Site-Specific Ecological Risk Assessment of Metal Mixtures in a Mining Influenced River: A Case Study of the Loddon River Basin, Australia

Mokhinabonu Mardonova[1], Ehsan Arzaghi[2], Bandita Mainali[1], Rouzbeh Abbassi[1], Muhammad Kashif Shahid[1]

[1]*School of Engineering, Macquarie University, Australia, mardonova.mokhinabonu@mq.edu.au, ORCID 0000-0001-6466-2909*

[2]*School of Mechanical, Medical & Process Engineering, Queensland University of Technology, Australia*

Abstract

Victoria, a southeastern state of Australia, has a long history of gold mining, leaving over 15,000 abandoned mine sites that continue to influence river water quality through elevated metal concentrations (Cu, Ni, Zn, Pb). Conventional guideline values (GVs) are often inadequate for mining-influenced rivers, as they do not account for site-specific water chemistry or metal bioavailability, potentially misrepresenting ecological risk.

This study presents an integrated ecological risk assessment (ERA) framework that combines bioavailability modelling and probabilistic mixture toxicity to evaluate multi-metal risks. The Loddon River basin was selected as a case study. Chronic toxicity data (NOEC) were compiled from the USEPA ECOTOX database to develop Species Sensitivity Distributions (SSDs). Site-specific HC5 values were derived using the Bio-Met tool based on local pH, DOC, and Ca conditions. Potentially Affected Fractions (PAFs) were calculated and integrated into mixture risk (msPAF) using a concentration addition (CA) approach.

Results revealed clear spatial variability, with downstream sites exhibiting greater ecological susceptibility due to increased metal bioavailability, despite comparable or lower concentrations. While HC5-based assessments indicated generally low risk, msPAF identified episodic high-risk conditions, particularly at upstream and midstream sites. These findings demonstrate a decoupling between metal concentrations and ecological risk, driven by site-specific bioavailability and mixture effects.

Overall, the proposed framework provides a more realistic and sensitive approach to ecological risk assessment by integrating bioavailability, species sensitivity, and mixture toxicity. Although demonstrated using the Loddon River, the approach is transferable to other mining-influenced catchments, supporting improved risk evaluation and water management in complex environmental systems.

Keywords: Mixture toxicity, freshwater ecosystems, ecological risk assessment, bioavailability, mining influenced river, species sensitivity distributions

Introduction

Generic water quality guideline values (GVs) are widely used to assess ecological risk by comparing measured concentrations to predefined thresholds (USEPA 1992). However, they do not account for influence of water chemistry on metal bioavailability. Key parameters such as pH, hardness, and dissolved organic carbon (DOC) can alter metal speciation and organism sensitivity, potentially leading to misrepresentation of ecological risk (Quality *et al.* 2014).

These limitations are particularly relevant in mining-influenced rivers, where metal contamination occurs under spatially variable physicochemical conditions (Macklin *et al.* 2023). While site-specific Species Sensitivity Distributions (SSDs) provide a robust probabilistic framework, their application is data-intensive(Quality *et al.* 2014; Rong *et al.* 2022). Bioavailability-based tools such as Biotic Ligand Models (BLMs) and simplified approaches like Bio-Met offer a practical alternative by adjusting toxicity thresholds to local water chemistry (European Copper Institute 2023).

Conventional ecological risk assessments also assume independent metal effects, despite co-occurrence and interactions in mining systems (Nys *et al.* 2017). SSDs enable estimation of the Potentially Affected Fraction (PAF), which can be extended to mixture conditions through mixture toxicity PAF (msPAF), providing a probabilistic measure of combined toxicity (Traas *et al.* 2001; Nys *et al.* 2017; Rong *et al.* 2022).

To address these challenges, this study presents an integrated ecological risk assessment framework that combines site-specific bioavailability corrections with probabilistic msPAF. The framework is applied to the Loddon River basin (Victoria, Australia), demonstrating its broader applicability to similar mining-influenced systems.

