Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Deriving and Transferring the SPEARpesticide Bioindicator to a New Region: an Ecotoxicity-Based, Indigenous-Fauna Sensitivity Index Field-Validated in the Seomjin River, South Korea

Jong-Hyeon Lee

j.lee@ehrnc.com

EH Research & Consulting Co., Ltd.

Jang-Yeon Park

EH Research & Consulting Co., Ltd.

Su-Hyun Kim

EH Research & Consulting Co., Ltd.

Seung-Been Heo

EH Research & Consulting Co., Ltd.

Research Article

Keywords: pesticide, agricultural streams, trait-based bioindicator, macroinvertebrates, SPEAR, biomonitoring, effect-based assessment

Posted Date: October 23rd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10749043/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Abstract

Trait-based bioindicators such as SPEAR$_{pesticide}$ promise stressor-specific diagnosis of pesticide impacts, but field validation outside their region of origin is limited, and intermittent, diffuse residues make exposure hard to quantify. We derived an ecotoxicity-based, indigenous-fauna sensitivity index for the family-level SPEAR$_{pesticide}$ bioindicator — augmenting the published sensitivity basis with new acute-toxicity tests on 20 indigenous Korean species — and field-validated it at 19 agricultural stream sites in the Seomjin River watershed (South Korea) against two exposure anchors: residue-based summed toxic units (ΣTU) and a whole-organism, effect-based ex situ median lethal time (LT50) of sediment organic extracts. The index correlated with the effect-based endpoint (r = 0.53), whereas the raw index–ΣTU association was masked by a co-varying organic-enrichment gradient; controlling for water quality and for the national organic-pollution index (BMI) unmasked a strong, correction-robust association (partial r up to −0.75) that survived multiple-comparison correction and separated the index from BMI. Generic richness and diversity metrics showed no such response. Incorporating indigenous-species toxicity data was decisive in bringing the effect-based signal to significance. The result is a transferable, confounder-controlled workflow that converts routine macroinvertebrate monitoring into pesticide-specific diagnosis, applicable to other regions and to other intermittently applied contaminants.

1. Introduction

Pesticide contamination is a pervasive stressor of agricultural stream ecosystems, altering macroinvertebrate community structure and impairing ecological function (Schäfer et al. 2012); at regional and continental scales it has been linked to marked declines in freshwater invertebrate biodiversity (Beketov et al. 2013; Malaj et al. 2014; Stehle and Schulz 2015). Diagnosing such impacts is challenging because ecological risk assessment integrates prospective chemical assessment, ecotoxicological monitoring and field bioassessment, whose combination provides the multiple lines of evidence needed to attribute observed impairment to specific causes. Traditional indices based on richness, diversity or aggregate tolerance scores document the presence and severity of impairment, but they assume that taxa contribute to community structure largely independently of their trait-specific sensitivities, which can obscure the signature of a particular stressor.

Trait-based bioindicators address this limitation. The SPEcies-At-Risk (SPEAR) framework classifies taxa as “at risk” from biological traits — physiological sensitivity, generation time, exposure-related habitat, and migration ability — and weights community composition accordingly, targeting the taxa most vulnerable to a defined stressor (Liess and von der Ohe 2005). Its pesticide-specific variant, SPEAR $_{pesticide}$, was established in European agricultural streams, progressively refined, and calibrated for faunas in Australia, North America, South America and East Africa, where it discriminates pesticide effects from confounding environmental gradients (Liess and von der Ohe 2005; Schäfer et al. 2007, 2011; Liess et al. 2008; Beketov et al. 2009; Chiu et al. 2016; Hunt et al. 2017; Knillmann et al. 2018; Ganatra et al. 2021); the same logic underlies SPEAR variants for other stressors (Beketov and Liess 2008; Malaj et al. 2012; von der Ohe and Goedkoop 2013; Liess et al. 2017; Wood et al. 2019). The Benthic Macroinvertebrate Index (BMI), by contrast, scores tolerance to organic enrichment and low dissolved oxygen and serves as Korea’s national index of biological water quality, responding to organic pollution rather than to specific toxicants (Kong et al. 2018).

Despite the promise of trait-based indices, their stressor-specific application to field datasets remains limited, and several obstacles are particular to pesticides. Pesticides are applied intermittently, degrade rapidly and enter streams from spatially diffuse sources distributed across the catchment, so residue concentrations measured at a single time point are weak surrogates for the toxic exposure communities actually experience. Region-specific sensitivity data are also scarce, and indices calibrated on non-native species may misrepresent the sensitivities of local fauna. In agricultural catchments pesticide signals co-occur with — and are often dominated by — nutrient, sediment and hydromorphological pressures, which confound stressor-specific interpretation (Bighiu et al. 2020; Bray et al. 2021); and where the index has been transferred to non-temperate faunas without local calibration, it has sometimes failed to register pesticide effects that locally adapted indices detected (Cornejo et al. 2019). Consequently, an ecotoxicity-based SPEAR $_{pesticide}$ sensitivity index has yet to be derived and field-validated for the Korean fauna, nor has it been examined whether such an index, applied to national monitoring data, provides stressor-specific information beyond generic indices.

A further consideration concerns the data the framework demands. Its at-risk classification rests on two distinct taxon properties: physiological sensitivity, quantifiable from standardized acute toxicity tests, and ecological life-history traits — generation time, exposure-related habitat, and migration ability — that toxicity testing does not measure and that must be compiled from autecological knowledge. For much of Korea's stream fauna the latter are unavailable even at family level, whereas physiological sensitivity can be strengthened directly by testing indigenous taxa. We therefore base the index on an ecotoxicity-only sensitivity measure, computed from acute toxicity data at both species and family resolution, rather than on the original multi-trait classification; family-level resolution, at which monitoring and toxicity records are most complete, retains the pesticide signal (Beketov et al. 2009). Deriving a sensitivity-index distribution for the indigenous fauna is an unavoidable prerequisite for any regional application of SPEAR $_{pesticide}$, so the procedure adopted here — compiling available acute toxicity records, augmenting them with targeted tests on indigenous species, and aggregating to species and family level — is framed as a transferable method, with Korea as the case study in which it is derived and field-validated.

The present study addresses these gaps in the Seomjin River watershed, a diffuse-source agricultural system. We (i) develop an ecotoxicity-based sensitivity index for SPEAR $_{pesticide}$ at species and family resolution — adopting an aquatic-insect, family-level formulation as the primary index — by augmenting the published sensitivity basis with newly generated acute toxicity data for 20 indigenous Korean species, and recalculate the index nationally; (ii) validate it against two exposure anchors, residue-based summed toxic units (Σ TU) and the effect-based *ex situ* LT50 of sediment organic extracts, the latter included because intermittent, degradable residues are poorly captured by single-time-point concentrations; and (iii) test stressor-specificity relative to generic indices and to BMI using partial correlation that removes co-varying organic-enrichment confounders, multivariate ordination and indicator concordance, with multiple-comparison control throughout. The study thereby complements concentration-anchored validations in point-source systems (Hwang et al. 2024; Chung et al. 2024) and offers a transferable method for deriving indigenous-fauna SPEAR $_{pesticide}$ and advancing national assessment from status documentation toward stressor attribution.

2. Materials and methods

2.1. Study area and site selection

The study area is the Seomjin River watershed in southwestern South Korea (Fig. 1). Nineteen monitoring sites were selected from the national aquatic ecosystem health assessment database by comparing the SPEAR $_{pesticide}$ index of Liess and von der Ohe (2005) with BMI to identify sites of suspected pesticide impact: sites with BMI > 50 but SPEAR < 0.3 were treated as potentially pesticide-impacted and were complemented by control sites, yielding 19 stations (national context and overall study design in Fig. S1). The watershed drains through a dendritic network of nested sub-basins, and the 19 sites are shown with their codes on the detailed drainage map (Fig. 1), and the stations span this network from northern headwater tributaries (e.g., SDC, ISC) down to the southern outlet near the river mouth (JG), capturing the full upstream–downstream gradient rather than a single reach.

2.2. Biological monitoring

Benthic macroinvertebrate data were obtained from the Korean National Aquatic Ecosystem Monitoring Program (NAEMP). Samples were collected by standard Surber-net methods following the national protocol (Kong et al. 2018); organisms were identified to the lowest practicable level (mostly species or genus) and enumerated. The national dataset comprised 347,559 records across approximately 3,034 sites, with 718 unique species.

2.3. Residual pesticide analysis

Sediments were collected at the 19 sites on 18–21 August 2024 and stored at − 20°C until analysis. Residual pesticides were extracted by a modified QuEChERS method (Anastassiades et al. 2003) and quantified by GC–MS/MS and LC–MS/MS; target analytes comprised nine fungicides, six insecticides and two herbicides (extraction and clean-up detail in Supplementary Methods; per-site results in Table S1).

