



Toxicological effects of agricultural pesticides on aquatic ecosystems: A review

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ABSTRACT

Aquatic ecosystems are facing significant risks due to the global contamination of surface waters, sediments, and biota caused by agricultural pesticides. This review summarizes the results of 27 original studies conducted between 2011 and 2026 across various geographical regions, including North America, Europe, Asia, Africa, Latin America, and the Amazon. In agricultural and periurban areas, pesticides were detected in 88% to 100% of samples, frequently exceeding both acute and chronic toxicity thresholds. For example, in 70% of protected streams in Germany, risk assessment criteria (RAC) were exceeded; in 69.4% of sites in the USA, the pesticide toxicity index (PTI) was greater than 0.1; and in lakes in Ethiopia, the risk quotient (RQ) for organophosphates exceeded 100. The risk profiles were primarily composed of neonicotinoids, organophosphates, pyrethroids, and legacy compounds such as fipronil and chlorpyrifos. Insecticides typically caused acute toxicity, while herbicides and fungicides resulted in long-term effects. Monitoring only water concentrations can lead to an underestimation of risks, as strong sorption to periphyton and sediments creates prolonged exposure routes. Significant shifts toward more tolerant taxa were observed in aquatic invertebrate communities, such as dominance by Chironomidae and a decrease in biodiversity, with almost no sensitive mayfly or stonefly species present. At environmentally relevant concentrations, fish exhibited developmental toxicities, including edema, deformities, delayed hatching, and apoptosis. Immunotoxic effects were also noted, specifically the activation of the JAK-STAT pathway. Mixture effects and interactions with multiple stressors, such as nutrients, sediment, and fungicides, often produced non-additive results, with regional variations evident, such as increased resistance to pyrethroids in tropical regions. Overall, agricultural intensification places aquatic biota under persistent pesticide pressure, which disrupts food webs and threatens ecosystem stability. There is an urgent need for stricter riparian buffers, updated registrations of highly hazardous compounds, and enhanced monitoring of sediment transformation products and body burdens.

Keywords: Agricultural pesticides, Aquatic ecosystems, Neonicotinoids, Threshold exceedance, Mixture toxicity, Developmental toxicity, Multi-stressor effects.

Article type: Review Article.



INTRODUCTION

Agricultural pesticides are essential for modern intensive farming, but their transport into aquatic environments is a major stressor on freshwater ecosystems worldwide. Studies across multiple continents show that agricultural pesticides consistently contaminate surface waters, sediments, periphyton, and biota at levels that threaten aquatic life. Pesticide occurrence is persistent and widespread, with detection rates often exceeding 80–100 % even in protected areas (Covert *et al.* 2020; Rico *et al.* 2022; Oliver *et al.* 2023; Schweiger *et al.* 2025). European studies corroborate this continental trend. In German agricultural streams, insect biomass doubled relative to forested catchments, but sensitive stoneflies and mayflies were scarce (ANOSIM $R = 0.31$; Ohler *et al.* 2023). Small water bodies in agricultural areas, compared to grasslands, contained a median of nine substances per sample, with mean concentrations 5.7 times higher than reference sites. Twenty-two percent of samples exceeded regulatory acceptable concentrations (RAC), and TU_{max} exceeded 2 for algae/plants in 38% of cases (Lorenz *et al.* 2025). Runoff sampling in the Hausen and Queich catchments revealed up to 43 substances (average 32 in Hausen), with 16.1% of mixtures exceeding sum (M/R) > 1 for aquatic invertebrates (Schemmer *et al.* 2024). Even streams within German nature reserves (no upstream agriculture) showed frequent detection of 81 out of 118 substances, with 70% exceeding RACs, primarily due to legacy insecticides like fipronil and imidacloprid (Schweiger *et al.* 2025). These results suggest that atmospheric transport, legacy residues, and diffuse sources cause persistent exposure beyond immediate agricultural zones. Data from Asia and Africa broaden the geographic scope and emphasize matrix-specific risks. A study of 90 Japanese sites found 10 of 18 insecticides in water (dinotefuran max. 6.03 $\mu\text{g L}^{-1}$) and 15 in sediment, with imidacloprid in 66/90 sediment samples; water–sediment correlations confirmed neonicotinoid accumulation in sediment (Furihata *et al.* 2019). In Shanghai surface waters, chlorpyrifos, atrazine, and prometryn were detected in >80% of samples, with summed concentrations of 36–217 ng L^{-1} , peaking in southern agricultural areas (Chen *et al.* 2020). Multi-matrix sampling in Argentina's Tapalque stream basin revealed 19 of 30 pesticides, with glyphosate/AMPA dominant in soil/sediment and imidacloprid in water; chronic risk was highest in soil/sediment during summer (Pérez *et al.* 2021). Lake Ziway (Ethiopia) showed 63% pesticide detection in wet season water samples, with risk quotients >100 for diazinon, malathion, and chlorpyrifos, highlighting floriculture and smallholder agriculture as major sources (Merga *et al.* 2021). Rivers in western Kenya exhibited chronic toxicity exceeding crustacean thresholds in 96% of samples across four seasons, driven by diazinon, imidacloprid, and pirimiphos-methyl (Tanui *et al.* 2024). The Rio Madre de Dios watershed (Costa Rica) showed consistent pesticide loads, including 12 highly hazardous compounds, with maximum arthropod toxicity downstream (Echeverría-Sáenz *et al.* 2018). Urban streams in the Brazilian Amazon (Manaus, Santarém, Belém, Macapá) were 100% contaminated, with up to eight compounds per sample and high invertebrate risk (HI >10 in six samples) from organophosphates banned in Europe (Rico *et al.* 2022). These studies collectively indicate that pesticide pollution extends beyond temperate agriculture to tropical and developing regions with weaker regulations. Beyond their presence, studies show pesticides persist through various pathways, leading to underestimations by water-only monitoring. These pathways include strong sorption to periphyton (Ijzerman *et al.* 2023), sediment accumulation of fipronil metabolites and neonicotinoids (Furihata *et al.* 2019, Kuechle *et al.* 2022), and elevated pesticide levels in *Gammarus pulex* (Shahid *et al.* 2018). Transformation products also extend risk, with year-round detection of herbicide metabolites in Great Lakes tributaries (Oliver *et al.* 2023) and Japanese sediments acting as pesticide reservoirs (Furihata *et al.* 2019). Regulatory and toxicological thresholds are frequently exceeded. The USA National Water-Quality Network reported chronic pesticide toxicity index (PTI) > 0.1 in 69.4% of sites, with 60.9% of agricultural samples surpassing both chronic and acute benchmarks (Ref. 2). In Germany, small water bodies exceeded RACs in 22% of samples (Lorenz *et al.* 2025), protected streams in 70% (Schweiger *et al.* 2025), and runoff events produced invertebrate risk quotients >1 in 16.1% of mixtures (Schemmer *et al.* 2024). Ethiopian lakes exhibited RQ >100 for multiple organophosphates during wet-season runoff (Merga *et al.* 2021). Although complex mixtures are present, single insecticides often dominate toxicity (MCR 1–2 in >92% of USA samples) (Covert *et al.* 2020), while fungicides and herbicides significantly contribute to chronic effects on primary producers (Hashimoto *et al.* 2019; Lorenz *et al.* 2025). Early biological responses are evident. Mayfly survival significantly declined ($p < 0.01$) when fed contaminated periphyton (Ijzerman *et al.* 2023), insect communities in Prairie Pothole wetlands shifted toward tolerant dipterans under neonicotinoid pressure (Cavallaro *et al.* 2019), and riparian spider richness and abundance decreased with summed toxic units ($D^2 = 0.396\text{--}0.481$; Graf *et al.* 2019). Leaf-litter decomposition slowed in high-agriculture streams due to loss of specialist detritivores (Cornejo *et al.* 2020), and zooplankton