Methods

Data sources and study area

The ecological risk assessment framework is summarised in Fig. 1. Water quality data for Cu, Pb, Ni, and Zn, along with key physicochemical parameters (pH, DOC, Ca), were obtained for the Loddon River basin (Victoria, Australia) from government database for 1997–2025. Five monitoring sites were analysed: Newstead (NS), Laanecoorie (LC), Serpentine Weir (SW), Kerang (KR), and Swan Hill (SH) (Fig.2). As concentrations were reported as totals, dissolved metals were estimated using total suspended solids (TSS) and literature-based partition coefficients (log K_d): Cu (0.768), Ni (0.255), Pb (2.07), and Zn (2.33) (Sedeño-Díaz *et al.* 2019).

Chronic toxicity data (NOECs) for freshwater species were compiled from the USEPA ECOTOX database, focusing on growth and reproduction endpoints. Based on data availability, 7–8 species per metal were included across multiple taxa (fish, crustaceans, molluscs, and insects). NOEC values ranged from 0.46–422 µg/L, with the lowest values for Cu and highest for Zn. The most sensitive records were selected, and data were normalised to site conditions.

Bioavailability adjustment and SSD development

Toxicity data were normalised to reference water chemistry using the Bio-Met tool (European Copper Institute 2023). Reference conditions were defined as pH 7.0, hardness 30 mg/L $CaCO_3$, DOC 2 mg/L, and Ca 12 mg/L. The hazardous concentration protecting 95% of species (*HC5*) was derived

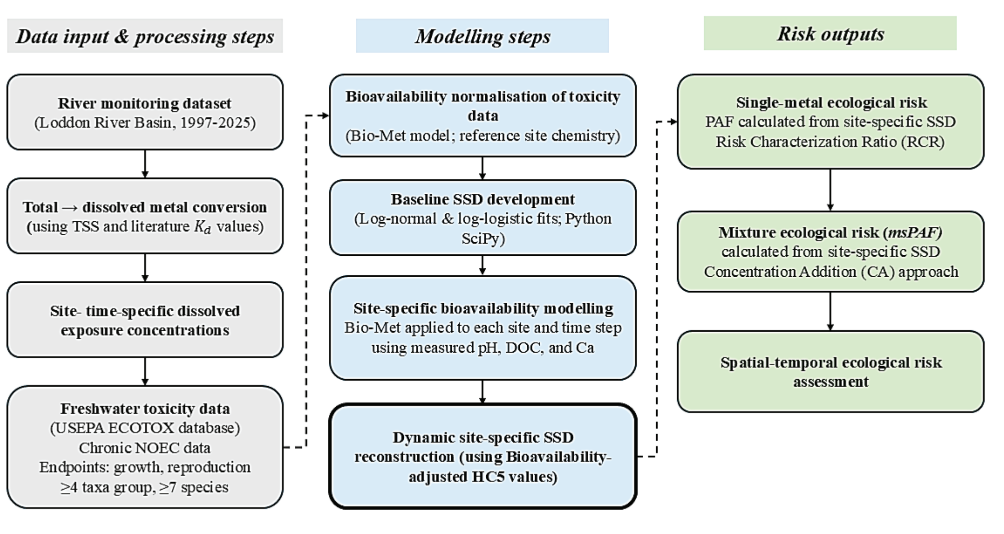

***Figure 1** Conceptual framework of the proposed ecological risk assessment methodology.*

as the 5th percentile of fitted distributions, and normalisation factors based on *HC5* ratios were applied to adjust *NOEC* values. SSDs were fitted using log-normal cumulative distribution functions in *SciPy* with parameters estimated via maximum likelihood. Sensitivity analysis confirmed robust model performance. Site- and time-specific *HC5* values were derived using measured water chemistry inputs.

Site-specific SSD reconstruction and PAF estimation

To account for spatial and temporal variability in bioavailability, *SSDs* were reconstructed for each metal across sites and time using site-specific *HC5* values, while retaining the original distribution shape. Mean μ values were derived for each site and used to reconstruct *SSDs*. Potentially Affected Fractions (*PAFs*) were then calculated from dissolved metal concentrations based on the fitted log-normal distributions (Traas *et al.* 2001).

Mixture ecological risk was quantified using the *msPAF*. Mixture ecological risk was evaluated using the Concentration Addition (CA) approach (Nys *et al.* 2017). Individual metal *PAFs* were combined as:

$$msPAF_{site\,(t)} = 1 - \prod_{i=1}^{n}(1 - PAF_{i,site\,(t)})$$

where *n* is the number of metals (Cu, Pb, Zn, Ni), representing the cumulative probability of species being affected by at least one toxicant.

