Science of the Total Environment 912 (2024) 169312

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

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Pesticide screening of surface water and soil along the Mekong River in Cambodia

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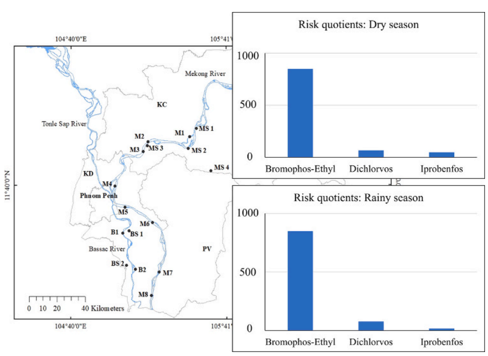
HIGHLIGHTS

- Screening 64 pesticides in 276 samples of surface water and soil along the Mekong River, Cambodia, both seasons
- 56 and 43 pesticides were detected in the surface-water and soil samples, respectively.
- Of the detected pesticides, 10 % presented a high or very high risk to aquatic organisms in the Mekong River in Cambodia.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Dimitra A Lambropoulou

Keywords:
Pesticides
Surface water
Soil
Mekong River
SPE
QuEChERS
LC-MS/MS
Ecological risk assessment

ABSTRACT

Widespread use of pesticides globally has led to serious concerns about environmental contamination, particularly with regard to aquatic and soil ecosystems. This work involved investigating concentrations of 64 pesticides in surface-water and soil samples collected in four provinces along the Mekong River in Cambodia during the dry and rainy seasons (276 samples in total), and conducting semi-structured interviews with local farmers about pesticide use. Furthermore, an ecological risk assessment of the detected pesticides was performed. In total, 56 pesticides were detected in surface water and 43 in soil, with individual pesticides reaching maximum concentrations of 1300 ng/L in the surface-water samples (tebufenozide) and 1100 ng/g dry weight in the soil samples (bromophos-ethyl). The semi-structured interviews made it quite evident that the instructions that farmers are provided regarding the use of pesticides are rudimentary, and that overuse is common. The perceived effect of pesticides was seen as an end-point, and there was a limited process of optimally matching pesticides to pests and crops. Several pesticides were used regularly on the same crop, and the period between application and harvest varied. Risk analysis showed that bromophos-ethyl, dichlorvos, and iprobenfos presented a very high risk to aquatic organisms in both the dry and rainy seasons, with risk quotient values of 850 for both seasons, and of 67 in the dry season and 78 in the rainy season for bromophos-ethyl, and 49 in the dry season and 16 in the rainy season for dichlorvos. Overall, this work highlights the occurrence of pesticide residues in surface water and soil along the Mekong River in Cambodia, and emphasizes the urgent need for monitoring and improving pesticide practices and regulations in the region.

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https://doi.org/10.1016/j.scitotenv.2023.169312
Received 21 September 2023; Received in revised form 5 December 2023; Accepted 10 December 2023
Available online 16 December 2023

1. Introduction

Pesticides are used to protect crops and improve production yields by reducing losses due to insect pests, plant pathogens, and weeds (Boye et al., 2019; Bureau-Point, 2021; Kazimierczak et al., 2022; Lee and Choi, 2020; Louati et al., 2023; Louca Christodoulou et al., 2018; Matsukawa et al., 2016; Sulaiman et al., 2019). Approximately 3 million tonnes of pesticides are utilized annually worldwide: 18 % of this is used in Europe, 15 % in the United States, 10 % in China, and 0.58 % in Cambodia (FAOSTAT, 2020). Pesticides are subdivided into several subgroups: 53 % are herbicides, 23 % fungicides and bactericides, 18 % insecticides, and 3 % other pesticides (FAOSTAT, 2020). However, this massive use of pesticides has caused great concern regarding global environmental contamination, in particular in relation to aquatic ecosystems and water, soil, etc. (Bureau-Point, 2021; Ciglasch, 2006; Derbalah et al., 2020; Devault et al., 2009; Donets et al., 2022; Ecobichon, 2001; Eissa et al., 2021; Freixo et al., 2015; Ha et al., 2010; Khammanee et al., 2020; Kruawal et al., 2005; Lee and Choi, 2020; Olisah et al., 2022; Rapp-Wright et al., 2023; Salem et al., 2021; Sangchan et al., 2014; Shehu et al., 2022; Silva et al., 2019; Şimşek Uygun and Albek, 2022; Stehle et al., 2019; Tan et al., 2020; Toth et al., 2022; Troger et al., 2021; Vittoria Barbieri et al., 2021; Xu et al., 2020; Yang et al., 2019; Zarei-Choghan et al., 2022).

Pesticides also have a strong ecotoxic effect on aquatic flora and organisms (Daramola et al., n.d.; Huang et al., 2020; Sharma et al., 2022; Ullah and Zorriehzahra, 2014) and cause several adverse effects, such as changes in behaviour (Ren et al., 2013; Sharbidre and Patode, 2012), oxidative stress (Bertrand et al., 2016; Bonifacio et al., 2017; Ezeoyili et al., 2019; Sharma et al., 2022; Sule et al., 2022; Vellingiri et al., 2022; Wang et al., 2023), neurological damage (Banaee et al., 2012; Bertrand et al., 2016; Bonifacio et al., 2017; Cooper and Bidwell, 2006; Sharbidre and Patode, 2012; Vellingiri et al., 2022; Xuereb et al., 2009), and genotoxicity (Ismail et al., 2018; Sharma et al., 2022; Ullah and Zorriehzahra, 2014). For example, carbofuran and oxamyl affect the behaviour of Chinese rare minnow (*Gobiocypris rarus*) (Ren et al., 2013) and carbendazim induces oxidative stress leading to apoptosis, causes multiorgan toxicity in various animals models, and is teratogenic, mutagenic, and aneugenic (Sharma et al., 2022). In the range of 0.22–0.64 mg/L, concentrations of carbendazim have been shown to inhibit the activity of acetylcholinesterase and cause oxidative stress in the brain of the African cat fish (*Clarias gariepinus*) (Ezeoyili et al., 2019).

Due to the environmental effects of pesticides they are heavily regulated in many countries; in the European Union this began in the 1960s, and in the United States in 1972 (Delaplane, 2000). In highly regulated markets all pesticides are continuously evaluated, and usage can be either banned or restricted. However, in less regulated markets pesticides with strong ecological effects are still used, and this is a particular problem in less wealthy developing countries with predominantly agricultural economy (Ecobichon, 2001; Rahman, 2021; Sadique Rahman, 2022).

Cambodia is one of the poorest countries in the world, and has a largely rural population where most workers are farmers (Asian Development Bank, 2021; United Nations Population Division, 2022; World Population Review, 2022). Pesticide usage in Cambodia is high, reflecting the importance of agriculture (Schreinemachers et al., 2015, 2017). There is no pesticide manufacturing in Cambodia, however, so all pesticides are imported from Vietnam, Thailand, and China. Moreover, estimated usage of pesticides in Cambodia is predicted to increase by 61 % annually (Schreinemachers et al., 2015, 2017). All pesticides that are legally imported must follow Cambodian legislation regarding quality assurance and labelling in the Khmer language, which reduces the risk of misuse. However, it has been shown that illegally imported pesticides are used, and these pose a greater risk because farmers are unable to read instructions written when these are in the language of the origin of the pesticide (Schreinemachers et al., 2017). In spite of this, screening of pesticides in environmental matrices such as water and soil has never been conducted in Cambodia, and published studies have instead focused on vegetables and rice (Chhay et al., 2017; Malacrino et al., 2020; Mohammad et al., 2018; Schreinemachers et al., 2015, 2017, 2020), severely limiting the possibility of targeted public-health interventions.

This study analysed surface-water and soil samples collected in two seasons at 16 sites along the Mekong River in Cambodia for 64 pesticides in order to identify pesticide use. In addition, semi-structured interviews and an environmental risk assessment for the pesticides detected in the surface-water samples were undertaken by calculating risk quotients.

2. Material and methods

2.1. Chemicals

The pesticide standards and internal standards used in this study were classified as analytical grade (95–99.8 %); chemical abstract numbers and supplier information are provided in the supplementary material (see Table S1). Stock solutions of each pesticide were prepared in methanol or acetonitrile and stored at -18 °C. The 64 pesticides were selected in relation to current legislation and usage in Cambodia, based on previous studies and information from local authorities (Kang and Visarto, 2015; Sokcheng et al., 2021). LC/MS grade methanol and acetonitrile (Lichrosolv – hypergrade) were purchased from Merck (Darmstadt, Germany). Purified water was prepared using a Milli-Q Advantage system and ultraviolet radiation source (Millipore, Billerica, USA). Formic acid (Sigma-Aldrich, Steinheim, Germany) was used (at 0.1 %) to prepare the mobile chromatographic phases.

2.2. Sampling

We collected samples from four provinces along the Mekong River: Kompong Cham, Thbong Khmum, Kandal, and Prey Veng (see Fig. 1; coordinates for these sampling sites are provided in Table S2 and S3 in the supplementary material). With regard to the soil sample collection, the criteria were that both dry- and rainy-season crops had been planted at the site.

A total of 96 surface-water samples and 180 soil samples were collected; 48 surface-water samples and 90 soil samples during the dry season between May 20 and 27, 2020, and 48 surface-water samples and 90 soil samples during the rainy season between July 15 and 20, 2020.

Surface-water samples were collected using the manual grab method in triplicate, at 15–60 cm depth in order to avoid the top surface layer at each sampling site. Most of the samples were taken 3 m (+/− 1 m) from the river bank using a telescopic water sampler, and a subset of the samples was taken in the centre of the main flow from either a boat or bridge (see Table S2 in the supplementary material). Sampling was conducted by first rinsing a 500 mL polyethylene bottle three times with surface water from the sampling site, then submerging and filling it to approximately 490 mL he samples were immediately stored in ice coolers at approximately 4 °C, and transported to the Chemistry Laboratory at the Royal University of Phnom Penh in Cambodia within 5 h of sampling. The samples were kept at -18 °C until they were analysed at the Environmental Chemistry Laboratory at Umeå University in Sweden.

Soil samples were taken using a random systematic approach at depths of 0 to 20 cm using a steel scoop. At each sampling location triplicate samples were collected, and each sample consisted of five individual core soil samples (each weighed about 100 g); these were placed in well-labelled, clean polyethene zip-lock bags, and were mechanically mixed by shaking. For storage and transportation, the same protocol was followed as for the surface-water samples. The 500 g of mixed soil samples were sieved using a sieve (mesh size, 2 mm) and mixed by a stainless-steel spoon then approximately 30 g of sieved soil samples were placed in well-labelled and clean polyethene zip-lock bags.