2.4. Sediment organic-extract ecotoxicity test

Acute toxicity was assessed on the **sediment organic extracts** obtained by the acetonitrile extraction of Section 2.3 — the organic-solvent-extractable fraction that concentrates the residual pesticides, rather than whole sediment, pore water or aqueous elutriate. Extracts were concentrated 30-fold or 60-fold (a correction factor of 2 was applied to the LT50 values of the 30-fold samples) and dosed directly into the aqueous test medium to a final solvent concentration of 10% (v/v). Tests used *Moina macrocopa* (< 24 h post-hatch) following OECD Test Guideline 202 (OECD 2004), with LT50 (median lethal time) as the endpoint; a solvent-carrier control produced no mortality, confirming that the observed lethality reflected the extracted toxic burden rather than the carrier solvent (full test conditions in Supplementary Methods).

2.5. Pesticide exposure: toxic units

Chemical pesticide exposure at each site was expressed in toxic units (TU), which rescale a compound's concentration by its toxicity so that chemicals of differing potency can be combined on a common, additive scale (Schäfer et al. 2012). For each detected compound, C_i is its concentration in the *ex situ* test medium (mg L⁻¹), derived from the measured sediment residue together with the extraction and dosing protocol of Section 2.4, and $LC50_i$ is its acute median lethal concentration, so that $\mathrm{TU}_i = C_i / LC50_i$ is dimensionless. Assuming concentration addition across the detected mixture, site-level exposure is the summed toxic unit (Eq. 4),

$$\Sigma\,\mathrm{TU} = \sum_i \frac{C_i}{LC50_i}$$

where higher Σ TU denotes greater combined acute chemical pressure; sites with no detected residues fall below the analytical floor.

2.6. Pesticide sensitivity index

The original SPEAR $_{pesticide}$ index of Liess and von der Ohe (2005) classifies taxa by combining a physiological sensitivity to organic toxicants — a relative-sensitivity ranking against a standard test species derived from acute toxicity data (von der Ohe and Liess 2004) — with life-history traits. Independently of that trait database, we took the physiological-sensitivity component from the published SPEAR calculator (Helmholtz Centre for Environmental Research (UFZ), 2023), whose per-taxon values derive from a compilation of more than 15,000 acute toxicity records for more than 800 compounds (Wogram and Liess 2001; von der Ohe and Liess 2004), and regionalised it for the Korean fauna with newly generated acute toxicity tests (48 h LC50/EC50) on 20 indigenous species (Table S3) through the same relative-sensitivity procedure. Records were normalized, standardized per compound and aggregated by a linear mixed-effects model; family-level values were the arithmetic mean of member species, with hierarchical taxonomic extrapolation for families lacking records (Supplementary Methods). The "at-risk" threshold was the median of family-level values confirmed in the monitoring database, and the resulting family coverage is reported in Section 3.1. All values derive exclusively from acute lethal/immobilization endpoints (LC50/EC50), with no acute-to-chronic ratio applied, so the index characterises *relative acute* physiological sensitivity — a transparent simplification dictated by the scarcity of chronic toxicity data (Section 4.5). These values are the sole basis of the at-risk classification used here (Section 2.8): unlike the original SPEAR formulation, the index does not additionally filter taxa by life-history traits (generation time, exposure-related habitat, migration ability), for which reliable regional records are unavailable. Physiological sensitivity is therefore the single diagnostic trait, assigned a priori from toxicity data independently of the field observations.

2.7. Community indices

Taxon richness (TR), Ephemeroptera–Plecoptera–Trichoptera richness (EPT), Shannon diversity ($H\prime$) and BMI were computed for each site. TR and EPT richness are generic, non-stressor-specific descriptors of community condition that decline under general impairment without isolating a particular stressor. BMI (NAEMP protocol, Kong et al. 2018) weights taxa by field-derived organic-pollution tolerances (saprobic values; Sládeček 1973); the defining equations for $H\prime$ and BMI are given in Supplementary Methods.

2.8. SPEAR $\varvec{}_{\varvec{p}\varvec{e}\varvec{s}\varvec{t}\varvec{i}\varvec{c}\varvec{i}\varvec{d}\varvec{e}}$ index

In the original framework, taxa are classified as “at risk” only if they meet all four SPEAR traits — physiological sensitivity above threshold, generation time above threshold, epibenthic habitat, and no documented rapid migration (Liess and von der Ohe 2005; Knillmann et al. 2018) — a definition requiring species-level life-history information unavailable for most of the regional fauna. Here the at-risk classification rested solely on the ecotoxicity-only sensitivity index (Section 2.6): a taxon was classified “at risk” when its sensitivity exceeded the threshold, applied identically at species and family resolution, dispensing with the life-history filters so that the classification could be assigned from toxicity data alone. As the primary index we compute SPEAR $_{pesticide}$ over the aquatic-insect assemblage, aggregated at family level for compatibility with national family-level monitoring (Beketov et al. 2009); species-level and whole-assemblage variants are retained for robustness comparison (Section 3.1). The index (Eq. 3) was:

$$\mathrm{SPEAR}_{\mathrm{pesticide}} = \frac{\sum_i \log\left(x_i + 1\right)\, y_i}{\sum_i \log\left(x_i + 1\right)}$$

where x_i is the abundance of taxon i and $y_i = 1$ if at risk, else 0; lower values indicate greater pesticide stress.

2.9. Statistical analysis

The analyses were structured to test pesticide-specificity through convergent lines of evidence rather than a single contrast: an exposure–response between the index and a residue-independent, effect-based toxicity measure, its persistence under organic-matter control, and the index’s separation along the toxic dimension in ordination.

Pearson and Spearman correlations related the two exposure anchors — the effect-based LT50 and the residue-based log(Σ TU), with log(TU $_{max}$) as a single-compound robustness metric — to the indices. Because pesticide pressure in agricultural catchments co-varies with organic enrichment, partial correlations tested the independence of the index–exposure association: rather than entering the six correlated water-quality variables (DO, BOD, COD, TN, TP, TOC) individually, we summarized them by principal component analysis and controlled for the first component (WQ-PC1) and the first two (WQ-PC1 + PC2), conserving degrees of freedom (raw-variable and WQ-purged-residual controls gave concordant results). To test whether SPEAR $_{pesticide}$ carried exposure information beyond the signal it shared with the national organic-pollution index, the associations were also recomputed with BMI partialled out (specificity control), and with WQ-PC1 and BMI together. The stability of all estimates was assessed by bootstrap resampling of sites (3,000 resamples, 95% confidence intervals). Because the index was tested against three exposure metrics (LT50, Σ TU, TU $_{max}$) under several confounder-control sets, the resulting eleven-test battery was corrected for multiple comparisons by the Benjamini–Hochberg false-discovery-rate procedure (Benjamini and Hochberg 1995) and reported as survival at q < 0.05; because the eleven statistics share the index and use nested covariate sets they are positively dependent, a condition under which the procedure validly controls the false-discovery rate (Benjamini and Yekutieli 2001).

Principal component analysis (PCA) on standardized indices and exposure variables used the broken-stick criterion for axis retention (Jackson 1993). No-mortality LT50 values were treated as right-censored (= 100 h). Analyses used Python 3.13 (pandas, scikit-learn, scipy, matplotlib).

3. Results

3.1. Refinement of pesticide sensitivity values and recalculation of the index

We adopted an aquatic-insect, family-level SPEAR $_{pesticide}$ formulation as the primary index on two grounds: its at-risk threshold is invariant to taxonomic aggregation, and it gave the strongest, most correction-robust association with residue-based exposure once organic-enrichment confounders were removed. Aquatic insects were markedly more sensitive than non-insect taxa (median − 0.35 vs − 1.82; Mann–Whitney $p \approx 2\times10^{-13}$), and fixing the sensitivity unit at the family level held the aquatic-insect at-risk threshold invariant across aggregation levels (− 0.35 at species, genus and family) while the all-taxa threshold swung by ~ 0.38 (Fig. S2); the exposure-specific advantage is established by the confounder-controlled analyses of Sections 3.3 and 4.3. Augmenting the published sensitivity basis with 20 indigenous species increased family coverage from 126 (75.4%) to 132 (79.0%) of 167 families and shifted the family sensitivity median downward (Fig. S3); because the added native taxa are generally

more sensitive, additional families crossed the at-risk threshold, lowering the national SPEAR $_{pesticide}$ median from 0.366 to 0.252 (Fig. S4) and yielding a more precautionary baseline against which impairment is scored.

At the 19 study sites both database versions matched the same 77 of the 87 families present (88.5%); incorporating the native species therefore did not extend field-level coverage but refined the sensitivity values of 11 families — all toward greater sensitivity, including Baetidae, Ephemeridae, Heptageniidae, Hydropsychidae, Gammaridae and Chironomidae (the off-diagonal families in Fig. S3; per-family values in Table S2). The remaining 66 families were identical between versions, so the field index was structurally robust to the update, yet the refined insect families are what sharpened the aquatic-insect variant's effect-based signal (Section 3.3).