Results and Discussion

Metal concentrations and water chemistry patterns

Dissolved metal concentrations showed clear spatial variability along the Loddon River. Zn exhibited the highest levels, particularly at LC,

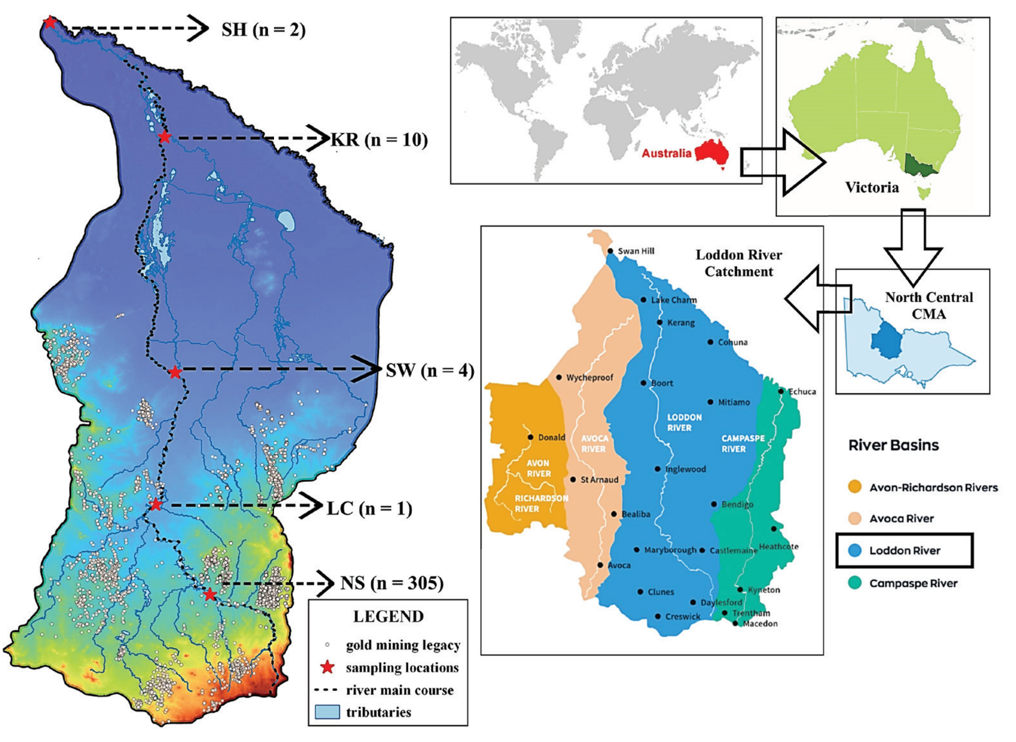

Figure 2 *Location of the Loddon River catchment (Victoria, Australia), showing historical gold mining legacy, monitoring sites (SH = Swan Hill, KR = Kerang, SW = Serpentine Weir, LC = Laanecoorie, NS = Newstead), and sampling density. The background colour gradient of the basin represents catchment topography, with elevation decreasing from brown (high elevation) to blue (low elevation).*

while Pb increased toward downstream sites (KR and SH). Cu and Ni remained relatively low. This longitudinal pattern from upstream (NS) to downstream suggests progressive metal mobilisation or accumulation along the river.

These trends align with contrasting physicochemical conditions. Upstream (NS) is characterised by higher hardness (≈210 mg/L $CaCO_3$), alkalinity (≈180 mg/L), and DOC (≈14 mg/L), which reduce metal bioavailability. In contrast, downstream sites show lower hardness (≈30 mg/L), lower DOC (≈5 mg/L), and higher turbidity (≈92 NTU), conditions that may enhance metal mobility and exposure. This highlights the role of water chemistry in controlling metal bioavailability.