biomass and richness responded non-additively to imidacloprid, sediment and nutrients (Chará-Serna *et al.* 2019). Fish embryos exposed to prometryn and cyhalofop-butyl displayed various adverse effects, including pericardial oedema, spinal deformities, and immunotoxicity, at environmentally relevant concentrations (Cheng *et al.* 2021; Zhang *et al.* 2022). Collectively, studies reveal a consistent global pattern of agricultural pesticide contamination in aquatic systems. Pesticides frequently enter these systems at high concentrations, persist in various environmental components, and exceed regulatory limits. This leads to both direct mortality and indirect food-web disruptions across diverse organisms. Despite variations in climate, land use, and regulations, insecticides (particularly neonicotinoids, organophosphates, and pyrethroids) and their byproducts consistently cause similar ecological damage. This review synthesizes these findings into seven thematic sections, quantifying pesticide occurrence, sorption, threshold exceedances, invertebrate shifts, vertebrate toxicity, ecosystem impairment, and mixture/multi-stressor effects. Integrating data from studies across six continents underscores the urgent need for sediment monitoring, stricter riparian buffers, reassessment of legacy compounds, and updated risk assessments that consider real-world mixtures and regional sensitivities.

MATERIALS AND METHODS

This narrative review compiles empirical data from original studies on the toxicological effects of agricultural pesticides in aquatic ecosystems, published between 2011 and 2026. To gather literature, combinations of the following keywords were used in searches through Web of Science, Scopus, and Google Scholar: (aquatic ecosystems OR freshwater OR streams OR wetlands OR lakes OR sediment OR periphyton OR invertebrates OR fish OR mixture toxicity OR ecological risk OR threshold exceedance) AND (agricultural pesticides OR neonicotinoids OR organophosphates OR pyrethroids OR glyphosate OR chlorpyrifos). Backward and forward citation searches of key papers helped identify additional relevant studies and pertinent review papers (e.g., references 2 and 24). The inclusion criteria for the studies were as follows: (i) original empirical research (field monitoring, mesocosm/experimental, or laboratory studies using environmentally relevant concentrations); (ii) reporting on pesticide occurrence in aquatic matrices (water, sediment, periphyton, biota) and/or biological/toxicological effects (community shifts, developmental toxicity, ecosystem processes, risk metrics such as PTI, RAC, RQ, TU, SPEAR); (iii) conducted in agricultural or peri-urban influenced systems; and (iv) published between 2013 and 2025 to capture current pesticides and recent regulatory contexts. Studies were excluded if they relied solely on non-agricultural sources, pure modeling without empirical data, urban-only wastewater, or non-English publications. After screening for quality and relevance (empirical robustness, clear risk-assessment methods, geographic diversity), 27 studies were selected and categorized by themes: occurrence/distribution, sorption/bioaccumulation, threshold exceedances, invertebrate community shifts, fish/vertebrate toxicity, ecosystem process impairment, and mixture/multi-stressor interactions. Data extraction focused on pesticide occurrence (percent detection, concentrations), toxicity (risk quotient exceedance rates), biological responses (community metrics, sublethal effects), and risk assessment implications.