3.2. Residual pesticides and *ex situ* toxicity across the watershed

Residues were at or below the analytical floor at several sites, with Σ TU exceeding the floor at 13 of 19 sites; detected compounds spanned fungicides (azoxystrobin most frequent, 8 sites), insecticides and herbicides, with fungicides predominating (Table S1). Summed toxic units were low and spatially patchy, and the *ex situ* LT50 of the sediment organic extracts ranged from 16.4 to > 100 h, with two no-mortality sites right-censored at 100 h.

The predominance of fungicides over insecticides is consistent with the cropping pattern of the watershed and with the tendency of a single sediment grab to capture moderately persistent compounds while missing short-lived insecticide pulses. Consistent with this decoupling, *ex situ* toxicity was measurable at three of the five residue-free sites, whereas the two no-mortality sites were themselves residue-free, illustrating the mismatch between instantaneous residue concentration and the integrated toxic burden retained in the sediment.

A site-level cross-classification of the three diagnostic layers made this decoupling explicit and defined the site classification carried through the rest of the analysis (Fig. 2; markers are coloured by each site's final impact class). Against effect-based toxicity, residue exposure was a poor screen (panel a): of the ten acutely toxic sites (LT50 < 48 h), six fell below Σ TU = 1, whereas the highest-exposure site (HD; Σ TU = 72) showed no acute toxicity; the same decoupling holds when exposure is quantified as TU $_{max}$. Biological impairment was decoupled from residues in the same way (panel b): only five of the fifteen sites with SPEAR $_{pesticide}$ < 0.30 exceeded Σ TU = 1, while the four reference sites (GCC, HGC, NSC, YDC) clustered at high SPEAR and low exposure (Σ TU $\leq$ 0.011). The two condition measures were themselves positively associated (Fig. S5), concordantly impacted sites falling in the lower-left quadrant and concordant reference sites in the upper-right. Colouring the same panels by BMI value (Fig. S6) shows that benthic condition co-varies with SPEAR $_{pesticide}$ across both the exposure and the toxicity axes, visualising the shared general-condition component that the confounder control isolates (Section 4.3). Under a SPEAR-anchored weight-of-evidence rule (SPEAR < 0.30 with at least one corroborating stressor line for impaired, and its clean mirror for reference; alternative rules are compared in Fig. S7), 12 sites were classified as impacted, four as reference and three as intermediate — the classification carried forward to the concordance and spatial analyses (Section 3.4); the same cross-classification computed with the generic community metrics in place of SPEAR $_{pesticide}$ is given in Fig. S8. Because Σ TU was computed only from quantified target analytes, acute toxicity at low- Σ TU sites implies that unquantified compounds, transformation products or non-additive mixture effects contributed — a burden the whole-extract LT50 integrates but a fixed residue panel cannot.

3.3. Index–exposure associations and their robustness to confounders

The index was validated against both exposure anchors, and the decisive pattern was that removing the co-varying organic-enrichment gradient strengthened rather than weakened the index–exposure signal (Fig. 3; community-index scatterplots in Fig. S9).

Effect-based anchor (LT50). The primary index correlated with the *ex situ* LT50 at a modest but significant level (r = 0.53, p = 0.03; Fig. S5), as did BMI (r = 0.55, p = 0.02); the generic descriptors did not (all p > 0.05; Table 1). Controlling for water quality strengthened the index–LT50 association (partial r = 0.57–0.64, n = 13), confirming it was not an artifact of organic enrichment, but on its own it did not survive multiple-comparison correction (Benjamini–Hochberg q $\approx$ 0.06).

Residue-based anchor (Σ TU). The raw index– Σ TU correlation was weak (r = − 0.40, p = 0.09), reflecting suppression by the shared organic-enrichment gradient rather than a true absence of relationship. Controlling for water quality unmasked a strong

association (partial r = − 0.64, q = 0.03), controlling for BMI did likewise (partial r = − 0.57, q = 0.03), and removing both confounders strengthened it further (partial r = − 0.75, q = 0.01; full values and FDR results in Table 1, underlying partial correlations in Table S5). The single-compound metric log(TU $_{max}$) behaved identically (Table 1). Of the eleven-test battery, five associations — all Σ TU- or TU $_{max}$-based and all confound-controlled — survived FDR correction, whereas none of the raw or LT50-based tests did.

Specificity relative to BMI. Because pesticide and organic-enrichment pressures co-occur, SPEAR $_{pesticide}$ and BMI shared much of their exposure-related variance; the critical test is whether the index retains exposure information once BMI is held constant. It did for the residue anchor (Table 1), indicating a pesticide-related signal not attributable to the national organic-pollution index, but not for the effect-based anchor (SPEAR–LT50 | BMI r = 0.24, p = 0.35), consistent with the two indices tracking the *ex situ* toxicity of the extract in largely the same way. The pesticide-specificity of the index is therefore carried by the confound-controlled, correction-robust residue association together with convergent effect-based and multivariate evidence (Section 4.3; Fig. S10), rather than by any single zero-order contrast.

Robustness across index variants. The effect-based association was stable across index variants distinguished by taxonomic resolution and by sensitivity-database version: the updated variants correlated significantly with the *ex situ* LT50 at both species and family resolution (r = 0.53, p = 0.03), and the existing-database variants were only marginally weaker (r = 0.44, p = 0.08). A consolidated comparison of every variant and the generic metrics against the three endpoints (Fig. S11) shows that all SPEAR $_{pesticide}$ variants track the toxicity endpoints in the expected direction — significant only for the updated variants — whereas generic richness and diversity do not. This resolution-invariance indicates that the validated response is a property of trait-based community structure rather than of aggregation level, whereas the sensitivity database mattered mainly for the aquatic-insect index, whose effect-based signal reached significance only after refinement (Section 4.2). Table 1. Associations between the primary SPEAR $_{pesticide}$ index (aquatic-insect, family level) and the two exposure anchors, and generic-index responses to LT50. Zero-order Pearson *r*; partial correlations control for water quality summarized by principal components (WQ-PC) and/or BMI; *q* = Benjamini–Hochberg FDR across the eleven-test battery. Bold indicates FDR-surviving (*q* < 0.05); "—" not assessed.

Table 1
Associations between the primary SPEAR_pesticide index (aquatic-insect, family level) and the two exposure anchors, and generic-index responses to LT50. Zero-order Pearson r; partial correlations control for water quality summarized by principal component.

Association	**Zero-order *r***	**| WQ-PC**	**| BMI**	**| WQ-PC + BMI**	**FDR**
SPEAR – ΣTU	−0.40	**−0.64**	**−0.57**	**−0.75**	✓ (survives)
SPEAR – TU$_{max}$	−0.43	**−0.64**	−0.58	**−0.75**	✓ (survives)
SPEAR – LT50	0.53	0.64	0.24	0.30	— (n.s. after FDR)
BMI – LT50	0.55	0.52	—	—	—
EPT – LT50	−0.03	—	—	—	—
Taxon richness – LT50	−0.20	—	—	—	—
Shannon *H'* – LT50	0.11	—	—	—	—

Zero-order n* = 19 (ΣTU, TU$_{max}$) or 17 (LT50; two no-mortality sites right-censored and excluded); WQ-controlled *n* = 14 (ΣTU) or 13 (LT50). Species-level and existing-database variants gave concordant results.*

3.4. Concordance among diagnostic layers and threshold classification

The three diagnostic layers — the biological index (SPEAR $_{pesticide}$), the effect-based toxicity measure (*ex situ* LT50), and the chemical exposure measure (Σ TU) — were each used to rank the 19 sites from least to most affected, and the rankings were compared by Kendall's coefficient of concordance (W; 0 = no agreement among rankings, 1 = perfect agreement). Agreement was moderate (W = 0.56; χ^2 = 30.1, df = 18, p = 0.04) and was carried by all three layers (the site-level ranks are shown as a per-layer

concordance heatmap in Fig. S12; underlying data in Table S4). The impact classification defined above (decision rule D: SPEAR < 0.30 with at least one corroborating stressor line — LT50 < 48 h or ΣTU above the median — for impaired, and its clean mirror for reference; § 3.2, Fig. S7) — 12 impacted, four reference (GCC, HGC, NSC, YDC), and three intermediate sites — is mapped across the watershed in Fig. 4, its spatial pattern examined in Section 4.4.