SSD-derived thresholds and site-specific adjustments

SSDs produced consistent *HC5* estimates across distributions, with the log-normal model adopted. Zn showed the highest *HC5*, while Cu and Ni exhibited lower thresholds, indicating greater intrinsic toxicity (Fig. 3). Site-specific SSD reconstruction revealed a clear spatial gradient in toxicity thresholds (Fig. 3). Downstream sites exhibited lower *HC5* values, indicating higher bioavailability and ecological sensitivity, whereas the upstream NS site showed higher *HC5* values, reflecting reduced bioavailability under local water chemistry.

Comparison with guideline values (Tab. 1) demonstrated that generic thresholds may not adequately represent local ecological protection. SSD-derived HC5 threshold values for Cu and Ni were substantially lower than GVs, suggesting potential underestimation of risk when relying solely on conventional benchmarks. These differences emphasise the importance of incorporating bioavailability into ecological risk assessment and generic GVs may not adequately capture ecological risks in mining-affected river systems.

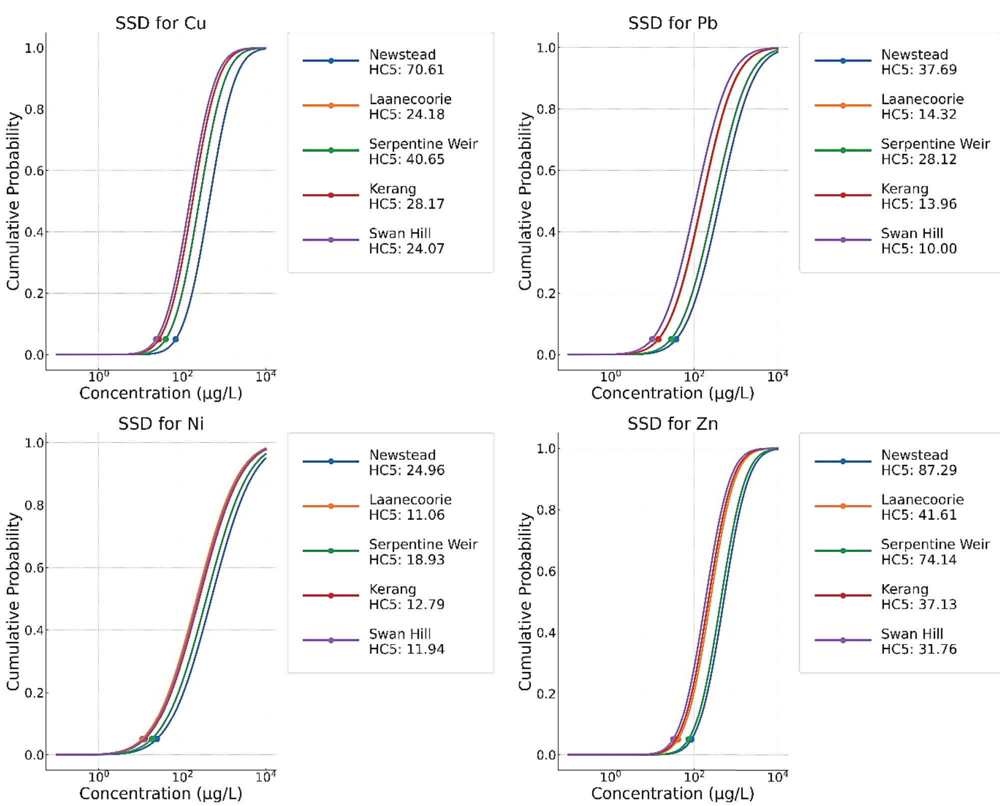

Figure 3 *Reconstructed site-specific log-normal SSDs for each metal across monitoring locations in the Loddon River.*

Table 1 Comparison of chronic protection thresholds (µg/L) derived in this study with USEPA hardness-based criteria and ANZG guideline values (hardness = 30 mg/L $CaCO_3$).

Metal	USEPA hardness-based guideline	ANZG guideline (95% protection)	This study HC5
Cu	3.2	1.0*	1.65
Pb	0.664	3.4	3.04
Ni	18.8	11.0	1.8
Zn	42.6	8.0	8.55

Mixture toxicity and msPAF patterns

Probabilistic metrics (*PAF* and *msPAF*) provided a more sensitive representation of ecological risk, particularly in identifying mixture effects and episodic risk conditions. Mean single-metal *PAF* values varied across sites, with Zn consistently contributing the highest values, followed by Ni, while Cu and Pb remained low (Fig. 4). However, *msPAF* values were consistently higher than individual-metal *PAFs*, demonstrating the importance of mixture effects.