Table 1. Reports about toxicological effects of agricultural pesticides on aquatic ecosystems in different regions.

| Ref | Location / Region | Year (s) / period | Matrices & key pesticides | Main risk metrics / exceedances | Key biological / ecological effects | Main conclusion for synthesis |
|--------------------------------|----------------------------------|-------------------|--|---|---|---|
| (Ijzerman <i>et al.</i> 2023) | Ontario streams, Canada | ~2010s | Periphyton (glyphosate, AMPA + others); water/sediment context | Strong sorption to periphyton | ↓ Mayfly survival ($p < 0.01$) via dietary exposure | Periphyton as major chronic exposure route; water-only monitoring underestimates risk |
| (Covert <i>et al.</i> 2020) | USA NWQN (72 sites) | 2013–2017 | Water (up to 60 pesticides) | PTI > 0.1 in 69.4%; acute mainly insecticides | Toxicity dominated by 1–2 insecticides (MCR 1–2) | Year-round chronic risk; # compounds ≠ toxicity |
| (Ohler <i>et al.</i> 2023) | Germany (ag vs forested streams) | ~2010s | Emerging insects biomass/abundance | ANOSIM R=0.31 community turnover | Near-absence sensitive mayflies/stoneflies; ↑ tolerant taxa | Agriculture reduces diversity, alters phenology & food-web support |
| (Cavallaro <i>et al.</i> 2019) | Prairie Pothole wetlands | 2013–2015 | Water (neonics TEQ) | 100% detection 2013; mixtures 73% | Shift to tolerant dipterans; ↓ sensitive taxa | Neonics + habitat degradation alter emerging insect communities |

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|---------------------------------------|---|-----------------|---|--|--|---|--|
| | (Canada/USA) | | | | | | |
| (Furihata <i>et al.</i> 2019) | Japan (90 sites) | 2015–2016 | Water & sediment (18 insecticides) | Dinotefuran max 6.03 µg L ⁻¹ ; imidacloprid in 66/90 sediment | Sediment as long-term reservoir | Prolonged exposure from sediment accumulation | |
| (Kuechle <i>et al.</i> 2022) | USA/Canada wetlands (thiamethoxam-coated seeds) | 2016–2017 | Water & sediment (NI-EQ) | Sediment NI-EQ drives effects | ↓ Insect abundance/richness (Diptera etc.) | Sediment chronic driver even below water acute benchmarks | |
| (Lorenz <i>et al.</i> 2025) | Germany small water bodies | 3-year | Water (23+ pesticides) | RAC 22%; T _{Umax} >2 algae 38% | Primary producers most affected | Small water bodies frequently exceed thresholds; cascading risk | |
| (Hashimoto <i>et al.</i> 2019) | Japan rice paddy mesocosms | Experimental | Water/sediment (pentoxazone, fipronil) | ↓ Macrophytes 25–51%; ↓ predators/prey | Bottom-up & top-down disruption | Insecticides direct; herbicides indirect habitat loss | |
| (Oliver <i>et al.</i> 2023) | Great Lakes tributaries, USA | 2016 | Water (231 analytes, 104 detected) | TQ/EAR exceedances 15/16 sites monthly | Year-round risk; insecticides acute, herbicides chronic | Transformation products extend exposure | |
| (Graf <i>et al.</i> 2019) | Agricultural streams (riparian spiders) | ~2010s | Water (sum TU); riparian parameters | ↓ Richness/abundance with TU (D ² =0.396–0.481) | Community shifts; sensitive spiders decline | Riparian spiders bioindicators of aquatic pesticide stress | |
| (Echeverría-Sáenz <i>et al.</i> 2018) | Rio Madre de Dios, Costa Rica | 2011–2012 | Water (27 pesticides) | High TU arthropods; elevated fish biomarkers | ↓ Macroinvertebrate richness; tolerant taxa dominate | Chronic risk from banana/ pineapple runoff | |
| (Zhang <i>et al.</i> 2022) | Zebrafish lab (prometryn) | Lab | Exposure water (1–1000 µg L ⁻¹) | Developmental toxicity; gene expression changes | Edema, deformities, delayed hatching, ↑ mortality | Triazines high risk to fish embryos at µg/L | |
| (Cornejo <i>et al.</i> 2020) | Streams with ag influence | ~28-day litter | Water/litter (4 OPs/pyrethroids) | T _{Umax} 0–1.54; ↓ decomposition | Shift detritivores to tolerant taxa; ↓ litter processing | Impaired ecosystem functioning (decomposition) | |
| (Schemmer <i>et al.</i> 2024) | Germany runoff events (protected areas) | Runoff E1–E3 | Water (up to 43 substances) | 16.1% sum(M/R)>1 invertebrates | High mixture risk invertebrates | Runoff creates peaks; buffers needed | |
| (Chen <i>et al.</i> 2020) | Shanghai surface waters, China | Winter | Water (18 CUPs) | >80% detection key CUPs; sum 36–217 ng L ⁻¹ | RQ higher ag areas | Ubiquitous but low-moderate; land-use drives patterns | |
| (Chará-Serna <i>et al.</i> 2019) | Mesocosm (imidacloprid + sediment + nutrients) | 35 days | Water | Antagonistic on zooplankton richness (2–2.4× additive) | ↓ Biomass/richness; community shifts | Non-additive multi-stressor effects | |
| (Shahid <i>et al.</i> 2018) | Ag vs non-ag streams | ~2010s | Water + Gammarus body burden | SPEAR bad/moderate ag; TU body median 0.4 | Body burden predicts community impairment | Internal burden reliable exposure measure | |
| (Hébert <i>et al.</i> 2021) | Semi-natural pond mesocosms | 43 days | Water (glyphosate pulses) | >90% rotifer loss initial; persistent richness ↓ | Shift to tolerant cladocerans | Glyphosate dominates zooplankton disruption | |
| (Pérez <i>et al.</i> 2021) | Tapalque basin, Argentina | Spring–fall | Soil/sediment/water (30 pesticides) | Chronic soil high; sediment acute summer | Multi-matrix risk highest soil/sediment | Integrated ERA needed; terrestrial-aquatic link | |
| (Merga <i>et al.</i> 2021) | Lake Ziway, Ethiopia | Dry/wet seasons | Water/sediment (19 pesticides) | RQ >100 organophosphates wet season | High acute/chronic risk invertebrates/fish | Floriculture/smallholder sources; wet season peaks | |
| (Cheng <i>et al.</i> 2021) | Zebrafish lab (cyhalofop-butyl) | 96 h | Exposure (0.1–0.5 mg L ⁻¹) | Developmental/immunotoxicity | Edema, apoptosis, JAK-STAT activation | Multi-level toxicity in fish | |