2.3. Pretreatment

In total, 276 samples of surface water and soil from 16 sampling sites were analysed for residues of 64 different pesticides. The pesticides were from three main groups – organophosphates, carbamates, and pyrethroids – and several smaller ones, including nitrophenol derivatives, triazine derivative pesticides, etc. Most pesticides are classified by the World Health Organization as being slightly to extremely hazardous and are thought to be currently in use in Cambodia (EJF, 2002; PPDB, 2023; WHO, 2020). The protocol of sample pretreatment of surface water and soil sample is in supplementary material.

2.4. LC-MS/MS analysis

Concentrations of the 64 selected pesticides in the surface-water and soil samples were measured using ultra-high-performance liquid chromatography and tandem mass spectrometry (UHPLC-MS/MS) (Lozowicka et al., 2016). Analysis was made using a Dionex UltiMate 3000 UHPLC system consisting of two LC pumps (Ultimate LPG 3400SD quaternary pump and HPG 3400RS binary UHPLC pump) with an analytical column (Thermo Scientific Hypersil GOLD aQ, 100 × 2.1 mm, 3 μm) equipped with a precolumn (Hypersil GOLD, 10 × 2.1 mm, 3 μm), connected to a TSQ Quantiva triple quadrupole mass spectrometer (Thermo Scientific). A heated-electrospray ionization (HESI) ion source was used, operating in positive or negative mode. Chromeleon Xpress (Thermo Scientific) was used to control the UHPLC system. Chromatographic separation was achieved using a gradient of Milli-Q water, acetonitrile, and methanol (LiChrosolv, Merck, Darmstadt, Germany). A gradient of 0.1 % formic acid in Milli-Q water and 0.1 % formic acid in acetonitrile was used as a mobile phase. The detailed gradient programme is shown in the supplementary information (see Table S4). The resolution for both quadrupoles was 0.7 full width of the peak at half its maximum height. The spray voltage was 3500 V, sheath gas 40 arbitrary units, sweep gas 0 arbitrary units, ion transfer tube temperature 350 °C, and vaporiser temperature 338 °C.

Two MS/MS transitions – one for quantification and one for qualification – were monitored for all analytes. MS/MS transitions, corresponding collision energies, associated internal standards, and retention times for each analyte are shown in Table S5 (supplementary material). Peak identification was performed by matching the sample and reference standard components using the Xcalibur™ 4.3 software (Thermo Fisher Scientific). An eight-point calibration curve for the range 1–500 ng/L was used for linearity evaluation and quantification. The results are presented as ng of pesticide compound per L of surface water or per g of dry weight (dw) for the surface-water and soil samples, respectively.

2.5. Quality assurance and quality control

Injection of several blank samples (procedural and instrumental blanks) was performed regularly during the analytical runs to detect contamination and sample carry-over. Carry-over effects were also evaluated by injecting standards at 500 ng/L followed by three mobile phase blanks. Two transitions were used, with the criterion that the ratio between the transitions could not deviate more than ±30 % from the ratio of the corresponding calibration standard. Retention times for all analytes also had to be within ±2.5 % of the retention time in the corresponding calibration standard. Together, this gave four identification

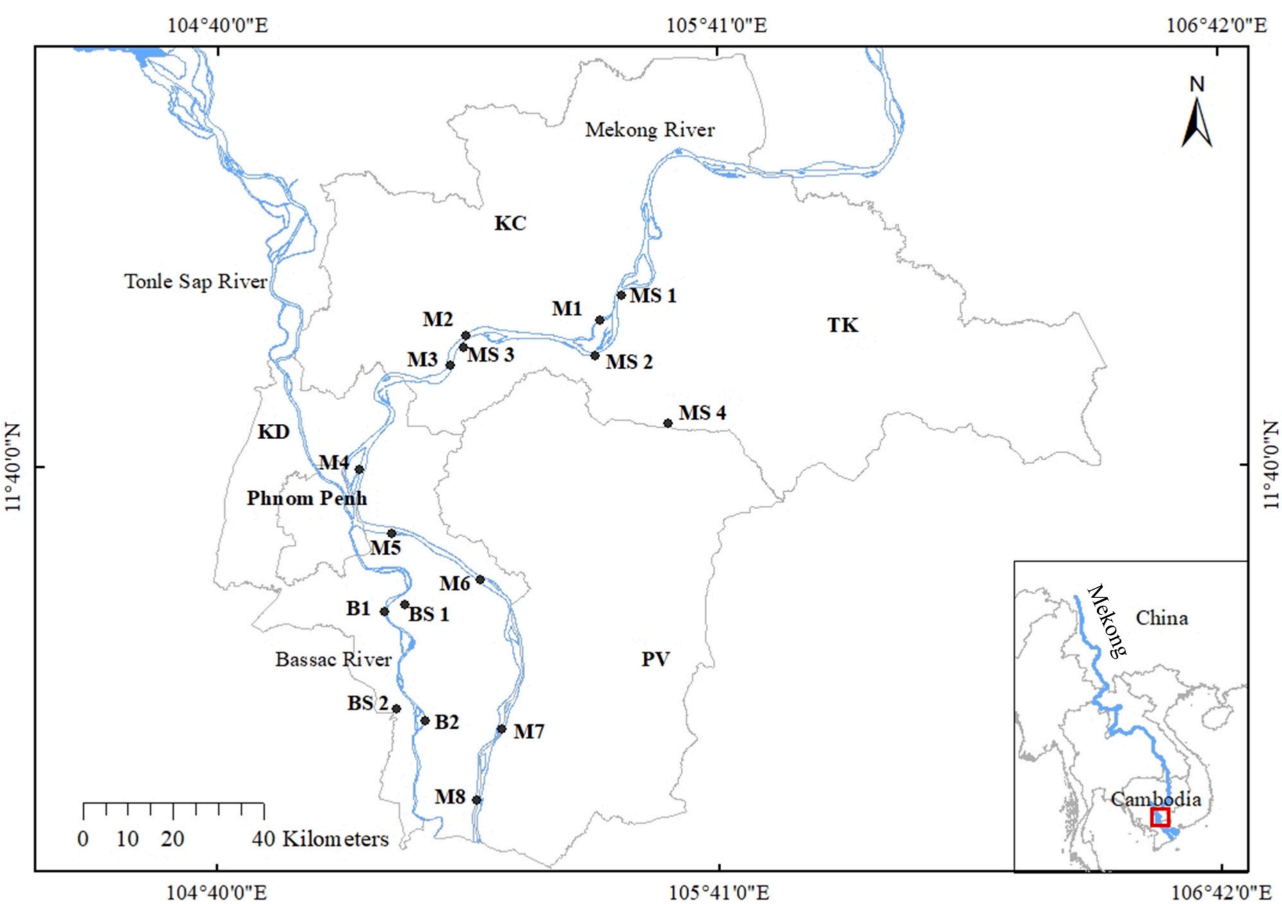

Fig. 1. Sampling sites in each province.
KC = Kompong Cham, TK = Thbong Khmum, KD = Kandal and PV = Prey Veng. M1–8 are sampling sites along the Mekong River. MS1–4 are sampling sites of streams near farms from the Mekong River. B1–2 are sampling sites along the Bassac River. BS1–2 are sampling sites of streams near farms from the Bassac River.

points (the highest possible number), as described in the Commission Decision 2002/657/EC (European Commision, 2002) concerning the performance of analytical methods and interpretation of results. The limit of quantification (LOQ) was determined from the second-lowest point within the linear range on the calibration curve.

2.6. Semi-structured interviews

Semi-structured interview is a data-collection method that involves asking questions which are not set in terms of order or phrasing, but are organised within a predetermined thematic framework (Adams, 2015). Fourteen informants – twelve farmers, who owned fields in the areas where soil samples were collected, and two key officials – were chosen as interviewees. During dry-season cropping from 20 to 27 May 2020 and rainy-season cropping from 15 to 24 July 2020, semi-structured interviews based on a question form were conducted in order to obtain information from the informants about the types of pesticides they use, duration for which they spray pesticides, and when they harvest produce (summarised in Table S6 in the supplementary material). During the interviews and field observations, product identification relating to pesticides and the language on the labels of pesticide packaging was also performed.

2.7. Environmental risk assessment

The environmental risk assessment for the pesticides detected in the surface-water samples for the dry and rainy seasons was performed by calculating the risk quotient (RQ) using Eq. (1).

$$\mathbf{RQ} = \frac{\mathbf{MEC}}{\mathbf{PNEC}} \tag{1}$$

where MEC is the maximum measured environmental concentration (detected maximum concentration of each pesticide at all sites) (Abraham Rivera-Jaimes et al., 2018). Where any pesticides were detected in the range between the limit of detection (LOD) and the LOQ, the MEC was expressed as zero. The lowest predicted no-effect concentration (PNEC) for fresh water was obtained from various databases, such as the Norman Ecotoxicology and Pesticide Properties Database (NORMAN Network, 2023; PPDB, 2023) (see Table S1 in the supplementary material). In the case that no lowest PNEC fresh water values were available in databases, this was calculated by dividing the no-observed-effect concentration (NOCE) value by an assessment factor (AF) obtained from Van Leeuwen and Vermeire (Van Leeuwen and Vermeire, 2007). When $RQ < 0.01$ the targeted pesticide has a very low risk to aquatic organisms, and when $0.01 \leq RQ < 0.1$, the ecological risk level is low. When $0.1 \leq RQ < 1$ the targeted pesticide has a moderate risk to aquatic organisms. When $1 \leq RQ < 10$, the targeted pesticide has a high risk to aquatic organisms, and when $RQ \geq 10$, the ecological risk level is very high (Van Leeuwen and Vermeire, 2007).

2.8. Data and statistical analysis

The data analysis of the pesticide concentrations in the surface-water (ng/L) and soil (ng/g dw) samples are presented as maximum and average values for each pesticide. The values below the LOQ for each pesticide in triplicate surface-water samples and individual soil samples for each site were replaced by LOQ/2 (Osorio et al., 2016) in order to get the average concentration for each pesticide. Detection frequency (%) is presented as the percentage of samples with pesticides detected above the LOQ based on average concentration of all of the samples analysed. Pearson correlation coefficients (values below LOQ were set as zero) were used to identify the correlation between average pesticide concentrations in surface water in the dry and rainy seasons, between average pesticide concentrations in soil in the dry and rainy seasons, and between average pesticide concentrations in surface water and in soil in the dry and rainy seasons. Welch's *t*-test (two tails, unequal variances) with a 95 % confidence interval was used to compare concentrations of each pesticide at each site for the dry and rainy seasons. In these statistical calculations, values below the LOQ for each pesticide were replaced by LOQ/2, and standard deviations were set to the theoretical maximum value. A p-value below 0.05 was considered to be statistically significant. In order to obtain an overview of the results, principal component analysis (PCA) was used. PCA is a latent vector-based method that compresses data into orthogonal vectors that summarise the variation and correlation patterns in the data. PCA is a very powerful tool, and typically two to five principal components (PCs) are sufficient to represent the structured information in a multivariate table. PCA was used to provide an overview of the possible correlations between soil concentrations and crop type.