Spatial patterns revealed low mixture risk at KR (*msPAF* < 0.001), moderate values at SW (≈0.004), and elevated levels at midstream sites such as LC and SW (up to ≈0.016). The highest variability occurred at NS, where *msPAF* ranged from near-zero to values exceeding 0.5, indicating episodic conditions under which a large proportion of species may be affected. These findings highlight that mixture toxicity can substantially amplify ecological risk beyond single-metal assessments, particularly under temporally variable conditions. A decoupling between concentration and ecological risk was observed, with some sites showing low risk despite high concentrations, highlighting the role of bioavailability and species sensitivity. Overall, ecological risk was low but spatially and temporally variable, with episodic peaks captured by *msPAF*, highlighting the importance of mixture toxicity.

The predominance of low baseline *msPAF* values with episodic high-risk events is consistent with previous studies showing that mixture effects are not captured by single-metal approaches (Nys *et al.* 2017). Spatial variability in risk along the Loddon River reflects patterns observed in other aquatic systems, where local physicochemical conditions strongly modulate toxicity. The observed decoupling between concentration and ecological risk highlights the importance of bioavailability and species sensitivity in probabilistic risk frameworks. While *msPAF* is widely applied to assess mixture toxicity, it is typically based on fixed toxicity thresholds that do not account for site-specific bioavailability (Nys *et al.* 2017; Rong *et al.* 2022; Tang *et al.* 2023). By integrating bioavailability-adjusted SSDs, this study enables toxicity thresholds to respond dynamically to local water chemistry, improving the ecological realism of mixture risk assessment in mining-influenced systems.

Conclusions

This study shows that ecological risk in mining influenced rivers is governed by site-specific bioavailability and mixture toxicity rather than metal concentrations alone. While *HC5*-based assessments indicated generally negligible risk across the Loddon River, probabilistic metrics (*PAF* and *msPAF*) revealed spatial variability and episodic high-risk conditions, particularly at upstream and midstream sites. The decoupling between concentration and ecological impact highlights limitations of conventional approaches. Overall, the proposed framework provides a more realistic and sensitive method for ecological risk assessment, with applicability to mining influenced systems beyond the case study.

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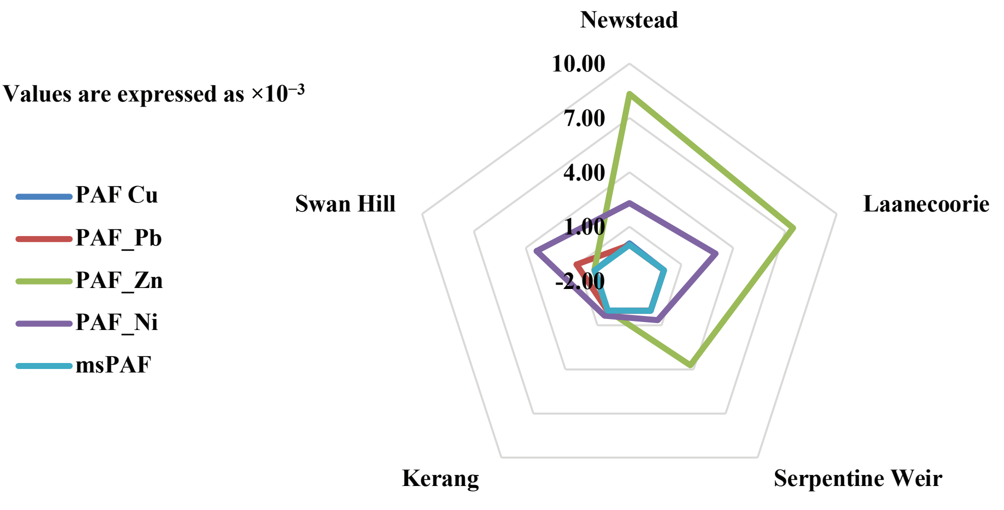

Figure 4 Mean single-metal PAF and msPAF values across monitoring sites along the Loddon River.

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