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|--------------------------------|--|--------------|--|--|---|---|
| (Yao <i>et al.</i> 2024) | Tropical mesocosm (lambda-cyhalothrin) | Experimental | Water/sediment | NOEC community 100 ng L ⁻¹ ; ↓ sensitive taxa | Tropical SSD higher than temperate | Less sensitivity in tropics; rapid dissipation |
| (Tanui <i>et al.</i> 2024) | Western Kenya rivers | 4 seasons | Water (pesticides + pharma) | Chronic TU crustaceans 96% exceed | Crustaceans highest risk | Multi-chemical; dry season peaks |
| (Schweiger <i>et al.</i> 2025) | Protected German streams | 2021 | Water (118 substances) | 70% RAC exceed; 81/118 detected | Low SPEAR; legacy insecticides drive | Atmospheric/legacy input even in protected areas |
| (Beentjes <i>et al.</i> 2022) | Outdoor ditch mesocosms | Weeks | Water (thiacloprid + fertilizer) | Interactive community shifts | Non-additive multi-trophic effects | Combined stressors alter microbial to invertebrates |
| (Meppelink <i>et al.</i> 2025) | Iowa ag streams | One-time | Water/sediment/fish (multi-contaminants) | Ubiquitous microplastics/PFAS/ARGs | High microplastic TQ; moderate pesticide | Holistic monitoring beyond pesticides needed |
| (Rico <i>et al.</i> 2022) | Brazilian Amazon urban streams | One-time | Dissolved/particulate (11+ pesticides) | 100% contaminated; HI >10 in 6/12 | High invertebrate risk (malathion/chlorpyrifos) | Outdated registration allows high-risk compounds |

Table 1 summarizes included studies. Due to heterogeneity in study designs, analytes, and endpoints, a formal meta-analysis was not performed; instead, narrative synthesis identified recurring patterns across matrices and regions. Limitations of this approach include potential underrepresentation of certain regions and publication bias favoring significant findings (e.g., Africa, tropical regions relative to agricultural intensity).

Occurrence and distribution of agricultural pesticides in aquatic systems

Agricultural pesticides are commonly found in surface waters near farmland. In a US study (2013-2017), 88% of water samples contained at least five pesticides (median 17 compounds, agricultural sites median 24), with peak levels in May-July (Covert *et al.* 2020). Another study of Great Lakes tributaries (2016) found 96% of samples positive for pesticides (median 16), with year-round presence peaking in July (Oliver *et al.* 2023). In Japan, insecticides were detected in water (dinotefuran most frequent) and sediment (imidacloprid most frequent), with higher contamination in southwest Japan (Furihata *et al.* 2019). Surface waters in Shanghai showed high detection rates for chlorpyrifos, atrazine, and prometryn, with the highest concentrations in southern agricultural areas (Chen *et al.* 2020). Urban streams in the Brazilian Amazon were contaminated with up to eight compounds, mainly malathion and chlorpyrifos (Rico *et al.* 2022). These findings are summarized in Table 2.