Microsoft Office Excel 2019 (WA, USA) was used to calculate concentrations of analytes, create all figures, and perform Pearson correlation-coefficient analysis. Simca-P 17 (Umetrics, USA) was used to perform PCA.

3. Results and discussion

3.1. Quality assurance and quality control

The analytical method performance was stable throughout the study. All retention times were within 1.5 % of the corresponding standards, no carry-over effects were observed, no pesticides were detected in the instrumental or procedural blank samples, and R^2 values were above 0.97 for all calibration curves in the given concentration ranges (see Table S5 in the supplementary material). LOQs for the analysed pesticides are shown in the supplementary material (Table S4).

3.2. Surface water

A total of 56 out of the 64 pesticides were detected in the 96 surface-water samples, with average concentrations for individual pesticides ranging from 0.05 to 1300 ng/L (see Fig. 2 and Table S7 in the supplementary material). Our results are comparable with those of previous published studies, e.g. a study of 24 pesticides at three different tributaries of the Mae Sa River in Northern Thailand detected pesticides in concentrations ranging from 10 to 590 ng/L (Ciglasch, 2006). In a research project that focused on 2362 contaminants, 586 pesticides were detected up to a maximum concentration of 1171 ng/L in the Danube River Basin, Europe's second largest river basin (Ng et al., 2023). Furthermore, a review article that focused on approximately 150 pesticides – mostly herbicides and insecticides – found that these were detected in a range from 7 to 322 ng/L at 609 urban surface water sites in the United States (Stehle et al., 2019).

The five pesticides with the highest detected concentration levels in surface water in our study were the insecticide tebufenozide (1300 ng/L), the herbicide isocarbamid (700 ng/L), the herbicide fluroxypyr (520 ng/L), the fungicide metabolite *N,N*-dimethyl-*N*-(4-methylphenyl) sulfamide (DMST; 200 ng/L), and the fungicide atrazine-desethyl (120 ng/L) (Fig. 2 and Table S7 in the supplementary material). This contrasts with previous studies, which detected the insecticide tebufenozide in the Rhone River delta (France) at a maximum concentration level of 120 ng/L (Comoretto et al., 2007) and in the River Thur in Switzerland at a concentration of 2.6 ng/L (NORMAN Network, 2023), and the herbicide fluroxypyr in the Rivers Derg and Finn in Northwest Ireland at a maximum concentration of 28.7 ng/L (Farrow et al., 2022) and in a river near Bruyères-le-Châtel in France at a concentration of 54 ng/L (NORMAN Network, 2023). In addition, in this study the highest detected concentration of DMST was 310 ng/L in surface water, contrasting with a value of 10–12 ng/L detected in Arcachon Bay in France (REPAR, 2011). Very few studies have screened for isocarbamid in surface water, so there is limited information on this. To the best of our knowledge we present the first record of the herbicide isocarbamid

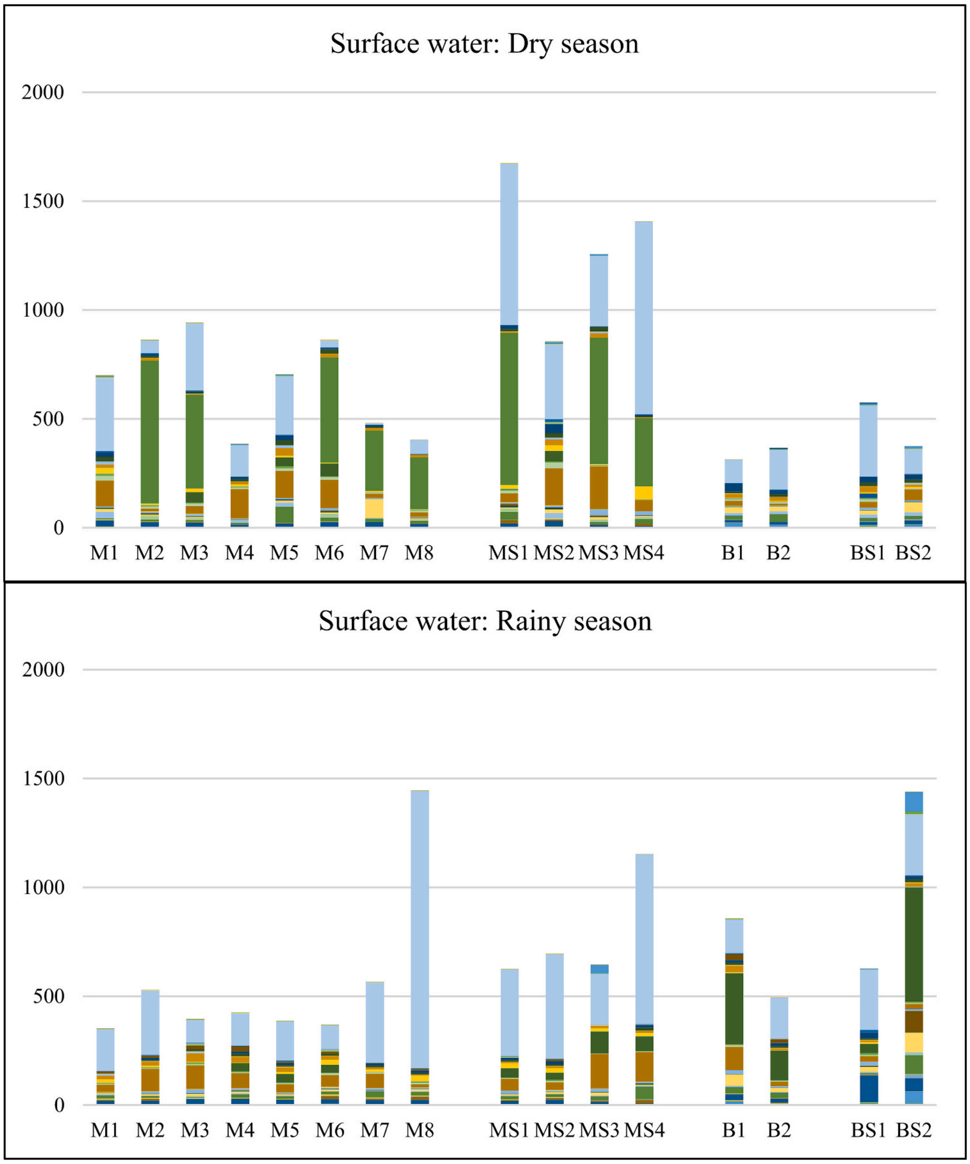

Fig. 2. Surface water average concentration in ng/L in both dry and rainy seasons.

2,6-Dichlorobenzamide	Acetamiprid	Ametryn	Atraton
Atrazine-desethyl	Azamethiphos	Aziprotryne	Azoxystrobin
Bentazon	Bromophos-ethyl	Bromoxynil	Bupirimate
Buprofezin	Butoxycarboxim	Carbendazim	Carbofuran
Cyclanilide	Cyproconazole	Desmetryne	Dichlorvos
Dicrotophos	Dimethametryn	Dimethomorph	DMST
DNOC	Etrimfos	Fenfuram	Fenobucarb
Flufenoxuron	Fluometuron	Fluroxypyr	Flutriafol
Furalaxyl	Imazamethabenz-methyl	Indoxacarb	Iprobenfos
Isocarbamid	Isomethiozin	Isoprothiolane	Isoxaben
Methabenzthiazuron	Methomyl	Metribuzin	Naptalam
Oxamyl	Phenmedipham	Pyrifenox	Pyriftalid
Pyroquilon	Quinoclamine	Simeton	Sulfentrazone
Tebufenozide	Tebutam	Thiacloprid	Triadimefon
Triazophos	Triazoxide	Triticonazole	

M1–8 are sampling sites along the Mekong River. MS1–4 are sampling sites of streams near farms from the Mekong River. B1–2 are sampling sites along the Bassac River. BS1–2 are sampling sites of streams near farms from the Bassac River. At each site, $n = 3$.

in surface water, with a maximum value of 1100 ng/L.

The average detection frequency for the 64 individual pesticides was 55 % for the dry season and 57 % for the rainy season. 13 of the 64 pesticides had 100 % detection frequencies in both the dry and rainy seasons at all investigated sites (see Fig. 2 and Table S7 in the supplementary material). These pesticides were six insecticides; carbofuran, indoxacarb, isoprothiolane, methomyl, tebufenozide, triazophos, five herbicides; 2,6-dichlorobenzamide, ametryn, atrazine-desethyl, DNOC, quinoclamine, and two fungicides; iprobenfos and metabolite DMST.

Fig. 2 shows the concentrations of pesticides for the dry and rainy seasons. The differences between the most and least polluted sites were not pronounced. Based on the total sum of average concentrations of all pesticides for both seasons, the most polluted site was a tributary of the Mekong River (Site MS4; 2600 ng/), and the least polluted site was found along the Mekong River (Site M4; 810 ng/L) (see Table S7 in the supplementary material). The main reason for the high levels at Site MS4 is likely to be high pesticide use in combination with the good drainage of the fields.

The statistical comparisons of pesticide residue concentrations in surface-water samples for the dry and rainy seasons show that 71 out of 1024 (64 pesticides at 16 sites) observations were significantly different (Welch's *t*-test, $p < 0.05$) (see Table S7 in the supplementary material). Three of the 64 pesticides showed a seasonal pattern: the herbicide ametryn increased in average concentration in the rainy season at 11 sites (M2, M4, M5, M6, M7, M8, MS1, MS3, MS4, B2, and BS1) according to Welch's t-test ($p < 0.05$) (see Table S7 in the supplementary material). This implies that this pesticide is used more in the rainy season at almost all of the sampling sites, and/or there is increased field run-off at this time. The insecticides butoxycarboxim and etrimfos had higher average concentrations in the dry season than the rainy season at three sites (B1, B2, and BS2) based on Welch's *t*-test ($p < 0.05$) (see Table S7 in the supplementary material). This indicates that these two insecticides are used more in the dry season, and/or there is increased field run-off in the Bassac region at this time.

Pearson's correlation coefficient was computed to evaluate the relationship between the dry and rainy seasons in terms of the average concentration ($n = 3$) of the 64 pesticides. Eleven out of the 64 had a strong positive correlation, including 2,6-dichlorobenzamide, azoxystrobin, triadimefon, acetamiprid, bentazon, bromoxynil, dimethomorph, methomyl, ametryn, DNOC, and thiacloprid. This indicates that if the concentration of these 11 pesticides was high in the dry season, it was also high in the rainy season. This in turn implies that these pesticides are used consistently, regardless of season.