4. Discussion

4.1. Effect-based versus concentration-based validation

In point-source metal systems, persistent chemical gradients give a strong concentration axis for bioindicator validation (Hwang et al. 2024; Chung et al. 2024). Pesticides differ — intermittent, rapidly degraded, and diffuse — and here residues were at or below the analytical floor at several sites and patchy elsewhere, so the *raw* index–log(Σ TU) correlation was weak despite the expected sign. Two features show this weakness to be largely a masking effect rather than a true absence of relationship: once the co-varying organic-enrichment gradient is removed, a strong, correction-robust index– Σ TU association emerges (Section 3.3); and the residue–effect decoupling was bidirectional (Fig. 2; Section 3.2), with acutely toxic sites carrying negligible residues and the most residue-laden site showing no acute toxicity. A single grab captures the moderately persistent fungicides that accumulate in sediment but misses the short-lived insecticide pulses driving acute toxicity (Ganatra et al. 2021), whereas SPEAR $_{pesticide}$ integrates months of community response, a temporal scale no instantaneous concentration matches (Liess et al. 2008). The *ex situ* LT50 of the sediment organic extract sidesteps both limitations by quantifying the realised toxic potential of the accumulated extractable burden rather than any single analyte, and yielded a directionally consistent, confound-robust association — as effect-based anchoring has elsewhere (Schäfer et al. 2012; Hunt et al. 2017). Two lessons follow for diffuse, degradable stressors. First, effect-based anchoring is a valuable complement to residue concentration, well suited to other intermittently applied, rapidly degrading organic contaminants — current-use pesticides, biocides, and pharmaceuticals — for which residue monitoring is chronically mismatched to the exposure communities experience. Second, where residue-based exposure is used, controlling for co-varying organic-enrichment confounders is essential: here it was the difference between an apparently null and a strongly significant, correction-robust association, and it is the residue anchor that ultimately provided the FDR-surviving evidence of specificity (Section 3.3).

Two clarifications bound this strategy. The extract LT50 is an acute lethal endpoint, matching the acute basis of the sensitivity index (Section 2.6), so the validation avoids an acute–chronic confound; it is also built independently of Σ TU — the realised toxicity of the integrated extractable burden rather than the summed toxic units of identified analytes — so it is not circular. The association therefore characterises the *acute toxic-pressure* dimension of contamination, and extending it to chronic or sublethal effects requires additional assumptions (Section 4.5).

This anchoring also distinguishes the study within the wider SPEAR $_{pesticide}$ literature, which has almost universally quantified exposure as concentration-derived toxic units — water or sediment residues, or passive samplers — rather than the measured toxicity of the field medium itself (Schäfer et al. 2012; Hunt et al. 2017; Bighiu et al. 2020; Ganatra et al. 2021). Whole-sample and whole-sediment toxicity tests have long correlated with benthic community structure (Chapman 1990; Day et al. 1995), but through generic descriptors rather than a trait-based, stressor-specific index; SPEAR itself has been benchmarked against bioanalytical in vitro assays only recently, and there judged complementary rather than a validation anchor (Rico et al. 2025). To our knowledge this is the first SPEAR $_{pesticide}$ field validation anchored to a whole-organism *ex situ* toxicity test of the field-collected medium, supplying evidence largely independent of the toxicity database from which the index is built.

4.2. Robustness of the field signal to the sensitivity database

The field association was insensitive to taxonomic resolution but, for the aquatic-insect primary index, reached significance only with the indigenous-species refinement (Section 3.3). The update altered only a few — but abundant — families (Baetidae, Heptageniidae, Hydropsychidae and Chironomidae among the eleven adjusted), all toward greater sensitivity; reclassifying such shared, high-abundance taxa shifts SPEAR scores in the same conservative direction across sites, improving protective classification and sharpening the among-site contrast that drives the effect-based correlation. The refinement's value thus lies in coverage, region-specific data and conservative classification, and it was against the residue anchor, not the effect-based one, that the aquatic-insect formulation showed its diagnostic advantage (Sections 3.1, 3.3).

The equivalence of species- and family-level resolution is itself instructive. Beketov et al. (2009) found family-level SPEAR $_{pesticide}$ only marginally — and not significantly — less effective than species-level, and our data reproduce that near-equivalence (Section 3.3). This matters because family resolution is what national biomonitoring routinely delivers; that it forfeits no discriminatory power against an integrative exposure measure is what makes the index deployable at scale. Its adequacy rests on within-family trait conservation, and in speciose, physiologically heterogeneous families — Chironomidae the canonical example — within-family variation could blunt the signal (Lenat and Resh 2001), so identifying which families warrant genus- or species-level resolution is a worthwhile refinement target.

Because the validated response is carried by family-level trait structure rather than any particular sensitivity values, the index can be deployed where exhaustive native toxicity data are lacking, provided family coverage is adequate and reported — the logic that let SPEAR $_{pesticide}$ perform well in Australia, South America and East Africa after only minor, locally informed adjustments (Schäfer et al. 2011; Hunt et al. 2017; Ganatra et al. 2021). The refinement remains worthwhile nonetheless: extending family coverage and adding the more sensitive native taxa yields a more conservative, regionally defensible classification and, for the aquatic-insect index, a stronger effect-based correlation. We therefore recommend that applications of trait-based indices report family coverage explicitly, so a score's reliability can be weighed against the completeness of its sensitivity basis.

4.3. Specificity and comparison with community indices

In the companion metal study, the non-response of BMI supported metal-specificity (Hwang et al. 2024); that argument does not transfer here. BMI responded to *ex situ* toxicity at least as strongly as SPEAR $_{pesticide}$ and shared most of its toxicity-related variance — expected in diffuse-source catchments, where pesticides are co-delivered with nutrients and fine sediment in the same runoff events. The decisive question is whether a pesticide-specific signal survives once that shared gradient is removed. For the residue anchor it did, and demonstrably beyond BMI: the index– Σ TU association held after controlling for BMI (partial r = − 0.57) — and more strongly for water quality and for both confounders together (Section 3.3; Fig. 3) — and survived false-discovery-rate correction, evidence that SPEAR $_{pesticide}$ carries pesticide-related exposure information the national organic-pollution index does not. This specificity has a mechanistic root: BMI's saprobic tolerances are calibrated from field co-occurrence with organic-pollution gradients (Sládeček 1973), whereas SPEAR $_{pesticide}$'s physiological sensitivities come from standardized laboratory acute-toxicity tests, fixed a priori and independently of any field distribution. Three consequences follow. Because BMI structurally indexes the organic-enrichment gradient it was calibrated on, the exposure information SPEAR $_{pesticide}$ carries beyond it is a toxicant-specific signal a field-calibrated index cannot isolate; because its sensitivity scale is external to the community data, the field association tested here is a genuine out-of-sample validation rather than a re-description of the calibration gradient; and because a field-derived scale absorbs whatever co-varies with organic pollution — including co-delivered pesticides — controlling for BMI is conservative, if anything understating the pesticide-specific signal. The effect-based LT50, by contrast, was largely shared with BMI (SPEAR–LT50 partialled on BMI, r = 0.24, n.s.), so on that anchor the two indices are not separable at this sample size; the residue anchor is what partitions them. Consistent with this, the index aligned on the toxicity axis in ordination while generic metrics did not, and cross-classifying each generic metric against the two exposure anchors reproduced the same contrast (Fig. S8); that generic descriptors can themselves co-respond is nonetheless documented (Knillmann et al. 2018).

We therefore rest specificity on convergence: a trait-mechanistic basis, a confound-controlled and correction-robust exposure–response that separates the index from BMI on the residue anchor, an independent and directionally consistent effect-based association, and multivariate separation from generic metrics. This both tempers and answers the caution of Bray et al. (2021), who showed that modest sensitivity-misclassification or co-occurring stressors can blur a single SPEAR contrast: here the residue association strengthened and survived multiple-comparison correction precisely as the co-occurring organic-enrichment stressor was removed. The convergence's force lies in the partial independence of the lines' weaknesses, and explaining the signal away would require one confounder acting on all three at once — which organic enrichment, already removed in the partial correlation and opposed to the toxicity axis in ordination, does not. Decisively partitioning the pesticide and organic-enrichment signals will nonetheless require designs that deliberately decouple the two gradients, for instance contrasting catchments with comparable nutrient loads but differing pesticide intensity.

4.4. Convergent diagnosis and the diffuse-source spatial pattern

Diagnostic-layer concordance was moderate (Kendall's W = 0.56; Section 3.4), driven by the biological and toxicity layers with the chemical layer weakest, as expected from patchy residues. Generic richness and diversity did not respond — unlike the metal study, where severe contamination showed even in generic metrics (Hwang et al. 2024) — consistent with pesticide effects being subtler and trait-mediated (Liess and von der Ohe 2005), though limited power cannot be excluded. Treating these layers as complementary lines of evidence rather than competing indicators is the appropriate framework for causal diagnosis in multi-stressor systems.