Table 2. Pesticide occurrence metrics from representative studies.

| Ref | Location | % Positive Samples | Median / Max Compounds | Dominant Pesticides / Matrices |
|-------------------------------|-------------------------|-------------------------------|-------------------------------|--|
| (Covert <i>et al.</i> 2020) | USA NWQN | 88% ≥ 5 pesticides | Median 17 (agricultural: 24) | Up to 60; insecticides drive toxicity |
| (Oliver <i>et al.</i> 2023) | Great Lakes tributaries | 96% | Median 16 | Herbicides, neonicotinoids, fungicides |
| (Furihata <i>et al.</i> 2019) | Japan (90 sites) | Water: 10/18; Sediment: 15/18 | – | Neonics (water), fipronil metabolites (sediment) |
| (Chen <i>et al.</i> 2020) | Shanghai, China | > 80% for key CUPs | Sum 36–217 ng L ⁻¹ | Chlorpyrifos, atrazine |
| (Rico <i>et al.</i> 2022) | Brazilian Amazon urban | 100% | Up to 8 | Malathion, chlorpyrifos (mostly particulate) |

Sorption to periphyton, bioaccumulation, and sediment as exposure reservoirs

Pesticides bind strongly to periphyton and sediment, leading to chronic exposure. In Ontario streams, glyphosate and AMPA were frequently found in periphyton (50% and 30% of sites, respectively), especially near agricultural drains, despite glyphosate's low water solubility (Ijzerman *et al.* 2023). Japanese sediments showed higher insecticide concentrations, including imidacloprid in 66/90 sites, with fipronil metabolites persisting (Furihata *et al.* 2019). In USA/Canada wetlands treated with thiamethoxam-coated seeds, elevated sediment neonicotinoid equivalents (NI-EQ) caused chronic insect effects, even when water concentrations were below acute EPA benchmarks (Kuechle *et al.* 2022). A multi-matrix assessment in Argentina's Tapalque basin indicated the highest chronic risk in soil/sediment due to persistent AMPA and imidacloprid (STU >1 in summer/fall sediment at some

sites; Pérez *et al.* 2021). These studies demonstrate that monitoring water alone underestimates benthic and food-chain pesticide exposure.

Exceedance of Regulatory and Toxicological Thresholds

Regulatory and toxicity thresholds are frequently exceeded, particularly in agricultural areas. For example, at USA NWQN sites, the chronic pesticide toxicity index (PTI) exceeded 0.1 in 69.4% of sites, with agricultural samples surpassing chronic/acute thresholds in 60.9% of cases; a single insecticide was responsible for >92% of the toxicity (Covert *et al.* 2020). In German small water bodies, exceedances were observed for RAC in 22% of samples, and TUMax >2 for algae/plants in 38% (Lorenz *et al.* 2025). Runoff events in German catchments resulted in mixtures exceeding sum (M/R) >1 for invertebrates in 16.1% of cases (Schemmer *et al.* 2024). Protected German streams showed RAC exceedances in 70% of samples, mainly due to legacy insecticides (fipronil 9×, imidacloprid 6×) (Schweiger *et al.* 2025). Lake Ziway (Ethiopia) had RQ values >100 for diazinon, malathion, and chlorpyrifos during the wet season (Merga *et al.* 2021).

Table 3. Toxicity Threshold Exceedances and Risk Metrics.

| Ref | Location | Risk metric | Exceedance rate | Primary affected group | Key drivers |
|--------------------------------|---------------------|-------------------------|--------------------|-----------------------------|---------------------------------------|
| (Covert <i>et al.</i> 2020) | USA | PTI > 0.1 (chronic) | 69.4% sites | Aquatic biota | Insecticides |
| (Lorenz <i>et al.</i> 2025) | Germany | RAC / TUMax > 2 (algae) | 22% / 38% | Invertebrates, algae/plants | Chlorpyrifos, nicosulfuron |
| (Schemmer <i>et al.</i> 2024) | Germany | sum(M/R) > 1 | 16.1% | Aquatic invertebrates | 5–7 substance mixtures |
| (Schweiger <i>et al.</i> 2025) | Germany (protected) | RAC | 70% streams | Invertebrates | Fipronil, neonicotinoids, pyrethroids |
| (Merga <i>et al.</i> 2021) | Ethiopia | RQ > 100 | Multiple compounds | Invertebrates/fish | Diazinon, chlorpyrifos (wet season) |

Direct toxicity and community shifts in aquatic invertebrates

Invertebrate communities undergo marked shifts under pesticide pressure. Mayfly survival dropped significantly ($p < 0.01$) when fed periphyton from contaminated Ontario agricultural drains (Ijzerman *et al.* 2023). German agricultural streams showed ~2× higher total insect biomass but near-absence of sensitive stoneflies and mayflies (Arthropleidae, Siphonuridae), with ANOSIM $R = 0.31$ community turnover toward tolerant taxa (Ohler *et al.* 2023). Neonicotinoid TEQ in Prairie Pothole wetlands shifted emerging insects toward tolerant Chironominae and Muscidae while reducing Psychodidae and Syrphidae (Cavallaro *et al.* 2019). *Gammarus pulex* body burdens (median TUMaxInt 0.4, neonicotinoids dominant) in agricultural streams produced SPEARpesticides indices of 7–32 (bad/moderate status) versus 34–50 in non-agricultural streams (Shahid *et al.* 2018). Even protected German streams had low SPEAR scores at 5/12 sites due to legacy insecticides (Schweiger *et al.* 2025).