A rough estimate of the daily environmental load of pesticides can be calculated using average values (from dry- and rainy season) at sampling point M8, which is the most downstream site on the Mekong River, included in our study. Average sum of all pesticides at sampling point M8 was 1800 ng/L (based on all measured samples, dry- and rainy season, Fig. 2). Based on the annual average flow in the Mekong River (16,000 m^3/s) and a conservative assumption of no degradation, a rough estimate would then be that approximately 2.6 t of all pesticides are discharged into the river every day.

3.3. Soil

Of the 64 pesticides, 43 were detected in concentrations ranging from 0.05 to 1100 ng/g dw in 180 soil samples obtained from sites along the Mekong River in Cambodia (Table S8 in the supplementary material). Several studies have measured pesticide concentrations in soil (e.g. (Braun et al., 2018; Khammanee et al., 2020; Salem et al., 2021; Tan et al., 2020). In a recent study, residues of 41 pesticides were measured at concentrations ranging from 0.2 to 675.4 ng/g wet weight (ww) in 256 tropical topsoil samples obtained from a riverside basin in China (Tan et al., 2020); another study found residue of 15 pesticides at concentrations ranging from 2.05 to 58.7 ng/g dw in 20 soil samples obtained from paddy fields in Thailand (Khammanee et al., 2020). Notably, our results were higher than most other studies, which could indicate that the Cambodian farmers apply larger amounts of pesticides per square metre than is recommend by manufacturers.

The five pesticides with the highest detected concentration levels in soil in our study were the insecticide bromophos-ethyl (1100 ng/g dw), the plant-growth regulator naptalam (610 ng/g dw), the fungicide azoxystrobin (480 ng/g dw), the herbicide phenmedipham (380 ng/g dw), and the fungicide dimethomorph (210 ng/g dw) (see Table S8 in the supplementary material). Previous studies have detected azoxystrobin in topsoil samples from paddy rice fields in the Red River Delta in Vietnam at a maximum concentration of 15.7 ng/g dw (Braun et al., 2018), the herbicide phenmedipham in arable soils in Finland at a maximum concentration of 60 ng/g dw (Laitinen et al., 2006), and the fungicide dimethomorph in China at a maximum concentration of 677 ng/g ww (Chen et al., 2018). Very few studies have screened for bromophos-ethyl and naptalam in soil, so there is still limited information on this. To the best of our knowledge, we present the first record of the insecticide bromophos-ethyl and the plant-growth regulator naptalam in soil, with maximum values of 1100 ng/g dw and 610 ng/g dw, respectively.

The average detection frequency based on average concentration was 16 % during the dry season and 22 % during the rainy season. The fungicide carbendazim was found in both the dry and rainy seasons at all investigated sites (see Fig. 3 and Table S8 in the supplementary material).

The most and least polluted sites, based on total sum average concentration of all pesticides for both seasons, were M7 and M3, respectively, and were both situated on the Mekong River (Site M7; 800 ng/g dw, and Site M3; 6 ng/g dw) (see Table S8 in the supplementary material). It should be noted that none, or only low levels, of the two pesticides that were detected at the highest amount in the most contaminated soil sampling site (M7) were detected in the corresponding surface-water samples, even though the surface-water samples were collected in close proximity to the soil-sampling sites. This could be due to either recent applications or restricted mobility on the part of the applied pesticides at these sites (Bhandari et al., 2020). A very strong smell of chemicals was noted during field sampling at Site M7, which indicates that the elevated levels were due to recent pesticide application.

The pattern of pesticide residues in soil samples differed from those in surface-water samples (see Figs. 2 and 3). This is likely because different pesticides were used at different sites and regions. The compound that was found in the highest average concentration was the insecticide bromophos-ethyl, which was present at almost all of the investigated sites in both the dry and rainy seasons (see Fig. 3).

The statistical comparisons of the pesticide residue concentrations in the soil samples between the dry and rainy seasons show that 22 of the 960 (64 pesticides at 15 sites) observations were significantly different (Welch's *t*-test, $p < 0.05$) (see Table S8 in the supplementary material). This indicates that there is no statistical difference between the dry and rainy season as regards the soil samples with a 95 % confidence interval.

Pearson's correlation coefficient was computed in order to evaluate the relationship between the dry and rainy seasons in terms of the average concentration of the 64 pesticides. Nine pesticides had a strong positive correlation; one explanation for this may be that farmers use the same pesticides across the two seasons.

PCA did not provide any information beyond there being no correlation between crop type and pesticide used. Two PCs were calculated that condensed only 27.7 % of the variation in the descriptor set (see Fig. S1 in the supplementary material).

3.4. Surface water vs. soil

Of the 64 pesticides included in this study, 56 and 43 were detected above the LOQ in the surface-water and soil samples, respectively (see Table S7 for surface-water and Table S8 for soil in the supplementary material), and a total of 59 out of the 64 pesticides were detected in

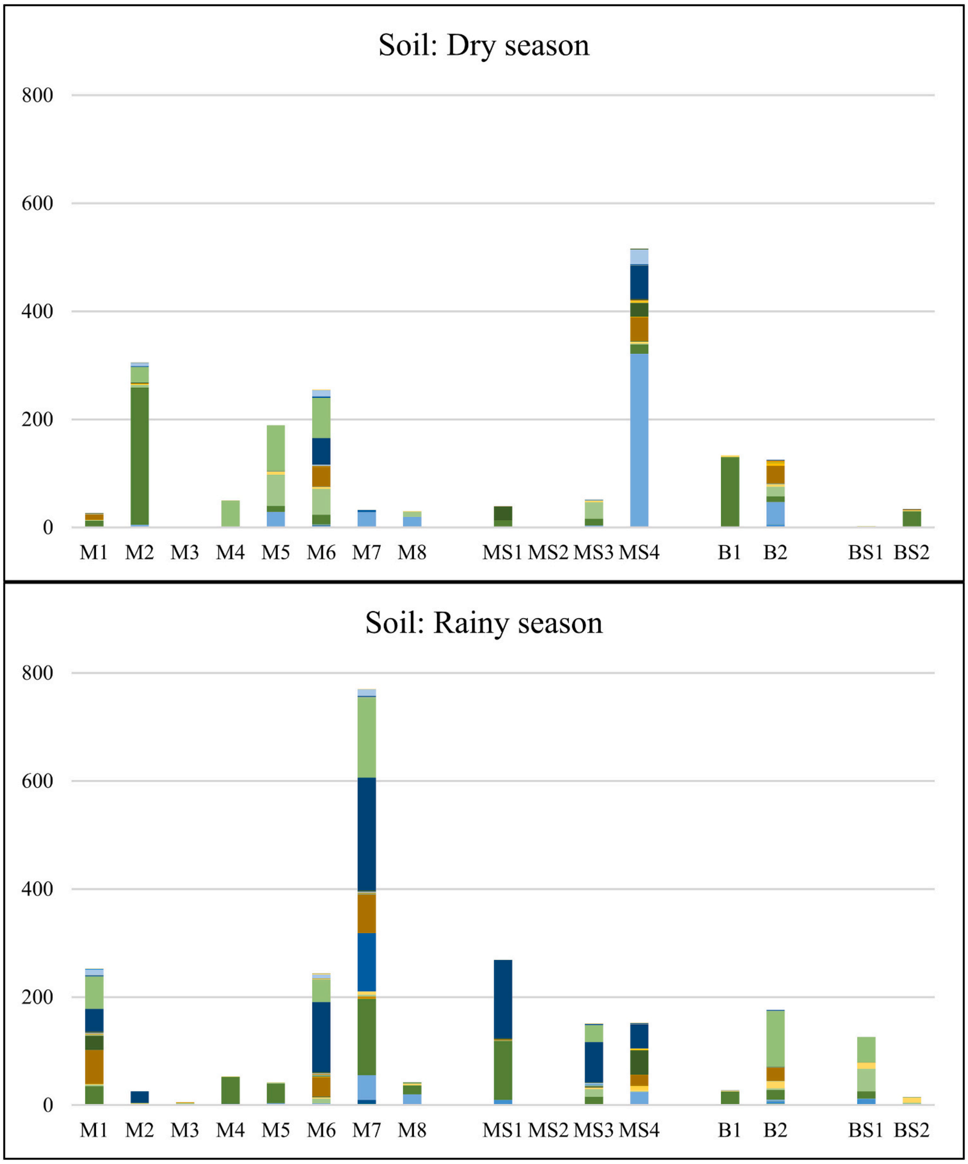

Fig. 3. Soil average concentration in ng/g dw in both dry and rainy seasons.

2,6-Dichlorobenzamide	Acetamiprid	Ametryn	Atraton
Atrazine-desethyl	Azamethiphos	Aziprotryne	Azoxystrobin
Bentazon	Bromophos-ethyl	Bromoxynil	Bupirimate
Buprofezin	Butoxycarboxim	Carbendazim	Carbofuran
Cyclanilide	Cyproconazole	Desmetryne	Dichlorvos
Dicrotophos	Dimethametryn	Dimethomorph	DMST
DNOC	Etrimfos	Fenfuram	Fenobucarb
Flufenoxuron	Fluometuron	Fluroxypyr	Flutriafol
Furalaxyl	Imazamethabenz-methyl	Indoxacarb	Iprobenfos
Isocarbamid	Isomethiozin	Isoprothiolane	Isoxaben
Methabenzthiazuron	Methomyl	Metribuzin	Naptalam
Oxamyl	Phenmedipham	Pyrifenox	Pyriftalid
Pyroquilon	Quinoclamine	Simeton	Sulfentrazone
Tebufenozide	Tebutam	Thiacloprid	Triadimefon
Triazophos	Triazoxide	Triticonazole	

M1–8 are sampling sites along the Mekong River. MS1–4 are sampling sites of streams near farms from the Mekong River. B1–2 are sampling sites along the Bassac River. BS1–2 are sampling sites of streams near the farm from the Bassac River. The number of samples (n) depends on the number of product types available at each site.

At M3, M4, MS3 and MS4 sites: n = 3; At M5, M6, M7, MS1, B2, BS1 and BS2 sites: $n = 6$; At M1, M2, M8 and B1: $n = 9$.

samples of either type. Forty pesticides were present in both surface-water and soil samples, 16 were detected only in surface-water samples, and three were found only in soil samples.

