snapshot of the environmental presence of cypermethrin. No sampling campaigns were previously undertaken in the region and the local environmental levels of cypermethrin were not yet known, so that the provided data provide an important first step. Still, follow-up sampling campaigns are needed to collect more in-depth data in order to fully characterize the environmental levels of cypermethrin in the region and its variation across space and time. For example, even though no cypermethrin could be detected in the temporary ponds, it is conceivable that these ponds may nevertheless become contaminated with pesticides given that these ponds may receive contaminated water from neighboring rivers during the frequent flooding events in the region (Zacharias and Zamparas, 2010), and given their vicinity to agricultural activity (Lahr et al., 2000; Lahr, 1998). Estimated risks for primary producers (*A. platensis*) of temporary ponds were low under both the lowest (0.01 ng/L) and highest (57.9 ng/L) detected cypermethrin concentration. This may not be unsurprising, given that pyrethroids (such as cypermethrin) often pose no great risk to aquatic plants or primary producers (Hill, 1989). For temporary-pond grazers (*S. lamellifer*), the calculated risk quotients revealed high risks under both exposure scenarios. The estimated risk for secondary consumers of temporary ponds (*N. neumanni*) was low at the lowest detected concentration of cypermethrin (0.01 ng/L), while the highest detected concentration (57.9 ng/L) lead to a moderate risk.

When using toxicity data of classic model species, the estimated risk quotients largely resulted in similar conclusions. Regardless of the assumed exposure scenario, calculated risk quotients indicated low risks to local primary producers (*A. platensis*) as well as to classic models (*A. obliquus*). Estimated risks were high to both specialized temporary pond invertebrates (*S. lamellifer*) and the classic model *D. magna*, both at high (57.9 ng/L) and low (0.01 ng/L) cypermethrin exposure. At a low exposure concentration, the estimated risks were low for both temporary-pond fish (*N. neumanni*) as well as classic fish models. These observations suggests that ERAs with classic models may be sufficiently conservative to also protect temporary pond systems. Similar ERA conclusions between temporary pond species and classic model species is attributed to comparable sensitivities to cypermethrin as observed in most several other aquatic animals (Giddings et al., 2019). Nevertheless, temporary-pond species may still be used as complimentary models to tailor regulations to specific habitats or specific conservation goals. Indeed, the high-concentration exposure scenario of 57.9 ng/L cypermethrin revealed high risks for classic fish models, whereas only moderate risks were estimated for the local *N. neumanni*.

In Tanzania, registration of pesticides is under the mandate of the Tropical Pesticide Registration Institute (TPRI) that oversees the efficacy of pesticide use and advices on possible phytotoxic effects. Still, apart from efficacy tests, no other ecotoxicological tests are carried out on pesticide formulations to inform risk managers of the possible short- and long-term effects of the compound on the natural environment. Currently, the country aims to enhance agricultural crop productivity to foster industrial commercialization (URT, 2021). This is envisioned to substantially increase the amount and diversity of pesticides imported and used in the country. Because environmental risks of pesticides may be compound-dependent (Almeida et al., 2021), follow-up research that considers the environmental fate and risks of different types of pesticides (e.g. organophosphates, carbamates) on temporary-pond systems is warranted. For pyrethroid pesticides, and cypermethrin in specific, we could confirm that ERAs using classic model species may be sufficiently accurate to inform environmental policies and regulations. While ERAs using local species could help to fine-tune these regulations depending on the conservation targets.

5. Conclusion

We report the occurrence of a synthetic pyrethroid, cypermethrin, in 46.7 % of the studied rivers and irrigation canals of the LMB. Although the current levels in contaminated rivers (lowest: 0.01 ng/L and highest: 57.9 ng/L) are too low to cause high risks on algae and fish from temporary ponds, substantial risks were derived for invertebrate grazers of temporary ponds (*S. lamellifer*) if they would become exposed to such concentrations, as well as for classic invertebrate models (*D. magna*). Additionally, at the highest detected concentration, cypermethrin appears to have moderate risk for fish when considering temporary-pond species (*N. neumanni*), but high risks when considering classic fish models. Our study suggests that ERAs based on cypermethrin toxicity data of classic models are sufficiently representative for ERAs based on local, temporary-pond species. We suggest that ERAs based on local temporary-pond species may be useful as a complementary method to tailor environmental regulations to local habitat conditions and specific conservation targets, and encourage follow-up research that considers different types of pesticides to assess the generality of our results.

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CRediT authorship contribution statement

Yusuph A. Kafula: Conceptualization, Methodology, Formal analysis, Writing – original draft. **Eli S. J. Thoré:** Methodology, Formal analysis, Writing – original draft, Review and Editing. **Charlotte Philippe:** Methodology, Formal analysis, Review and Editing. **Linus K. Munishi:** Funding acquisition, Supervision, Methodology, Review and Editing. **Francis Moyo:** Supervision, Review and Editing. **Bram Vanschoenwinkel:** Funding acquisition, Supervision, Review and Editing. **Luc Brendonck:** Funding acquisition, Supervision, Methodology, Formal analysis, Review and Editing.

Data availability

Data will be made available on request.

Declaration of competing interest

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

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