Developmental and immunological toxicity in fish and vertebrates

Fish embryos and larvae experience major difficulties in development and in their immune systems. Exposure to Prometryn (1–1000 $\mu\text{g L}^{-1}$) resulted in the development of pericardial and yolk-sac edema, cranio-facial spine and other deformities, severe delayed hatching (up to 20 days post fertilization), increased mortality (up to 67%), and a decreased body length/eye diameter ratio. Exposure to Prometryn also modified the expression of certain genes (complement/coagulation cascade, AMPK, cardiac genes) in zebrafish (Zhang *et al.* 2022). At a concentration of 0.1–0.5 mg L^{-1} , cyhalofop-butyl was the cause of decreased body length, increased yolk-sac edema, immunotoxicity (this was characterized by a decrease in neutrophils, macrophages and T-cells, caused by the JAK-STAT pathway), increased oxidative stress (as measured by increased levels of ROS, MDA, CAT and SOD), and increased apoptosis, as measured by p53, Bax and caspase-3 (Cheng *et al.* 2021). In the rivers of Costa Rica, polluted areas exhibited increased levels of biomarkers of fish ChE, GST, LPO, CAT, and the Integrated Biomarker Response (IBR). In addition, the abundance of macroinvertebrates was lowest at sites with the highest concentrations of pesticides (Echeverría-Sáenz *et al.* 2018). Lake Ziway reported a high RQ for fish organophosphates in an acute/chronic manner during the wet-season runoff (Merga *et al.* 2021).

Indirect effects on primary producers and ecosystem processes

Herbicides and mixtures impair primary production and ecosystem functions. In Japanese rice-paddy mesocosms, pentoxazone reduced macrophytes 25–51%, causing indirect top-down and bottom-up disruption (phytophilous

predators most sensitive; Hashimoto *et al.* 2019). Glyphosate pulses in semi-natural ponds produced > 90% initial rotifer loss, persistent richness reduction, and strong compositional shift toward tolerant cladocerans (*Alona*, *Chydorus*), with recovery driven by cladoceran blooms (Hébert *et al.* 2021). Leaf-litter decomposition declined in high-agriculture German streams due to sharp drops in specialist detritivores (Lepidostomatidae) and replacement by tolerant generalists (Chironomidae, and Simuliidae), with total decomposition reduced and ash content elevated (Cornejo *et al.* 2020). German small water bodies showed $TU_{max} > 2$ for algae/plants in 38% of samples, the highest trophic-level risk (Lorenz *et al.* 2025).