Pearson's correlation coefficient was computed in order to evaluate the relationship between the surface-water and soil samples for both the dry and rainy seasons. This statistical analysis was based on the average concentration of the 40 pesticides detected in both the surface-water and soil samples. Pearson's correlation coefficient showed that there were five pesticides with a strong positive correlation for the dry season; DNOC, iprobenfos, tebufenozide, triazoxide, and triticonazole. This correlation indicates that if the concentration of these five pesticides was high in the surface-water samples, it was also high in the soil samples obtained from nearby. One explanation for this may be that pesticides leach from the soil and contaminate the surface water. However, there was no strong correlation between average concentration in surface water and in soil in the rainy season, which indicates that pesticide dissipation during the rainy season is more complex, and that usage is the main factor.

3.5. Semi-structured interviews

All twelve of the interviewed farmers used pesticides and plant-growth regulators during their crop-production cycles. However, none knew the active ingredients in the pesticide products they used, either due to inadequate labelling (usually in Thai or Vietnamese) or their own literacy issues. Visual inspection of products used by the interviewed farmers and packages observed at sampling sites made it possible to determine 13 individual products containing 15 identified pesticides (see Table S9 in the supplementary information). Seven of the products were labelled in non-Khmer languages (38 % in Vietnamese and 8 % in Thai; see Table S9 in the supplementary material). Furthermore, the farmers described frequently mixing multiple pesticide products in spray tanks and applying this mixture without consideration of the kinds of pesticides being used. This finding is consistent with other research that has evaluated the actual status of pesticide use by farmers in rural Cambodia (Matsukawa et al., 2016). Twenty-five percent of the interviewed farmers reported that they use plant-growth regulators to support stem and leaf growth. Information regarding quantities to be used and time between application and harvest was described as being acquired from neighbours and pesticide sellers. Pesticides were described as being used to prevent pests damaging crops and to control diseases. Thirty-three percent of the interviewed farmers stated that they do not follow the advice obtained from their neighbours and/or pesticide sellers. They stated that, as a result of their produce being severely damaged by insects, the active ingredients are often applied in up to twice the recommended dose. In addition, pesticide application is influenced by the perceived effectiveness of the product: 16 % of the interviewed farmers mentioned that when applied pesticides have not prevented insects from damaging the crop, another type of pesticide is applied the next day. The time from last application to harvest also depends on the degree of damage caused by insects; when crops are severely damaged, the time to harvest can be shorter than recommended. Thirty-three percent of the interviewed farmers mentioned that when there is low demand for crops, the time to harvest is sometimes extended.

Based on the semi-structured interviews, it was clear that the instructions provided with pesticides are rudimentary, and that overuse is common. The perceived effect of pesticides was used as an end-point, and there was little evidence of processes of optimally matching pesticides to pests and crops. Several pesticides were used regularly on the same crop, and the time between application and harvest often varied.

3.6. Environmental risk assessment

All of the compounds detected in the surface-water samples collected from the Mekong River were subjected to an environmental risk assessment, and RQ values can be found in the supplementary material (see Table S10 in the supplementary material and Fig. 4). The RQ values of the detected pesticides ranged from below 0.001 to 850. More than half presented a very low risk ($RQ < 0.001$); nine presented a low risk ($RQ\ 0.01 \leq RQ < 0.1$) in both the dry and rainy seasons. Nine and eleven presented a moderate risk in the dry and rainy seasons, respectively. For the high-risk category ($1 \leq RQ < 10$), there were two insecticides and one fungicide in the dry season, and three insecticides in the rainy season. Three pesticides presented a very high risk, i.e., $RQ > 10$: the insecticides bromophos-ethyl and dichlorvos and the fungicide iprobenfos.

The insecticide bromophos-ethyl was associated with an extremely high risk ($RQ = 850$ in both the dry and rainy seasons), and is classified by the WHO as "highly hazardous" and currently banned in Cambodia (WHO, 2020) The insecticide dichlorvos presented a very high risk ($RQ = 67$ in the dry season and 78 in the rainy season), and is also classified by the WHO as "highly hazardous" and restricted in Cambodia (WHO, 2020). The fungicide iprobenfos presented a very high risk ($RQ = 49$ in the dry season, 16 in the rainy season), and is classified as "moderately hazardous" by the WHO and permitted in Cambodia (WHO, 2020). The result regarding the insecticide bromophos-ethyl is in agreement with the high RQ (≥ 10) reported for samples collected in the Ebro River in Presa De Pina in Spain ($RQ = 50$; 10 ng/L) (NORMAN Network, 2023). Similar to several previous studies, the insecticide dichlorvos was shown to be very high risk ($RQ \geq 10$) in the Tama River, Japan ($RQ = 22$; 13.2 ng/L) (Nakamura and Daishima, 2005); Yangtze River in China ($RQ = 2600$; 1552 ng/L) (Gao et al., 2009); Songhuajiang River in China ($RQ = 33$; 20 ng/L) (Gao et al., 2009; RQ based on PNEC values); major rivers in South Korea ($RQ = 250$; 147 ng/L) (Cho et al., 2014; RQ based on PNEC values); and Santa Maria River in California ($RQ = 14$; 8.3 ng/L) (Phillips et al., 2012; RQ based on PNEC values). However, other studies have assessed dichlorvos: as presenting a high risk in the Sarno River and Estuary in Southern Italy ($RQ = 2.6$; 1.5 ng/L) (Montuori et al., 2015; RQ based on PNEC values), and as presenting a very low risk in the Naseri artificial wetland in Iran (Zarei-Choghan et al., 2022). Studies have also investigated the fungicide iprobenfos and achieved a very high RQ: in the Han River Basin in South Korea it was found in a concentration of 460 ng/L ($RQ = 130$) (Kim et al., 2019; RQ based on PNEC values), and in the Ansikhola watershed in Nepal at a concentration of 4,000,000 ng/L ($RQ = 1{,}100{,}000$) (Kafle et al., 2015; RQ based on PNEC values). However, it was also found to be low risk ($RQ > 1$) in the Kurose and Ashida Rivers in Japan (Derbalah et al., 2020).

Four pesticides were classified as high-risk; the fungicide triticonazole ($RQ = 4.9$ in the dry season; and is classified by the WHO as "slightly hazardous") (WHO, 2020), the insecticide tebufenozide ($RQ = 4.5$ in the dry season, 2.6 in the rainy season; and is classified by the WHO as "unlikely to present acute hazard") (WHO, 2020), the insecticide acetamiprid ($RQ = 1.1$ in the dry season, 3.3 in the rainy season; and is classified by the WHO as "moderately hazardous") (WHO, 2020), and the insecticide azamethiphos ($RQ = 1.2$ in the dry season; and is classified by the WHO as "moderately hazardous") (WHO, 2020). Interestingly, the insecticide tebufenozide was the pesticide detected at the highest concentration (2600 ng/L, and presented a high risk ($1 \leq RQ < 10$). A few studies have shown that acetamiprid is a high-risk pesticide with an RQ value of 5 (based on surface-water samples collected from the Yangtze River in China) and 6.7 (samples collected from wastewater effluent in the Republic of Ireland) (Mahai et al., 2019; Rapp-Wright et al., 2023). In contrast, in the Louros River in Greece Kapsi et al. discovered that acetamiprid presented a low risk to zooplankton ($RQ < 0.001$) (Kapsi et al., 2019).

4. Conclusion

This study focused on the occurrence of 64 pesticides in surface-water and soil samples that were collected at 16 sites along the

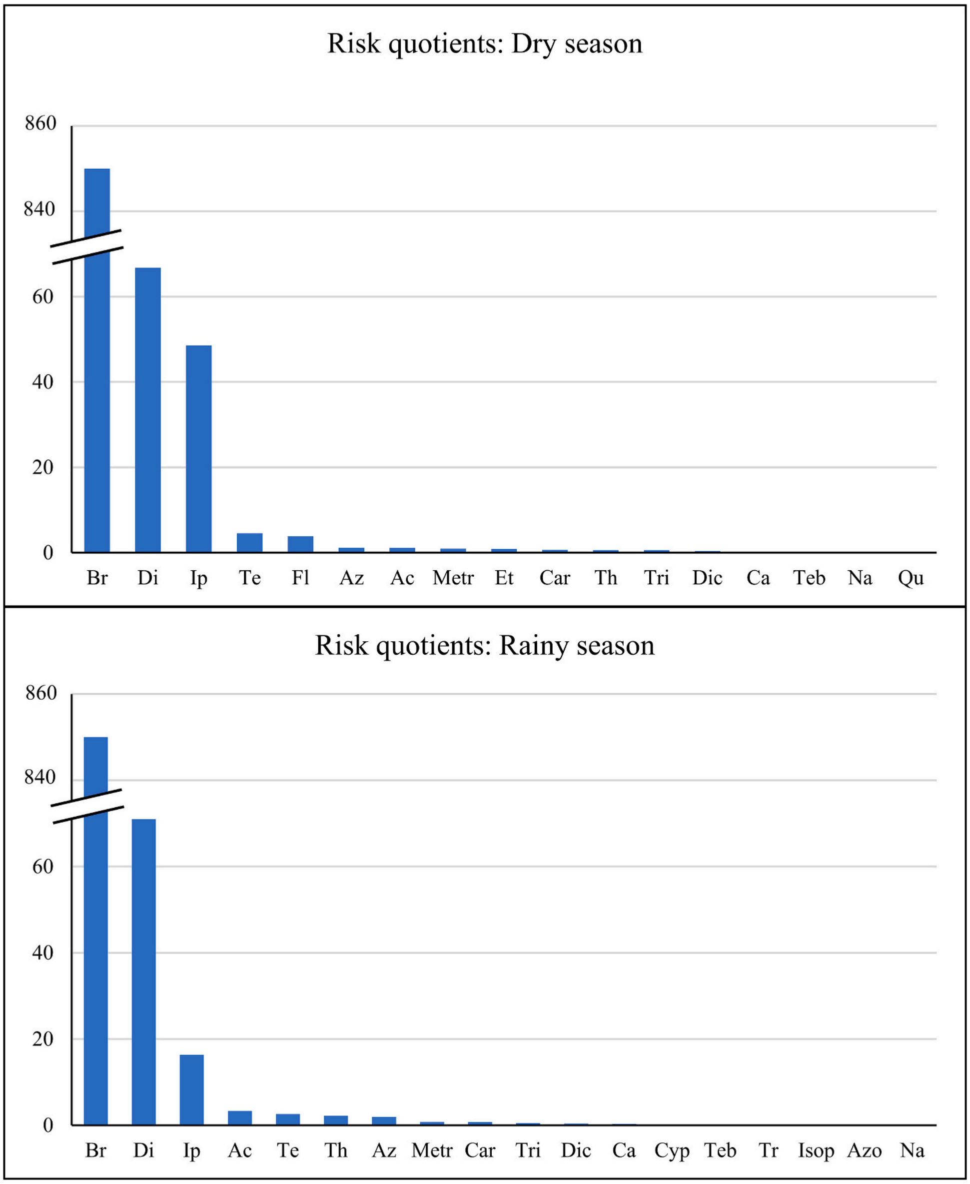

Fig. 4. Environmental risk assessment of detected pesticides at maximum concentration (as risk quotients) quantified in surface-water samples along the Mekong River in both dry and rainy seasons

Risk quotients were calculated based on the detected maximum concentration of each pesticide at all sites and PNEC values obtained from the NORMAN Ecotoxicology and Pesticide Properties DataBases. In the case that no lowest PNEC fresh water values in the NORMAN database, these values were calculated by dividing the median effective concentration (EC50) or no-observed-effect concentration (NOCE) by an assessment factor (AF) of 1000 or 100, respectively according to Van Leeuwen and Vermeire. The specific EC50/NOCE values were sourced from Pesticide Properties DataBase (PPDB), referencing aquatic toxicity studies on the most sensitive specie. The graph presents three categories: very high ($RQ \geq 10$), high ($1 \leq RQ < 10$), and moderate ($0.1 \leq, RQ < 1$) risk to aquatic organisms.