The spatial arrangement of the classified sites (Fig. 4) is consistent with diffuse rather than point-source pressure. The 12 impacted sites are dispersed across the watershed — mean pairwise separation 44 km (up to 97 km), spanning headwater, mid-watershed and downstream tributaries — rather than clustered below a single location, as expected for watershed-wide agricultural loading through the dendritic network. The four reference sites (GCC, HGC, NSC and YDC) lie instead on short tributaries or headwaters set back from the main channel, draining only small local sub-catchments and integrating neither a large agricultural source area nor the cumulative mainstem load — consistent with the low residue exposure ($\sum$ TU $\leq$ 0.011) observed there.

4.5. Implications, limitations, and future directions

Korea's national biomonitoring programme already generates the family-level macroinvertebrate data SPEAR $_{pesticide}$ requires, yet its indices — BMI foremost — grade overall biological quality rather than attribute impairment to a stressor. A trait-based, stressor-specific index can therefore be layered onto such a programme at essentially no additional sampling cost, converting a status-assessment dataset into a diagnostic one: where BMI flags impairment, a concurrently depressed SPEAR $_{pesticide}$ score tracking an independent toxicity measure implicates pesticide pressure, whereas a normal score redirects attention to organic enrichment or habitat degradation. Realising this will require region-specific reference conditions, periodic updating of the native sensitivity database, and pairing the index with time-integrated or effect-based exposure measures. The same architecture could extend to other SPEAR variants (Beketov and Liess 2008; Schäfer et al. 2011; Malaj et al. 2012), assembling a multi-stressor diagnostic suite from a single biological survey.

Several limitations bound these conclusions. The modest sample (17 sites with measurable toxicity, 13–14 with complete water-quality data) yielded wide bootstrap intervals and rested on a single survey with two right-censored sites; while the residue anchor separated SPEAR $_{pesticide}$ from BMI (Section 4.3), the two indices could not be resolved on the effect-based anchor, so their contributions to the *ex situ* toxicity signal remain partly entangled. Validation was anchored to the toxicity of a concentrated sediment organic extract — a time-integrated surrogate rather than in-situ bioavailable or water-column exposure; because the SPEAR $_{pesticide}$ exposure trait was conceived for water-column pulses, water-column or passive-sampler measures would test the correspondence directly, although sediment-anchored validation has proven informative elsewhere (Hunt et al. 2017). Two further boundaries constrain generalization: truly unimpacted reference reaches are scarce in intensively farmed landscapes, compressing the assessable gradient (Bighiu et al. 2020); and monsoon-driven, episodic high-flow regimes differ from the temperate-European setting in which the index was calibrated, potentially altering both pulse-exposure dynamics and the recovery-related traits that underpin it.

A further limitation concerns the sensitivity index itself, derived exclusively from acute lethal and immobilization data (Section 2.6). Acute data are generally adequate for a dichotomous at-risk classification — relative rankings are more conserved across endpoints than absolute values, and acute records far outnumber chronic ones — but three caveats follow: acute lethality need not track chronic and sublethal sensitivity, and acute-to-chronic ratios vary systematically among compounds and taxa (Roex et al. 2000; Roessink et al. 2013); many records rest on the standard crustacean *Daphnia*, which underrepresents insect-specific modes of action and can misrank the compounds most relevant to macroinvertebrate communities (Maltby et al. 2005); and even acute coverage is incomplete (21% of families required taxonomic extrapolation). The index is therefore best read as a transparent, conservative minimum-viable formulation.

Future work should expand the number and environmental range of sites — especially where pesticide and organic-pollution gradients diverge — add water-column and time-integrated exposure measures, and integrate chronic, sublethal, and mode-of-action-weighted endpoints into a regional trait–sensitivity database. Validated stressor-specific bioindicators provide the diagnostic basis to move national assessment from status documentation toward stressor attribution.

5. Conclusions

This study derived and field-validated an aquatic-insect, family-level SPEAR $_{pesticide}$ index in a diffuse-source agricultural watershed, addressing three obstacles that have limited the field application of trait-based pesticide indicators outside their region of origin. First, validation against two complementary exposure anchors — residue-based summed toxic units and the effect-based *ex situ* LT50 of sediment organic extracts — showed that the key to recovering a pesticide-specific signal from intermittent residues is to remove the co-varying organic-enrichment confounder: the raw index– Σ TU association was weak, but after controlling for water quality and for the national organic-pollution index it became strong and survived multiple-comparison correction, with the LT50 giving directionally consistent corroboration. Second, specificity rests on convergent evidence — the residue association separated the index from BMI under confounder control and the index aligned with the toxicity axis in ordination while generic metrics did not — even though the two indices remained entangled on the effect-based anchor at this sample size. Third, the field signal was robust to taxonomic resolution, while incorporating indigenous-species toxicity data brought the aquatic-insect index's effect-based signal to significance; the index is therefore transferable to data-limited regions when family coverage — and, where available, region-specific sensitivity data — are reported transparently. Layered onto existing national monitoring at negligible additional cost, such a trait-based index converts status assessment into stressor diagnosis; larger, multi-watershed studies with decoupled gradients and time-integrated exposure measures are the priority next step.

Declarations

Ethics approval and consent to participate

Not applicable.

Clinical trial number

Not applicable.

Consent for publication

Not applicable.

Competing interests

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.

Supplementary material

Supplementary data associated with this article include: Table S1 (residual pesticides, LC50, and toxic units per site), Table S2 (family-level pesticide sensitivity values), Table S3 (acute toxicity of 20 indigenous Korean species), Table S4 (site-level community indices, SPEAR variants, *ex situ* LT50, and ΣTU), Table S5 (zero-order and confounder-controlled partial correlations of the index with both exposure anchors), Table S6 (PCA loadings), and Table S7 (Benjamini–Hochberg FDR results for the eleven-test battery); Fig. S1 (study design and national context), Fig. S2 (family-level sensitivity distribution by taxonomic group), Fig. S3 (family-level sensitivity: existing versus updated), Fig. S4 (national SPEAR $_{pesticide}$ distribution shift), Fig. S5 (LT50 versus SPEAR $_{pesticide}$ cross-classification panel), Fig. S6 (site cross-classification coloured by BMI value), Fig. S7 (site classification under alternative decision rules), Fig. S8 (cross-classification of the generic community metrics), Fig. S9 (community indices vs the exposure anchors), Fig. S10 (PCA biplot), Fig. S11 (validation summary across index variants), Fig. S12 (per-layer concordance heatmap), and Fig. S13 (spatial distribution of exposure, effect-based toxicity, and impact class). Full analytical protocols are given in Supplementary Methods.

Funding

This research was supported by the Environmental Technology Development Project (R&D) for securing water ecosystem health of the Korea Environment Industry & Technology Institute (KEITI). (Ministry of Environment R&D project numbers: 2022003050005 and RS-2018-KE000099).

Author contributions

Jong-Hyeon Lee: Conceptualization, Methodology, Formal analysis, Supervision, Writing – original draft, Writing – review & editing, Funding acquisition. **Jang-Yeon Park**: Investigation, Data curation, Writing – original draft. **Su-Hyun Kim**: Data curation, Resources. **Seung-Been Heo**: Investigation, Data curation.

Acknowledgements

This research was administratively and technically supported by the Ministry of Environment, Korean government.

Declaration of generative AI use During the preparation of this work the authors used a generative AI assistant (Anthropic Claude) to support language editing and the drafting of figures and analysis code. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Data availability

The data and source code for the statistical analysis that support the findings of this study are available from the corresponding authors upon reasonable request.

References

1. Anastassiades M, Lehotay SJ, Štajnbaher D, Schenck FJ (2003) Fast and easy multiresidue method employing acetonitrile extraction/partitioning and dispersive solid-phase extraction for the determination of pesticide residues in produce. J AOAC Int 86:412–431. https://doi.org/10.1093/jaoac/86.2.412
2. Beketov MA, Liess M (2008) An indicator for effects of organic toxicants on lotic invertebrate communities: independence of confounding environmental factors over an extensive river continuum. Environ Pollut 156:980–987. https://doi.org/10.1016/j.envpol.2008.05.005
3. Beketov MA, Foit K, Schäfer RB, Schriever CA, Sacchi A, Capri E, Biggs J, Wells C, Liess M (2009) SPEAR indicates pesticide effects in streams — comparative use of species- and family-level biomonitoring data. Environ Pollut 157:1841–1848. https://doi.org/10.1016/j.envpol.2009.01.021
4. Beketov MA, Kefford BJ, Schäfer RB, Liess M (2013) Pesticides reduce regional biodiversity of stream invertebrates. Proc. Natl. Acad. Sci. U.S.A. 110:11039–11043. https://doi.org/10.1073/pnas.1305618110
5. Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc B 57:289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x
6. Benjamini Y, Yekutieli D (2001) The control of the false discovery rate in multiple testing under dependency. Ann Stat 29:1165–1188. https://doi.org/10.1214/aos/1013699998
7. Bighiu MA, Höss S, Traunspurger W, Kahlert M, Goedkoop W (2020) Limited effects of pesticides on stream macroinvertebrates, biofilm nematodes, and algae in intensive agricultural landscapes in Sweden. Water Res 174:115640. https://doi.org/10.1016/j.watres.2020.115640
8. Bray JP, O'Reilly-Nugent A, Kon Kam King G, Kaserzon S, Nichols SJ, Mac Nally R, Thompson RM, Kefford BJ (2021) Can species at risk of pesticides (SPEAR) indices detect effects of target stressors among multiple interacting stressors? Sci. Total Environ 763:142997. https://doi.org/10.1016/j.scitotenv.2020.142997