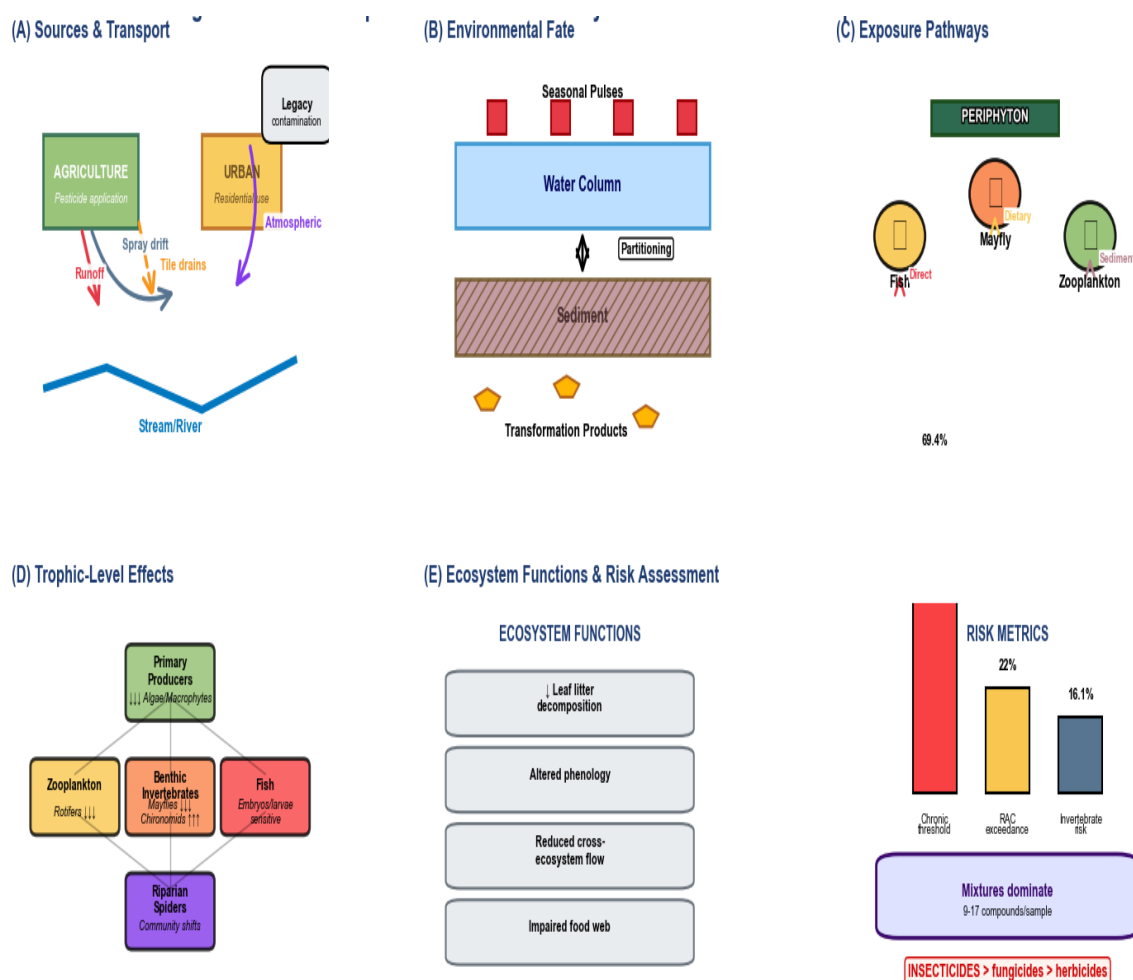


Fig. 1. Pesticide Impacts on Freshwater Ecosystems: A Multi-Level Conceptual Framework: (A) Pesticides enter aquatic systems through multiple pathways including runoff, tile drains, spray drift, and atmospheric transport from agricultural and urban sources. (B) Environmental fate includes partitioning between water column and sediment, seasonal pulses, and formation of transformation products. (C) Aquatic organisms are exposed via direct contact with water, dietary uptake through periphyton, and sediment ingestion. (D) Effects cascade through trophic levels, with primary producers and benthic invertebrates being most sensitive, while some tolerant taxa (e.g., Chironomids) increase in abundance. (E) Ecosystem functions including leaf litter decomposition, phenology, cross-ecosystem flows, and food web support are disrupted. (F) Risk metrics show 69.4% of sites exceed chronic thresholds, 22% exceed RAC (Regulatory Acceptable Concentration), and 16.1% of events show high invertebrate risk. Mixtures dominate environmental samples.

Mixture toxicity, multi-stressor interactions and regional differences

Mixtures and co-stressors produce non-additive effects that vary regionally. Despite 88% of USA samples containing ≥ 5 pesticides, toxicity was driven by 1–2 key insecticides (MCR 1–2) and 17 dominant compounds (Covert *et al.* 2020). Neonicotinoid + fungicide exposure reduced insect abundance/richness in wetlands, with sediment NI-EQ as the main chronic driver (Kuechle *et al.* 2022). Imidacloprid + sediment + nutrient mesocosms showed antagonistic interactions on zooplankton richness (2–2.4 \times higher than additive expected) and community shifts (Chará-Serna *et al.* 2019). Kenya rivers exhibited chronic TU exceedances for crustaceans in 96% of samples from mixed pesticides + pharmaceuticals (Tanui *et al.* 2024). Tropical mesocosms indicated higher NOEC

(100 ng L⁻¹) for lambda-cyhalothrin than temperate SSDs, yet sensitive taxa (*Dero digitata*) still declined (Yao *et al.* 2024). Amazon urban streams showed high invertebrate risk (HI >10 in 6/12 samples) from European-banned compounds (Rico *et al.* 2022). Riparian spider richness and abundance also declined significantly by summed toxic units ($D^2 = 0.396\text{--}0.481$; Graf *et al.* 2019; Fig. 1).