Ac = Acetamiprid; Az = Azamethiphos; Azo = Azoxystrobin; Br = Bromophos-Ethyl; Ca = Carbendazim; Car = Carbofuran; Cyp = Cyproconazole; Di = Dichlorvos; Dic = Dicrotophos; Et = Etrimfos; Fl = Flufenoxuron; Ip = Iprobenfos; Isop = Isoprothiolane; Metr = Metribuzin; Na = Naptalam; Qu = Quinoclamine; Te = Tebufenozide; Teb = Tebutam; Th = Thiacloprid; Tr = Triadimefon; Tri = Triazophos.

Mekong River in Cambodia during the dry and rainy seasons. The pesticide with the highest measured concentration in surface water was the insecticide tebufenozide, with a value of 1300 ng/L. This was detected at all of the investigated sites during both the dry and rainy seasons. The pesticide with the highest measured concentration in soil was the insecticide bromophos-ethyl, at 1100 ng/g dw, and was present in almost all of the sites in both the dry and rainy seasons. The pesticide concentrations in both surface water and soil from the Mekong River were higher than those previously reported in various rivers and lakes in China, Vietnam, and Thailand. To the best of our knowledge, these are the first residual concentrations of recently used pesticides detected in the Mekong River and its tributaries.

Two insecticides and one fungicide had a very high risk RQ, i.e., $RQ > 10$: the insecticides bromophos-ethyl and dichlorvos, and the fungicide iprobenfos. This emphasizes that the environmental impact of pesticides in relation to concentration in surface-water samples deserves more attention, and should be monitored regularly. This is particularly important given that two of the pesticides that pose a very high risk are either banned or restricted, and yet are clearly in use in Cambodia currently.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.169312.

CRediT authorship contribution statement

Putheary Ngin: Conceptualization, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Peter Haglund:** Conceptualization, Supervision, Validation, Writing – review & editing. **Sorya Proum:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing. **Jerker Fick:** Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing.

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.

Data availability

Data will be made available on request.

Acknowledgement

The authors would like to thank all involved the participants for the semi-structured interview and a master student at Royal University Phnom Penh for sampling surface water and soil along the Mekong River in Cambodia. The authors also thank Sophearith Yen, Earth Science Lecturer, at Phnom Penh Teacher Education College, Cambodia for his valuable contribution and support to this study with the extensive sampling carried out and processing authorized letters with different ministries in Cambodia for shipping samples from Phnom Penh, Cambodia to Umeå, Sweden. The authors also acknowledge Richard Lindberg at Umeå University for helping with instrumentation access and advising on extraction experiments with internal standards and different types of SPE cartridges.

Funding

This work was supported by the Swedish International Development Cooperation Agency (Sida) via the Swedish Bilateral Research Cooperation with the Royal University of Phnom Penh (RUPP), Cambodia, which is coordinated via ISP, Uppsala University.

References

Abraham Rivera-Jaimes, J., Postigo, C., Maria Melgoza-Aleman, R., Acena, J., Barcelo, D., Lopez de Alda, M., 2018. Study of pharmaceuticals in surface and wastewater from Cuernavaca, Morelos, Mexico: occurrence and environmental risk assessment. Sci. Total Environ. 613, 1263–1274. https://doi.org/10.1016/j.scitotenv.2017.09.134.

Adams, W., 2015. Conducting Semi-Structured Interviews. https://doi.org/10.1002/9781119171386.ch19.

Asian Development Bank. (2021). Cambodia agriculture, natural resources, and rural development sector assessment, strategy, and road map (0 ed.). Asian Development Bank doi:10.22617/TCS210256-2.

Banaee, M., Haghi, B.N., Ibrahim, A.T.A., 2012. Sub-lethal toxicity of chlorpyrifos on common carp, Cyprinus carpio (Linnaeus, 1758): biochemical response. Int. J. Aquat. Biol. https://doi.org/10.22034/ijab.v1i6.144.

Bertrand, L., Monferrán, M.V., Mouneyrac, C., Bonansea, R.I., Asis, R., Amé, M.V., 2016. Sensitive biomarker responses of the shrimp Palaemonetes argentinus exposed to chlorpyrifos at environmental concentrations: roles of alpha-tocopherol and metallothioneins. Aquat. Toxicol. 179, 72–81. https://doi.org/10.1016/j.aquatox.2016.08.014.

Bhandari, G., Atreya, K., Scheepers, P.T., Geissen, V., 2020. Concentration and distribution of pesticide residues in soil: non-dietary human health risk assessment. Chemosphere 253, 126594.

Bonifacio, A.F., Ballesteros, M.L., Bonansea, R.I., Filippi, I., Amé, M.V., Hued, A.C., 2017. Environmental relevant concentrations of a chlorpyrifos commercial formulation affect two neotropical fish species, Cheirodon interruptus and Cnesterodon decemmaculatus. Chemosphere 188, 486–493. https://doi.org/10.1016/j.chemosphere.2017.08.156.

Boye, K., Lindström, B., Boström, G., Kreuger, J., 2019. Long-term data from the Swedish National Environmental Monitoring Program of Pesticides in Surface Waters. J. Environ. Qual. 48 (4), 1109–1119. https://doi.org/10.2134/jeq2019.02.0056.

Braun, G., Sebesvari, Z., Braun, M., Kruse, J., Amelung, W., An, N.T., Renaud, F.G., 2018. Does sea-dyke construction affect the spatial distribution of pesticides in agricultural soils? - A case study from the Red River Delta. Vietnam. Environ. Pollut. 243, 890–899. https://doi.org/10.1016/j.envpol.2018.09.050.

Bureau-Point, E., 2021. Pesticides et récits de crise dans le monde paysan cambodgien. Anthropologie & Santé. Revue internationale francophone d'anthropologie de la santé 22 https://doi.org/10.4000/anthropologiesante.9054.

Chen, L., Jia, C., Li, F., Jing, J., Yu, P., He, M., Zhao, E., 2018. Dissipation and residues of fluazinam and dimethomorph in potatoes, potato plants, and soil, determined by QuEChERS ultra-performance liquid chromatography tandem mass spectrometry. Environ. Sci. Pollut. Res. 25 (32), 32783–32790. https://doi.org/10.1007/s11356-018-3281-7.

Chhay, N., Seng, S., Tanaka, T., Yamauchi, A., Cedicol, E.C., Kawakita, K., Chiba, S., 2017. Rice productivity improvement in Cambodia through the application of technical recommendation in a farmer field school. Int. J. Agric. Sustain. 15 (1), 54–69. https://doi.org/10.1080/14735903.2016.1174811.

Cho, E., Khim, J., Chung, S., Seo, D., Son, Y., 2014. Occurrence of micropollutants in four major rivers in Korea. Sci. Total Environ. 491–492, 138–147. https://doi.org/10.1016/j.scitotenv.2014.03.025.

Ciglasch, H., 2006. Insecticide Dynamics in the Soil Environment of a Tropical Lychee Plantation–A Case Study from Northern Thailand.

Comoretto, L., Arfib, B., Chiron, S., 2007. Pesticides in the Rhone river delta (France): basic data for a field-based exposure assessment. Sci. Total Environ. 380 (1–3), 124–132. https://doi.org/10.1016/j.scitotenv.2006.11.046.

Cooper, N.L., Bidwell, J.R., 2006. Cholinesterase inhibition and impacts on behavior of the Asian clam, Corbicula fluminea, after exposure to an organophosphate insecticide. Aquat. Toxicol. 76 (3), 258–267. https://doi.org/10.1016/j.aquatox.2005.09.012.

Daramola, I. O., Ojemaye, M. O., Okoh, A. I., & Okoh, O. O. (n.d.). Occurrence of herbicides in the aquatic environment and their removal using advanced oxidation processes: A critical review. Environ. Geochem. Health doi:https://doi.org/10.1007/s10653-022-01326-5.

Delaplane, K.S., 2000. Pesticide usage in the United States: History, benefits, risks, and trends. *The University of Georgia, College of Agricultural and Environmental Sciences. Bulletin 1121/Reprinted November*, 61.

Derbalah, A., Tahara, K., Sakugawa, H., 2020. Monitoring sources, discharges, and fluxes of, and assessing the risks from, pesticides in the Kurose and Ashida Rivers, Japan. Int. J. Environ. Sci. Technol. 17 (2), 1035–1050. https://doi.org/10.1007/s13762-019-02525-x.

Devault, D.A., Gérino, M., Laplanche, C., Julien, F., Winterton, P., Merlina, G., Delmas, F., Lim, P., Miguel Sánchez-Pérez, J., Pinelli, E., 2009. Herbicide accumulation and evolution in reservoir sediments. Sci. Total Environ. 407 (8), 2659–2665. https://doi.org/10.1016/j.scitotenv.2008.12.064.

Donets, M.M., Tsygankov, V.Y., Polevschikov, A., Kulshova, V., Elkhoury, J.A., Boyarova, M.D., Prozorova, L.A., Chernova, E.N., Bogatov, V.V., Lysenko, E., Quang, N.X., 2022. Organochlorine compounds in commercial bivalves from the Mekong and Saigon-Dong Nai River deltas (South Vietnam). Water Air Soil Pollut. 233 (2), 64. https://doi.org/10.1007/s11270-022-05540-w.

Ecobichon, D.J., 2001. Pesticide use in developing countries. Toxicology 160 (1), 27–33. https://doi.org/10.1016/S0300-483X(00)00452-2.

Eissa, F., Al-Sisi, M., Ghanem, K., 2021. Occurrence, human health, and ecotoxicological risk assessment of pesticides in surface waters of the River Nile's Rosetta Branch. Egypt. Environ. Sci. Pollut. Res. 28 (39), 55511–55525. https://doi.org/10.1007/s11356-021-14911-5.