9. Chiu M-C, Hunt L, Resh VH (2016) Response of macroinvertebrate communities to temporal dynamics of pesticide mixtures: a case study from the Sacramento River watershed. Calif Environ Pollut 219:89–98. https://doi.org/10.1016/j.envpol.2016.09.048
10. Chapman PM (1990) The sediment quality triad approach to determining pollution-induced degradation. Sci Total Environ 97–98:815–825. https://doi.org/10.1016/0048-9697(90)90277-2
11. Chung J, Kim SH, Hwang DS, Sung CG, Moon SD, Kim C, Choi M, Lee JH (2024) Integrated chemical and ecotoxicological assessment of metal contamination in the Andong watershed: identifying key toxicants and ecological risks. Water 16:3176. https://doi.org/10.3390/w16223176
12. Cornejo A, Tonin AM, Checa B, Tuñón AR, Pérez D, Coronado E, González S, Ríos T, Macchi P, Correa-Araneda F, Boyero L (2019) Effects of multiple stressors associated with agriculture on stream macroinvertebrate communities in a tropical catchment. PLoS ONE 14:e0220528. https://doi.org/10.1371/journal.pone.0220528
13. Day KE, Dutka BJ, Kwan KK, Batista N, Reynoldson TB, Metcalfe-Smith JL (1995) Correlations between solid-phase microbial screening assays, whole-sediment toxicity tests with macroinvertebrates and in situ benthic community structure. J Great Lakes Res 21:192–206. https://doi.org/10.1016/S0380-1330(95)71031-0
14. Ganatra AA, Kandie FJ, Fillinger U, McOdimba F, Torto B, Brack W, Liess M, Hollert H, Becker JM (2021) Calibration of the SPEARpesticides bioindicator for cost-effective pesticide monitoring in East African streams. Environ Sci Eur 33:58. https://doi.org/10.1186/s12302-021-00497-9
15. Helmholtz Centre for Environmental Research (UFZ) (2023) SPEAR calculator: indicator system for the effects of toxicants on aquatic invertebrate communities. https://www.systemecology.de/indicate/ (accessed 21 July 2026)
16. Hunt L, Bonetto C, Marrochi N, Scalise A, Fanelli S, Liess M, Lydy MJ, Chiu M-C, Resh VH (2017) Species at Risk (SPEAR) index indicates effects of insecticides on stream invertebrate communities in soy production regions of the Argentine Pampas. Sci Total Environ 580:699–709. https://doi.org/10.1016/j.scitotenv.2016.12.016
17. Hwang D-s, Kim J, Chung J, Lee J (2024) Refinement and validation of the SPEcies At Risk index for metals (SPEAR_metal index) for assessing ecological impacts of metal contamination in the Nakdong River, South Korea. Water 16:3308. https://doi.org/10.3390/w16223308
18. Jackson DA (1993) Stopping rules in principal components analysis: a comparison of heuristical and statistical approaches. Ecology 74:2204–2214. https://doi.org/10.2307/1939574
19. Knillmann S, Orlinskiy P, Kaske O, Foit K, Liess M (2018) Indication of pesticide effects and recolonization in streams. Sci Total Environ 630:1619–1627. https://doi.org/10.1016/j.scitotenv.2018.02.056
20. Kong D, Son S-H, Hwang S-J, Won D-H, Kim M-C, Park J-H, Jeon T-S, Lee J-E, Kim J-H, Kim J-S, Park S-J, Kwak S-J, Lee J-A, Kim Y-J, Park S-J, Lee S-W, Kang K-H, Moon J-S, Park H-K, Ham S-A, Bae Y-J, Yoon T-J, Shin H-S, Kim H-G, Choi J-S (2018) Development of benthic macroinvertebrates index (BMI) for biological assessment on stream environment. J Korean Soc Water Environ 34:183–201 (in Korean)
21. Lenat DR, Resh VH (2001) Taxonomy and stream ecology — the benefits of genus- and species-level identifications. J North Am Benthol Soc 20:287–298. https://doi.org/10.2307/1468323
22. Liess M, von der Ohe PC (2005) Analyzing effects of pesticides on invertebrate communities in streams. Environ Toxicol Chem 24:954–965. https://doi.org/10.1897/03-652.1
23. Liess M, Schäfer RB, Schriever CA (2008) The footprint of pesticide stress in communities — species traits reveal community effects of toxicants. Sci Total Environ 406:484–490. https://doi.org/10.1016/j.scitotenv.2008.05.054
24. Liess M, Gerner NV, Kefford BJ (2017) Metal toxicity affects predatory stream invertebrates less than other functional feeding groups. Environ Pollut 227:505–512. https://doi.org/10.1016/j.envpol.2017.05.017
25. Malaj E, Grote M, Schäfer RB, Brack W, von der Ohe PC (2012) Physiological sensitivity of freshwater macroinvertebrates to heavy metals. Environ Toxicol Chem 31:1754–1764. https://doi.org/10.1002/etc.1868
26. Malaj E, von der Ohe PC, Grote M, Kühne R, Mondy CP, Usseglio-Polatera P, Brack W, Schäfer RB (2014) Organic chemicals jeopardize the health of freshwater ecosystems on the continental scale. Proc. Natl. Acad. Sci. U.S.A. 111:9549–9554. https://doi.org/10.1073/pnas.1321082111

27. Maltby L, Blake N, Brock TCM, Van den Brink PJ (2005) Insecticide species sensitivity distributions: importance of test species selection and relevance to aquatic ecosystems. Environ Toxicol Chem 24:379–388. https://doi.org/10.1897/04-025R.1
28. OECD (2004) Test No. 202: Daphnia sp., Acute Immobilisation Test. OECD Guidelines for the Testing of Chemicals, Section 2. OECD Publishing, Paris. https://doi.org/10.1787/9789264069947-en
29. Rico A, Hommen U, Escher BI, Koch A, Bado-Nilles A, González-Gaya B, Cody E, Sylvester F, Treu G, Alurralde G, Hollert H, Alvarez-Mora I, Moe SJ, De Jonge J, Ng K, Soto M, Liess M, Muz M, Bundschuh M, Lopez-Herguedas N, Pucheux N, Alygizakis N, von der Ohe PC, Beaudouin R, Finckh S, Schulze T, Verhaegen Y, Van den Brink PJ (2025) The use of diagnostic tools to assess the risks of chemicals to freshwater ecosystems: towards a unified evaluation framework. Environ Manage 75:3433–3448. https://doi.org/10.1007/s00267-025-02265-4
30. Roessink I, Merga LB, Zweers HJ, Van den Brink PJ (2013) The neonicotinoid imidacloprid shows high chronic toxicity to mayfly nymphs. Environ Toxicol Chem 32:1096–1100. https://doi.org/10.1002/etc.2201
31. Roex EWM, van Gestel CAM, van Wezel AP, van Straalen NM (2000) Ratios between acute aquatic toxicity and effects on population growth rates in relation to toxicant mode of action. Environ Toxicol Chem 19:685–693. https://doi.org/10.1002/etc.5620190321
32. Schäfer RB, Caquet T, Siimes K, Müller R, Lagadic L, Liess M (2007) Effects of pesticides on community structure and ecosystem functions in agricultural streams of three biogeographical regions in Europe. Sci Total Environ 382:272–285. https://doi.org/10.1016/j.scitotenv.2007.04.040
33. Schäfer RB, Kefford BJ, Metzeling L, Liess M, Burgert S, Marchant R, Pettigrove V, Goonan P, Nugegoda D (2011) A trait database of stream invertebrates for the ecological risk assessment of single and combined effects of salinity and pesticides in South-East Australia. Sci Total Environ 409:2055–2063. https://doi.org/10.1016/j.scitotenv.2011.01.053
34. Schäfer RB, von der Ohe PC, Rasmussen J, Kefford BJ, Beketov MA, Schulz R, Liess M (2012) Thresholds for the effects of pesticides on invertebrate communities and leaf breakdown in stream ecosystems. Environ Sci Technol 46:5134–5142. https://doi.org/10.1021/es2039882
35. Sládeček V (1973) System of water quality from the biological point of view. Arch Hydrobiol Beih Ergebn Limnol 7:1–218
36. Stehle S, Schulz R (2015) Agricultural insecticides threaten surface waters at the global scale. Proc. Natl. Acad. Sci. U.S.A. 112:5750–5755. https://doi.org/10.1073/pnas.1500232112
37. von der Ohe PC, Liess M (2004) Relative sensitivity distribution of aquatic invertebrates to organic and metal compounds. Environ Toxicol Chem 23:150–156. https://doi.org/10.1897/02-577
38. von der Ohe PC, Goedkoop W (2013) Distinguishing the effects of habitat degradation and pesticide stress on benthic invertebrates using stressor-specific metrics. Sci Total Environ 444:480–490. https://doi.org/10.1016/j.scitotenv.2012.12.001
39. Wogram J, Liess M (2001) Rank ordering of macroinvertebrate species sensitivity to toxic compounds by comparison with that of Daphnia magna. Bull Environ Contam Toxicol 67:360–367. https://doi.org/10.1007/s001280133
40. Wood RJ, Mitrovic SM, Lim RP, Warne MSJ, Dunlop J, Kefford BJ (2019) Benthic diatoms as indicators of herbicide toxicity in rivers — a new SPEcies At Risk (SPEAR_herbicides) index. Ecol Indic 99:203–213. https://doi.org/10.1016/j.ecolind.2018.12.035