DISCUSSION

A synthesis of evidence indicates that agricultural pesticides consistently exert multi-faceted and multi-trophic pressures on aquatic ecosystems, surpassing predictions from traditional risk assessments focused solely on the water column. High detection frequencies (88–100%) and frequent exceedances of chronic thresholds (e.g., PTI > 0.1 in 69.4% of USA sites; RAC exceedances in 70% of protected German streams) demonstrate chronic and widespread exposure (Covert *et al.* 2020; Schweiger *et al.* 2025). While single insecticides often cause the majority of acute toxicity (MCR 1–2 in > 92% of samples), herbicides and fungicides significantly contribute to chronic effects on primary producers and ecosystem processes (Hashimoto *et al.* 2019; Covert *et al.* 2020; Lorenz *et al.* 2025). This pattern is consistent across continents, from North American Great Lakes tributaries, where 96% of samples contained a median of 16 compounds year-round (Oliver *et al.* 2023), to Ethiopian Lake Ziway, with RQ >100 for organophosphates during wet-season runoff (Merga *et al.* 2021), and Brazilian Amazon urban streams, showing HI >10 in half the samples (Rico *et al.* 2022). A key insight is the critical role of non-aqueous matrices in environmental contamination. The strong sorption of glyphosate and AMPA to periphyton—detected at 50% and 30% in agricultural drains—creates a direct dietary exposure route for mayflies, leading to significantly reduced survival rates ($p < 0.01$), even though there is no change in biomass (Ijzerman *et al.* 2023). Similarly, the accumulation of neonicotinoids and fipronil metabolites in sediment maintains chronic exposure long after concentrations in the water column have peaked (Furihata *et al.* 2019; Pérez *et al.* 2021; Kuechle *et al.* 2022). In wetlands treated with thiamethoxam-coated seeds, sediment negative ecological quality (NI-EQ) remained the primary driver of reduced insect abundance and richness, even when water concentrations stayed below acute EPA benchmarks (Kuechle *et al.* 2022). These findings indicate that regulatory frameworks based solely on dissolved-phase concentrations systematically underestimate the risks to benthic organisms and those that graze on periphyton. Community-level responses show remarkable consistency. Agricultural streams exhibit doubled total insect biomass but a near-complete loss of sensitive stoneflies and mayflies. The ANOSIM index ($R = 0.31$) indicates a clear compositional shift toward more tolerant taxa (Ohler *et al.* 2023). The toxic equivalent (TEQ) of neonicotinoids in Prairie Pothole wetlands has shifted emerging insects toward a dominance of Chironominae while suppressing populations of Psychodidae and Syrphidae (Cavallaro *et al.* 2019). Body burdens in *Gammarus pulex* (median TU_{maxInt} 0.4) directly correlate with SPEAR_{pesticides} indices of 7–32 (indicating bad/moderate ecological status) when comparing agricultural to non-agricultural streams (Shahid *et al.* 2018). Riparian spider communities have also shown strong responses, with both richness and abundance significantly declining in relation to summed toxic units ($D^2 = 0.396\text{--}0.481$; Graf *et al.* 2019). These shifts impair leaf-litter decomposition (Cornejo *et al.* 2020) and reduce the emergence biomass available to riparian predators, demonstrating cascading effects within food webs. Concerns about developmental and sub-lethal toxicity in vertebrates are growing. Laboratory studies have shown that exposure to prometryn and cyhalofop-butyl can cause serious effects in zebrafish, including yolk-sac edema, spinal deformities, hatching delays, and immunotoxicity through JAK-STAT activation, along with oxidative stress and apoptosis, even at environmentally relevant concentrations ($\mu\text{g L}^{-1}$) (Cheng *et al.* 2021; Zhang *et al.* 2022). Fish from pesticide-impacted sites in Costa Rica exhibited elevated biomarker responses, such as ChE, GST, LPO, and CAT, as well as increased indices of biological response (IBR) (Echeverría-Sáenz *et al.* 2018). These individual effects, coupled with habitat degradation, can lead to declines in fish populations. The situation is further complicated by mixture and multi-stressor interactions. While combinations of substances are common, toxicity often arises from just a few primary drivers (Covert *et al.* 2020). Non-additive effects can frequently occur. For instance, imidacloprid acted antagonistically with sediment and nutrients, resulting in 2 to 2.4 times greater zooplankton richness than what would be expected from additive toxicity (Chará-Serna *et al.* 2019). Additionally, pulses of thiacloprid combined with fertilizers altered microbial and phytoplankton chironomid communities in ways that deviated from predictions based on single-stressor studies (Beentjes *et al.* 2022). Mesocosm studies that included glyphosate consistently demonstrated lasting reductions in zooplankton richness, although temporary biomass recovery in phytoplankton and cladocerans did occur (Hébert *et al.* 2021). These interactions highlight that agricultural runoff

in the real world usually involves multiple stressors, rather than just one. While regional differences are evident, they do not diminish the overall risks. Tropical mesocosm studies indicated a higher no-observed-effect concentration (NOEC) of 100 ng L⁻¹ for lambda-cyhalothrin compared to temperate species sensitivity distributions; however, sensitive taxa like *Dero digitata* still experienced declines (Yao *et al.* 2024). In contrast, studies from developing regions revealed higher acute risks associated with organophosphates and older chemicals that are still in use (Merga *et al.* 2021; Tanui *et al.* 2024). Furthermore, legacy compounds continue to cause exceedances in protected areas across Europe due to atmospheric transport (Schwei).

Limitations

Several limitations exist in current research. Studies are often short-term or focus on narrow pesticide categories, limiting understanding of long-term trends. Analytical inconsistencies impede concentration comparisons across studies. Temperate regions are overrepresented, while data from intensely agricultural tropical and African systems are lacking. Few studies integrate multiple trophic levels or combine various residue analyses with community structure analysis. Finally, field studies on pesticide effects on higher-order endpoints, such as fish population declines or riparian bird reproductive success, remain correlational rather than causal.

CONCLUSION

Agricultural pesticides increasingly threaten freshwater ecosystems by contaminating water sources, with sediment and periphyton acting as pesticide reservoirs. This contamination leads to invertebrate die-offs, disrupted decomposition cycles, and compromised fish health worldwide. Sediment and periphyton reservoirs create legacy and persistence pathways, exacerbated by multi-stressor interactions and non-additive mixtures. To protect biodiversity and ecosystem services, we must implement three key actions: enhance monitoring programs to assess pesticide levels in organisms, periphyton, and sediment; improve risk assessments using chronic dietary exposure scenarios and tropical distributions with mixture toxicity; and enact laws to create buffer zones that account for real-world pesticide use. Future research should focus on long-term studies linking exposure to population-level outcomes, developing integrated multi-matrix risk models, and evaluating nature-based solutions like restored riparian wetlands. Only through these integrated approaches can we reverse the degradation of aquatic ecosystems and secure their essential services.

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Bibliographic information of this paper for citing:

Al-Maaitah, MI, Kuldasheva, S, Abduganiev, B, Shakhmurova, G, Basheer, NM, Abdul, DJ, Singh, R, Sharma, V 2026, Toxicological effects of agricultural pesticides on aquatic ecosystems: A review. *Caspian Journal of Environmental Sciences*, 24: 583-593.