EJF, 2002. Death in Small Doses: Cambodia's Pesticides Problems and Solutions.

European Commision, 2002, August 12. *Commission Decision 2002/657/EC of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. Off. J. Eur. Commun. L221/8*. Publications Office of the European Union. (accessed 14 March 2023) 2022. http://op.europa.eu/en/publication-detail/-/publication/ed928116-a955-4a84-b10a-cf7a82bad858/language-en.

Ezeoyili, I.C., Mgbenka, B.O., Atama, C.I., Ngwu, G.I., Madu, J.C., Nwani, C.D., 2019. Changes in brain acetylcholinesterase and oxidative stress biomarkers in African catfish exposed to carbendazim. J. Aquat. Anim. Health 31 (4), 371–379.

FAOSTAT, F. and A. organization of the U. N, 2020. Pesticide Use: Agricultural Use. https://www.fao.org/faostat/en/#data/RP.

Farrow, L.G., Morton, P.A., Cassidy, R., Floyd, S., McRoberts, W.C., Doody, D.G., Jordan, P., 2022. Evaluation of Chemcatcher? Passive samplers for pesticide monitoring using high-frequency catchment scale data. J. Environ. Manag. 324, 116292 https://doi.org/10.1016/j.jenvman.2022.116292.

Freixo, J.L., Dores, E.F.G. de C., Villa, R.D., 2015. Sampling and analysis of pesticides in the gas phase of air: method validation using a volatilization chamber. Anal. Methods 7 (19), 8255–8261. https://doi.org/10.1039/C5AY01691F.

Gao, J., Liu, L., Liu, X., Zhou, H., Lu, J., Huang, S., Wang, Z., 2009. The occurrence and spatial distribution of Organophosphorous pesticides in Chinese surface water. Bull. Environ. Contam. Toxicol. 82 (2), 223–229. https://doi.org/10.1007/s00128-008-9618-z.

Ha, T., Hojer, J., Nguyen, T., 2010. Potentially hazardous environmental factors for poisoning in rural Vietname> a community based survey. Southeast Asian J. Trop. Med. 41, 1021–1027.

Huang, X., Cui, H., Duan, W., 2020. Ecotoxicity of chlorpyrifos to aquatic organisms: A review. Ecotoxicol. Environ. Saf. 200, 110731 https://doi.org/10.1016/j.ecoenv.2020.110731.

Ismail, M., Ali, R., Shahid, M., Khan, M.A., Zubair, M., Ali, T., Mahmood Khan, Q., 2018. Genotoxic and hematological effects of chlorpyrifos exposure on freshwater fish *Labeo rohita*. Drug Chem. Toxicol. 41 (1), 22–26. https://doi.org/10.1080/01480545.2017.1280047.

Kafle, B., Pokhrel, B., Shrestha, S., Raut, R., Dahal, B.M., 2015. Determination of Pesticide Residues in Water and Soil Samples From Ansikhola Watershed, Kavre. Nepal 5, 2277–2081.

Kang, S., Visarto, P., 2015. Current use of pesticides in the agricultural products of Cambodia. FFTC Agricultural Policy Platform (FFTC-AP) 192 (3), 1441–1449. https://ap.fftc.org.tw/article/986.

Kapsi, M., Tsoutsi, C., Paschalidou, A., Albanis, T., 2019. Environmental monitoring and risk assessment of pesticide residues in surface waters of the Louros River (N.W. Greece). Sci. Total Environ. 650, 2188–2198. https://doi.org/10.1016/j.scitotenv.2018.09.185.

Kazimierczak, R., Średnicka-Tober, D., Golba, J., Nowacka, A., Hołodyńska-Kulas, A., Kopczyńska, K., Góralska-Walczak, R., Gnusowski, B., 2022. Evaluation of pesticide residues occurrence in random samples of organic fruits and vegetables marketed in Poland. Foods 11(13), Article 13. https://doi.org/10.3390/foods11131963.

Khammanee, N., Qiu, Y., Kungskulniti, N., Bignert, A., Meng, Y., Zhu, Z., Teffera, Z.L., 2020. Presence and health risks of obsolete and emerging pesticides in Paddy Rice and soil from Thailand and China. Int. J. Environ. Res. Public Health 17 (11), 3786. https://doi.org/10.3390/ijerph17113786.

Kim, C.-S., Lee, H.-D., Son, K.-A., Lee, E.-Y., Oh, J.-A., 2019. Occurrence of pesticide residues in Han River basin in 2012 and 2014. Kor. J. Environ. Agric. 38 (4), 338–351. https://doi.org/10.5338/KJEA.2019.38.4.45.

Kruawal, K., Sacher, F., Werner, A., Muller, J., Knepper, T.P., 2005. Chemical water quality in Thailand and its impacts on the drinking water production in Thailand. Sci. Total Environ. 340 (1–3), 57–70. https://doi.org/10.1016/j.scitotenv.2004.08.008.

Laitinen, P., Siimes, K., Eronen, L., Ramo, S., Welling, L., Oinonen, S., Mattsoff, L., Ruohonen-Lehto, M., 2006. Fate of the herbicides glyphosate, glufosinate-ammonium, phenmedipham, ethofumesate and metamitron in two Finnish arable soils. Pest Manag. Sci. 62 (6), 473–491. https://doi.org/10.1002/ps.1186.

Lee, G.-H., Choi, K.-C., 2020. Adverse effects of pesticides on the functions of immune system. Comp. Biochem. Physiol. Part C: Toxicol. Pharmacol. 235, 108789 https://doi.org/10.1016/j.cbpc.2020.108789.

Louati, K., Kolsi, F., Kallel, R., Gdoura, Y., Borni, M., Hakim, L.S., Zribi, R., Choura, S., Maalej, A., Sayadi, S., Chamkha, M., Mnif, B., Khemakhem, Z., Boudawara, T.S., Boudawara, M.Z., Safta, F., 2023. Research of pesticide metabolites in Human brain tumor tissues by Chemometrics-based gas chromatography-mass spectrometry analysis for a hypothetical correlation between pesticide exposure and risk factor of central nervous system tumors. ACS Omega 8 (32), 29812–29835. https://doi.org/10.1021/acsomega.3c04592.

Louca Christodoulou, D., Kourouzidou, O., Hadjigeorgiou, M., Hadjiloizou, P., Constantinou, M., Constantinou, P., Kika, K., Klavarioti, M., 2018. Multi-residue analysis of pesticide residues in fruits and vegetables using gas and liquid chromatography with mass spectrometric detection. Accred. Qual. Assur. 23 (3), 145–175. https://doi.org/10.1007/s00769-018-1320-1.

Lozowicka, B., Ilyasova, G., Kaczynski, P., Jankowska, M., Rutkowska, E., Hrynko, I., Mojsak, P., Szabunko, J., 2016. Multi-residue methods for the determination of over four hundred pesticides in solid and liquid high sucrose content matrices by tandem mass spectrometry coupled with gas and liquid chromatograph. Talanta 151, 51–61. https://doi.org/10.1016/j.talanta.2016.01.020.

Mahai, G., Wan, Y., Xia, W., Yang, S., He, Z., Xu, S., 2019. Neonicotinoid insecticides in surface water from the Central Yangtze River, China. Chemosphere 229, 452–460. https://doi.org/10.1016/j.chemosphere.2019.05.040.

Malacrino, A., Seng, K.H., An, C., Ong, S., O'Rourke, M.E., 2020. Integrated pest management for yard-long bean (Vigna unguiculata subsp. Sesquipedalis) in Cambodia. Crop Prot. 135, 104811 https://doi.org/10.1016/j.cropro.2019.05.005.

Matsukawa, M., Ito, K., Kawakita, K., Tanaka, T., 2016. Current status of pesticide use among rice farmers in Cambodia. Appl. Entomol. Zool. 51 (4), 571–579. https://doi.org/10.1007/s13355-016-0432-5.

Mohammad, N., Abidin, E.Z., How, V., Praveena, S.M., Hashim, Z., 2018. Pesticide management approach towards protecting the safety and health of farmers in Southeast Asia. Rev. Environ. Health 33 (2), 123–134. https://doi.org/10.1515/reveh-2017-0019.

Montuori, P., Aurino, S., Nardone, A., Cirillo, T., Triassi, M., 2015. Spatial distribution and partitioning of organophosphates pesticide in water and sediment from Sarno River and Estuary, Southern Italy. Environ. Sci. Pollut. Res. 22 (11), 8629–8642. https://doi.org/10.1007/s11356-014-4016-z.

Nakamura, S., Daishima, S., 2005. Simultaneous determination of 64 pesticides in river water by stir bar sorptive extraction and thermal desorption-gas chromatography-mass spectrometry. Anal. Bioanal. Chem. 382 (1), 99–107. https://doi.org/10.1007/s00216-005-3158-8.

Ng, K., Alygizakis, N., Nika, M.-C., Galani, A., Oswald, P., Oswaldova, M., Čirka, Ľ., Kunkel, U., Macherius, A., Sengl, M., Mariani, G., Tavazzi, S., Skejo, H., Gawlik, B. M., Thomaidis, N.S., Slobodnik, J., 2023. Wide-scope target screening characterization of legacy and emerging contaminants in the Danube River Basin by liquid and gas chromatography coupled with high-resolution mass spectrometry. Water Res. 230, 119539 https://doi.org/10.1016/j.watres.2022.119539.

NORMAN Network, 2023. NORMAN Ecotoxicology Database. https://www.norman-network.com/nds/ecotox/lowestPnecsIndex.php (accessed 06 December 2022).

Olisah, C., Rubidge, G., Human, L.R.D., Adams, J.B., 2022. Organophosphate pesticides in South African eutrophic estuaries: spatial distribution, seasonal variation, and ecological risk assessment. Environ. Pollut. 306, 119446 https://doi.org/10.1016/j.envpol.2022.119446.

Osorio, V., Larrañaga, A., Aceña, J., Pérez, S., Barceló, D., 2016. Concentration and risk of pharmaceuticals in freshwater systems are related to the population density and the livestock units in Iberian Rivers. Sci. Total Environ. 540, 267–277. https://doi.org/10.1016/j.scitotenv.2015.06.143.

Phillips, B.M., Anderson, B.S., Hunt, J.W., Siegler, K., Voorhees, J.P., Tjeerdema, R.S., McNeill, K., 2012. Pyrethroid and organophosphate pesticide-associated toxicity in two coastal watersheds (California, USA). Environ. Toxicol. Chem. 31 (7), 1595–1603. https://doi.org/10.1002/etc.1860.

PPDB, 2023. Pesticide Properties DataBase. http://sitem.herts.ac.uk/aeru/ppdb/en/index.htm (accessed 15 February 2023).