Figures

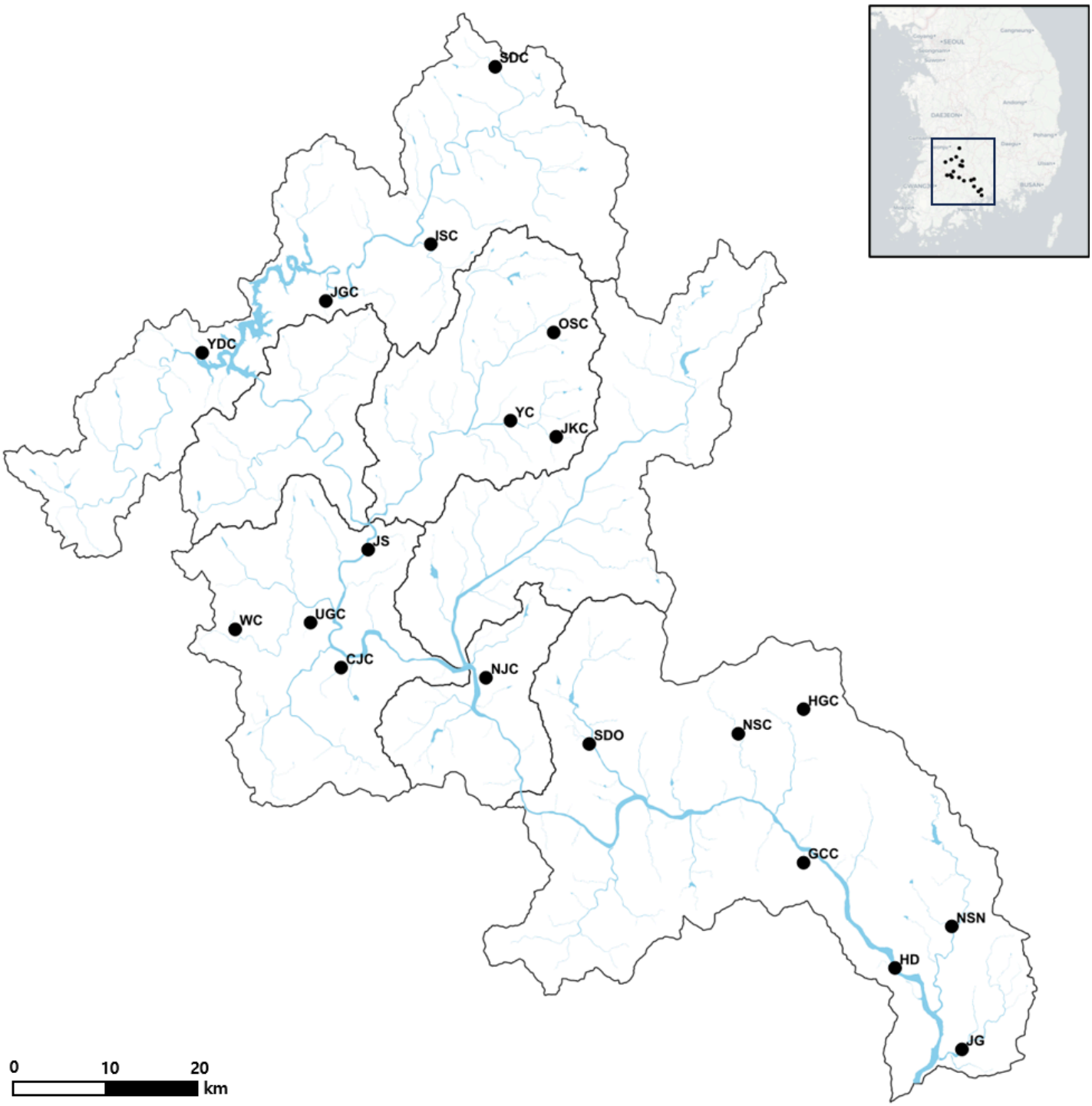

Figure 1

Study area and the 19 monitoring sites in the Seomjin River watershed, southwestern South Korea, shown on the drainage network with sub-basin boundaries and labelled with site codes (inset: location of the watershed within the Republic of Korea). Sites span the dendritic network from northern headwater tributaries (upstream; e.g., SDC) to the southern outlet near the river mouth (downstream; JG).

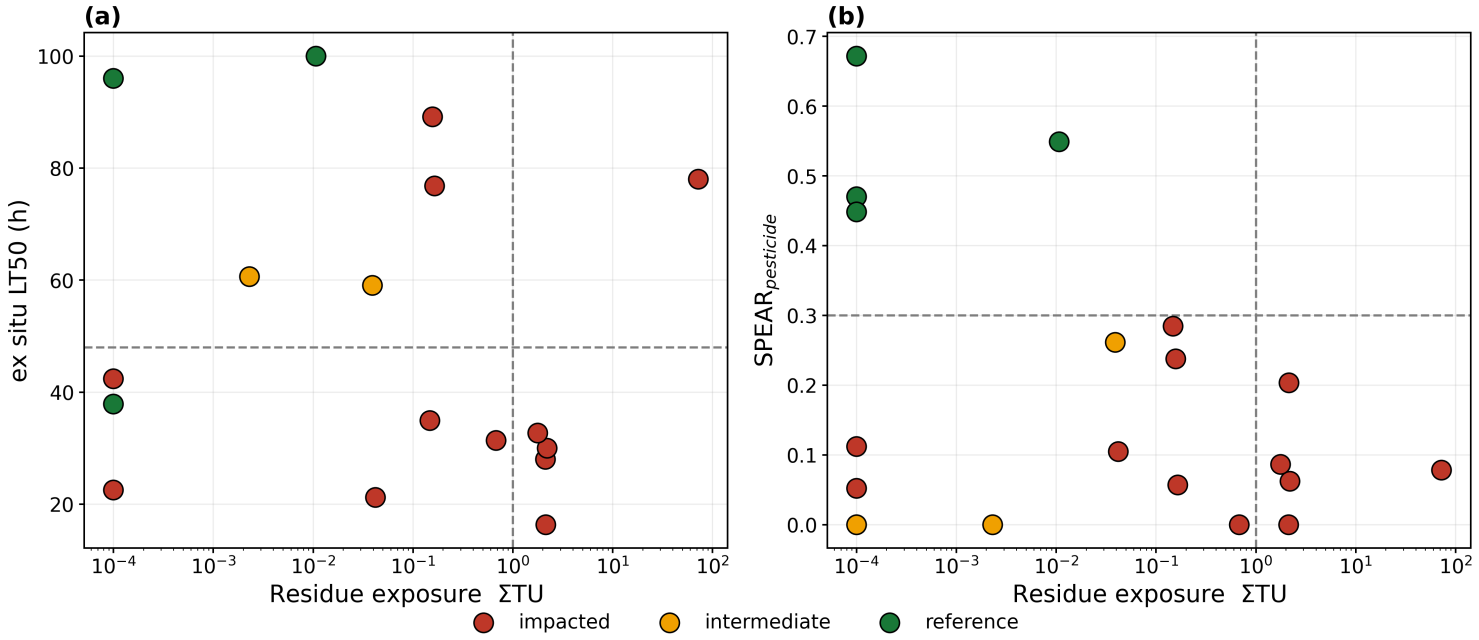

Figure 2

Site-level cross-classification of the 19 sites by residue-based exposure (ΣTU) against (a) effect-based toxicity (ex situ LT50) and (b) biological impairment (SPEAR_pesticide). Dashed lines are classification thresholds; markers are coloured by each site's final impact class (impacted, reference, intermediate). The corresponding LT50 versus SPEAR_pesticide panel is given in Fig. S5 and a BMI-value-coloured version in Fig. S6.