Rahman, Md.S., 2021. Determinants of integrated pest management (IPM) practices dis-adoption in vegetables production in Bangladesh. Int. J. Trop. Insect Sci. 41 (1), 487–494. https://doi.org/10.1007/s42690-020-00231-7.

Rapp-Wright, H., Regan, F., White, B., Barron, L.P., 2023. A year-long study of the occurrence and risk of over 140 contaminants of emerging concern in wastewater influent, effluent and receiving waters in the Republic of Ireland. Sci. Total Environ. 860, 160379 https://doi.org/10.1016/j.scitotenv.2022.160379.

Ren, Z., Liu, L., Fu, R., Miao, M., 2013. The stepwise behavioral responses: behavioral adjustment of the Chinese rare minnow (Gobiocypris rarus) in the exposure of carbamate pesticides. Biomed. Res. Int. 2013, 697279 https://doi.org/10.1155/2013/697279.

REPAR, 2011. REseau Pesticides Bassin d'Arcachon Action 2: Quantification de la présence. Résultats des analyses chimiques sur prélèvements ponctuels 2011. University of Bordeaux.

Sadique Rahman, M., 2022. Farmers' perceptions of integrated pest management (IPM) and determinants of adoption in vegetable production in Bangladesh. Int. J. Pest Manag. 68 (2), 158–166. https://doi.org/10.1080/09670874.2020.1807653.

Salem, S.H., Abd-El Fatah, S., Abdel-Rahman, G.N., Fouzy, A.S.M., Marrez, D.A., 2021. Screening for pesticide residues in soil and crop samples in Egypt. Egypt. J. Chem. 64 (5), 2525–2532. https://doi.org/10.21608/EJCHEM.2021.64117.3374.

Sangchan, W., Bannwarth, M., Ingwersen, J., Hugenschmidt, C., Schwadorf, K., Thavornyutikarn, P., Pansombat, K., Streck, T., 2014. Monitoring and risk assessment of pesticides in a tropical river of an agricultural watershed in northern Thailand. Environ. Monit. Assess. 186 (2), 1083–1099. https://doi.org/10.1007/s10661-013-3440-8.

Schreinemachers, P., Afari-Sefa, V., Heng, C., Dung, P., Praneetvatakul, S., Ramasamy, S., 2015. Safe and sustainable crop protection in Southeast Asia: status, challenges and policy options. Environ. Sci. Pol. 54, 357–366. https://doi.org/10.1016/j.envsci.2015.07.017.

Schreinemachers, P., Chen, H.-P., Nguyen, T.T.L., Buntong, B., Bouapao, L., Gautam, S., Le, N.T., Pinn, T., Vilaysone, P., Srinivasan, R., 2017. Too much to handle? Pesticide dependence of smallholder vegetable farmers in Southeast Asia. Sci. Total Environ. 593–594, 470–477. https://doi.org/10.1016/j.scitotenv.2017.03.181.

Schreinemachers, P., Grovermann, C., Praneetvatakul, S., Heng, P., Nguyen, T.T.L., Buntong, B., Le, N.T., Pinn, T., 2020. How much is too much? Quantifying pesticide overuse in vegetable production in Southeast Asia. J. Clean. Prod. 244, 118738 https://doi.org/10.1016/j.jclepro.2019.118738.

Sharbidre, A., Patode, P.S., 2012. Behavioural changes and acetylcholinesterase activity in guppy fish (poecilia reticulata) exposed to chlorpyrifos. Nat. Environ. Pollut. Technol. 11, 487–492.

Sharma, M., Maheshwari, N., Khan, F.H., Mahmood, R., 2022. Carbendazim toxicity in different cell lines and mammalian tissues. J. Biochem. Mol. Toxicol. 36 (12), e23194 https://doi.org/10.1002/jbt.23194.

Shehu, Z., Nyakairu, G.W.A., Tebandeke, E., Odume, O.N., 2022. Overview of African water resources contamination by contaminants of emerging concern. Sci. Total Environ. 852, 158303 https://doi.org/10.1016/j.scitotenv.2022.158303.

Silva, V., Mol, H.G.J., Zomer, P., Tienstra, M., Ritsema, C.J., Geissen, V., 2019. Pesticide residues in European agricultural soils—A hidden reality unfolded. Sci. Total Environ. 653, 1532–1545. https://doi.org/10.1016/j.scitotenv.2018.10.441.

Şimşek Uygun, B., Albek, E.A., 2022. Seasonal monitoring of organochlorine pesticides in water, soil, and sediment in a small pond and determining ecotoxicological risk assessment. Environ. Qual. Manag. 32 (2), 295–307. https://doi.org/10.1002/tqem.21844.

Sokcheng, S., Keo, S., & Molideth, S. (2021). Pesticide Use Practices in Cambodia's Vegetable Farming. CDRI, Cambodia Development Resource Institute. https://cdri.org.kh/storage/pdf/WP128_1631156847.pdf (accessed 10 October 2023).

Stehle, S., Bline, A., Bub, S., Petschick, L.L., Wolfram, J., Schulz, R., 2019. Aquatic pesticide exposure in the US as a result of non-agricultural uses. Environ. Int. 133, 105234 https://doi.org/10.1016/j.envint.2019.105234.

Sulaiman, N.S., Rovina, K., Joseph, V.M., 2019. Classification, extraction and current analytical approaches for detection of pesticides in various food products. J. Consum. Protect. Food Safe. 14 (3), 209–221. https://doi.org/10.1007/s00003-019-01242-4.

Sule, R.O., Condon, L., Gomes, A.V., 2022. A common feature of pesticides: oxidative stress—the role of oxidative stress in pesticide-induced toxicity. Oxidative Med. Cell. Longev. 2022, 5563759. https://doi.org/10.1155/2022/5563759.

Tan, H., Li, Q., Zhang, H., Wu, C., Zhao, S., Deng, X., Li, Y., 2020. Pesticide residues in agricultural topsoil from the Hainan tropical riverside basin: determination, distribution, and relationships with planting patterns and surface water. Sci. Total Environ. 722, 137856 https://doi.org/10.1016/j.scitotenv.2020.137856.

Toth, G., Hahn, J., Szoboszlay, S., Harkai, P., Farkas, M., Rado, J., Gobolos, B., Kaszab, E., Szabo, I., Urbanyi, B., Kriszt, B., 2022. Spatiotemporal analysis of multi-pesticide residues in the largest central European shallow lake, Lake Balaton, and its sub-catchment area. Environ. Sci. Eur. 34 (1), 50. https://doi.org/10.1186/s12302-022-00630-2.

Troger, R., Ren, H., Yin, D., Postigo, C., Nguyen, P.D., Baduel, C., Golovko, O., Been, F., Joerss, H., Boleda, M.R., Polesello, S., Roncoroni, M., Taniyasu, S., Menger, F., Ahrens, L., Lai, F.Y., Wiberg, K., 2021. What's in the water? - target and suspect screening of contaminants of emerging concern in raw water and drinking water from Europe and Asia. Water Res. 198, 117099 https://doi.org/10.1016/j.watres.2021.117099.

Ullah, S., Zorriehzahra, J., 2014. Ecotoxicology: A review of pesticides induced toxicity in fish. Adv. Anim. Vet. Sci. 3, 40–57. https://doi.org/10.14737/journal.aavs/2015/3.1.40.57.

United Nations Population Division, 2022. *Cambodia Population (Live)- Worldometer, the United Nations, Department of Economic and Social Affairs, Population Division. World Population Prospects: The 2022* Revision. https://www.worldometers.info/world-population/cambodia-population/ (accessed 20 October 2022).

Van Leeuwen, C.J., Vermeire, T.G., 2007. *Risk Assessment of Chemicals: An Introduction* (2nd, illustrated ed.). Springer Science & Business Media, 2007.

Vellingiri, B., Chandrasekhar, M., Sabari, S.S., Gopalakrishnan, A.V., Narayanasamy, A., Venkatesan, D., Iyer, M., Kesari, K., Dey, A., 2022. Neurotoxicity of pesticides-A link to neurodegeneration. Ecotoxicol. Environ. Saf. 243, 113972 https://doi.org/10.1016/j.ecoenv.2022.113972.

Vittoria Barbieri, M., Peris, A., Postigo, C., Moya-Garces, A., Simon Monllor-Alcaraz, L., Rambla-Alegre, M., Eljarrat, E., Lopez de Alda, M., 2021. Evaluation of the occurrence and fate of pesticides in a typical Mediterranean delta ecosystem (Ebro River Delta) and risk assessment for aquatic organisms. Environ. Pollut. 274, 115813 https://doi.org/10.1016/j.envpol.2020.115813.

Wang, X., Chen, F., Lu, J., Wu, M., Cheng, J., Xu, W., Li, Z., Zhang, Y., 2023. Developmental and cardiovascular toxicities of acetochlor and its chiral isomers in zebrafish embryos through oxidative stress. Sci. Total Environ. 896, 165296 https://doi.org/10.1016/j.scitotenv.2023.165296.

WHO, 2020. WHO recommended classification of pesticides by hazard and guidelines to classification, 2019 edition. Geneva: World Health Organization; 2020. Licence: CC BY-NC-SA 3.0 IGO. https://www.who.int/publications-detail-redirect/9789240005662 (accessed 20 October 2022).

World Population Review, 2022. Poorest Asian Countries. https://worldpopulationreview.com/country-rankings/poorest-asian-countries (accessed 20 October 2022).

Xu, L., Granger, C., Dong, H., Mao, Y., Duan, S., Li, J., Qiang, Z., 2020. Occurrences of 29 pesticides in the Huangpu River, China: highest ecological risk identified in Shanghai metropolitan area. Chemosphere 251, 126411. https://doi.org/10.1016/j.chemosphere.2020.126411.

Xuereb, B., Lefèvre, E., Garric, J., Geffard, O., 2009. Acetylcholinesterase activity in Gammarus fossarum (Crustacea Amphipoda): linking AChE inhibition and behavioural alteration. Aquat. Toxicol. 94 (2), 114–122. https://doi.org/10.1016/j.aquatox.2009.06.010.

Yang, M., Zhao, X., Meng, T., 2019. What are the driving factors of pesticide overuse in vegetable production? Evidence from Chinese farmers. China Agric. Econ. Rev. 11 (4), 672–687. https://doi.org/10.1108/CAER-08-2018-0170.

Zarei-Choghan, M., Jorfi, S., Saki, A., Jaafarzadeh, N., 2022. Spatial distribution, ecological and health risk assessment of organophosphorus pesticides identified in the water of Naseri artificial wetland. Iran. Mar. Pollut. Bull. 179, 113643 https://doi.org/10.1016/j.marpolbul.2022.113643.