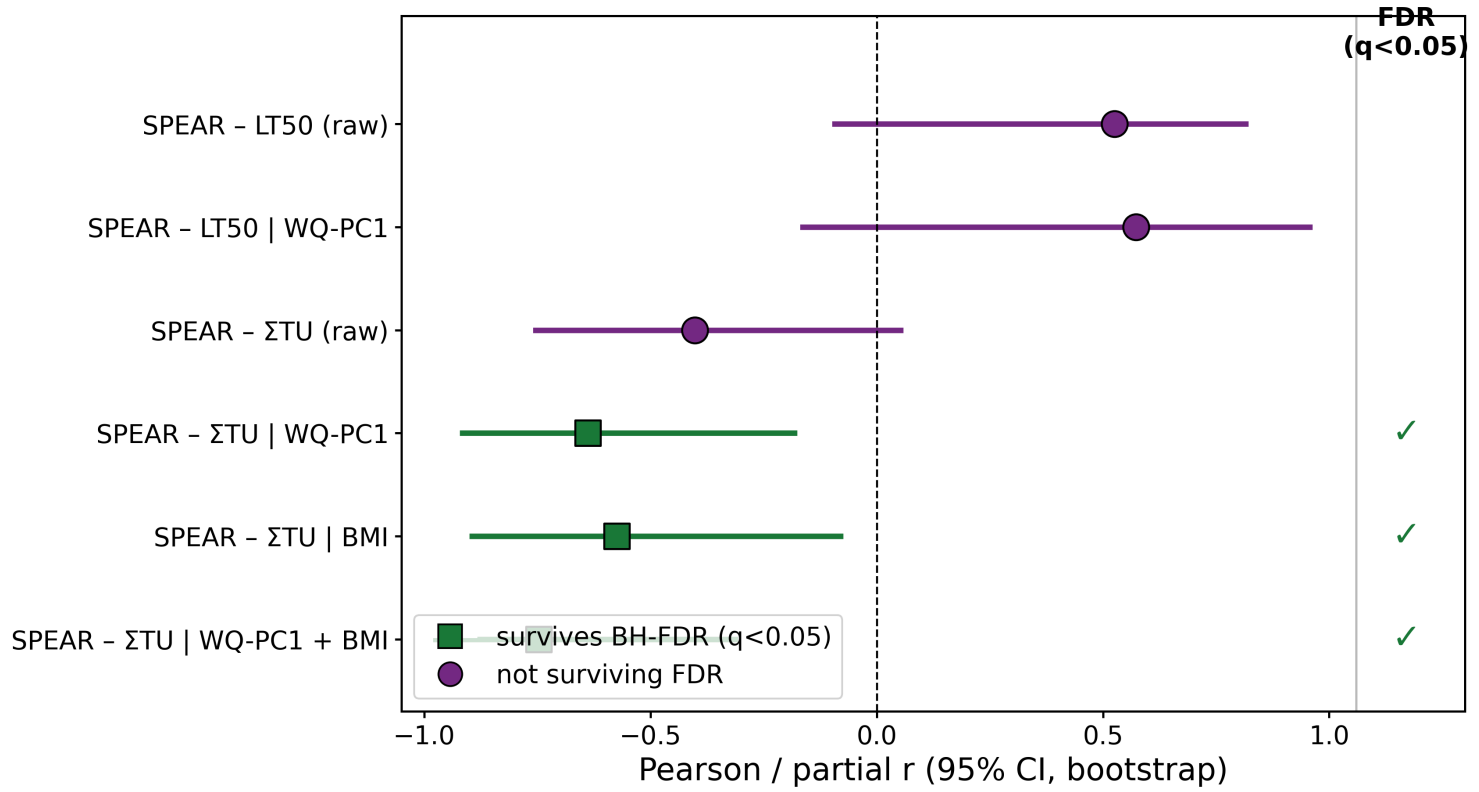

Figure 3

Zero-order Pearson correlations (filled circles) and confounder-controlled partial correlations (open squares) of the index with both exposure anchors (LT50 and ΣTU), and of generic indices with LT50 (forest plot; whiskers 95% CI; symbols mark associations

surviving Benjamini–Hochberg FDR at q < 0.05).

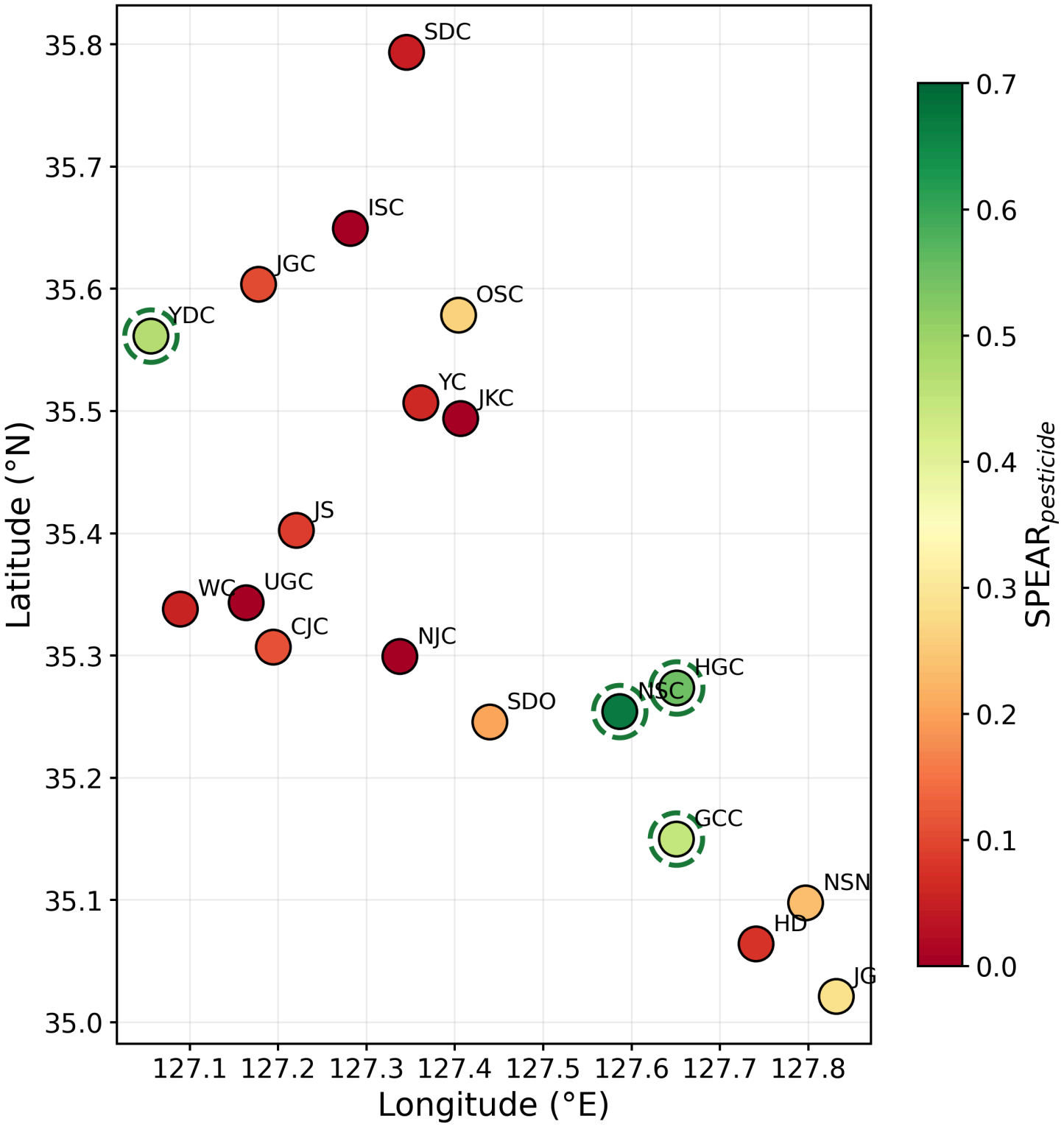

Figure 4

Spatial distribution of the primary SPEAR_pesticide index across the Seomjin watershed (2023–2025); lower values indicate greater pesticide impairment (impaired below 0.30). Impacted sites are dispersed across headwater, mid-watershed and downstream tributaries rather than clustered below a single location. Corresponding maps of residue exposure (ΣTU) and effect-based toxicity (LT50) are given in Fig. S13.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- 03Highlights.docx
- GraphicalAbstract.png
- FigS1.png
- FigS10.png
- FigS11.png
- FigS12.png
- FigS13.png
- FigS2.png
- FigS3.png
- FigS4.png
- FigS5.png
- Supplementarymaterial.docx
- FigS6.png
- FigS7.png
- FigS8.png
- FigS9.png