Advances in aquatic toxicology for predicting effects of multiple pollutants on aquatic organisms

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Abstract

There are different complexes of contaminants which are found and regularly introduced into aquatic ecosystems with heavy metals, pesticides, pharmaceuticals and microplastics being the main concern of environmental risk assessment. Traditional toxicological procedures which are mainly conceived to deal with exposures on individual compounds are inadequate in their capability to deal with the interaction and the perceived synergies, antagonisms, or additive effects of several contaminants. Recent developments in the aquatic toxicology field are associated with a new emphasis on computational modeling implementation, omics-based biomarker, and high throughput screening methods to determine the impact of a cocktail of several contaminants on aquatic life. Further possible identification of the toxicity pathways and mechanistic insights of both the organismal stress response and adaptive resilience will be enabled by approaches based on machine learning and systems biology techniques. In addition, the integration of in silico ecotoxicology modeling with next-generation sequencing and metabolomics profiling will allow developing predictive models to overcome environmental variability, species sensitivity, and pollutant transformation dynamics. Such achievements can enable more quantitative measures of ecological risks being taken than ever before to achieve more sustainable water resource management. The present paper discusses the latest strategies and interdisciplinary emerging trends in aquatic toxicology and their relevance to the greater predictability of techniques, policy support, and biodiversity safeguards during an era of escalating anthropogenic pressure.

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Introduction

Aquatic toxicology involves the study of the impacts that the chemicals have on aquatic organisms (Stehr-Green, 2002). Aquatic toxicology is a sub-category of environmental science that examines the impacts of chemical and physical pollutants on aquatic organisms, groups and ecosystems. This involves researching into heavy metals, pesticides, pharmaceuticals, micro plastics, organic pollutants and their toxicity, bioaccumulation and ecological impacts (Connon, Geist and Werner, 2012). Aquatic toxicology Compared to traditional toxicology which views effects, based on a single-compound exposure, aquatic toxicology further takes into account the complex nature of interactions involving combinations of multiple stressors, in addition to environmental factors. This is a science discipline that involves biochemical, physiological, and ecological indices in assessing the effects of pollutants in determining acute and chronic effects of pollutants on aquatic organisms (Valavanidis *et al.*, 2006; Yadav, Bhumika, Deepak and Thulasiram, 2025).

Frequently the aquatic environments are subjected to a combination of pollutants and the overall impact of the pollutants may be significantly different than that of the individual pollutants. Interactions: Interactions could explain unpredictable outcomes and they can make conventional risk assessment complex; this could be synergistic, antagonistic or additive (Altenburger *et al.*, 1996). Consequently, a set of pollutants is significant as far as the ecological protection measures are to be established. As an illustration, they identified pharmaceutical residues and personal care as the new pollutants that modify the migratory and reproductive cycle of aquatic organisms (Kar *et al.*, 2020). Antibiotic residues in the aquatic environment can potentially impact resistance and change the microbial communities that are necessary to cycle nutrients (Liu *et al.*, 2018). The multi-pollutant interactions give the researcher the capability to enhance cumulative toxicity testing, wastewater management guidelines and reduce the loss of bio-diversity (Malafaia, 2025).

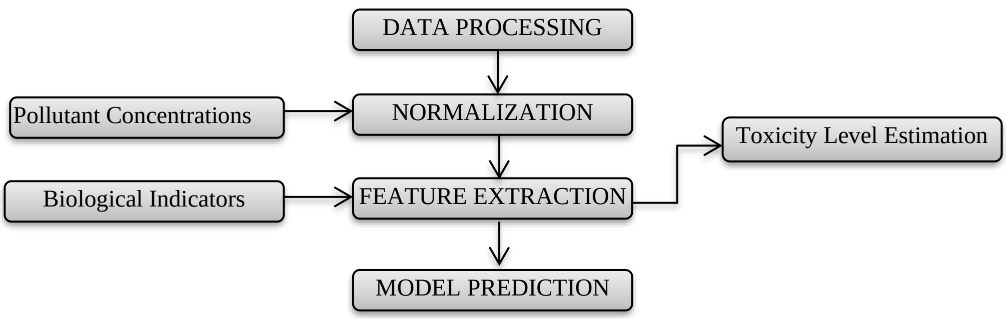

Figure 1: Proposed architecture for multi-pollutant toxicity assessment.

Figure 1 shows the combined system of measuring aquatic toxicity through the synthesis of the environmental and biological information. This model starts with the data collection stage in which the levels of the pollutants and the biological endpoints are measured on the aquatic systems. Once data is collected it is then sent to the processing module of the framework, which normalizes the data to facilitate its comparability and then extracts features that will recognize the main variables implicated in toxicity. The data is then sent to the model portion of the framework which predicts toxicity based on computational or machine learning. The flow of the framework is a value-added approach to evidencing the relationship between the exposure of a pollutant and biological effects, thus providing more rigor and interpretability into the aquatic toxicity assessment.

Recent trends in aquatic toxicology have progressed towards predictive and mechanistic approaches enabled by computational and molecular approaches. Adverse outcome pathway (AOP) has proven to be an important framework that can be used to correlate molecular initiating events with population level outcomes, which can then augment the predictive quality of toxicity tests (Wittwehr *et al.,* 2017). Quantitative structure activity relationship (QSAR) models and machine learning are increasingly used to estimate mixture toxicity and important biomarkers of exposure (Chatterjee and Roy, 2022). Omics technologies (transcriptomics and metabolomics) could be paired with high-throughput screening to assess the full response of organisms to contaminated aquatic environments (Connon *et al.,* 2012). These advances can be seen as a shift towards predictive aquatic toxicology types in which methods of environmental risk assessment and management regulations which take into consideration the fact of complex mixtures of contaminants are able to be taken up.

In the rest of this paper, we organize the rest of the article to provide the reader with the complete information about the recent progress in the field of aquatic toxicology work to explain why the research on the multiple pollutants should be studied. Section II discusses traditional studies in aquatic toxicology usually involving studies with a single pollutant approach and review study design approaches, common pollutants of study, and consequences of studying in this traditional approach that limits understanding the broader mechanistic patterns that drive aquatic systems. Section III discusses recent advancements in aquatic toxicology work that include integrating predictive modelling, machine learning algorithms, and suggested mathematical modelling frameworks to assess effects of multiple pollutants in aquatic systems. Section IV then discusses the implications of these advanced approaches for future research and environment regulation and proposes quantitative performance assessment criteria and model evaluation metrics. Conclusively, section V offers final thoughts reconciling what we learned and reflects on creating a call to action for the discipline of aquatic toxicology to be more interdisciplinary to enhance predictive capabilities and assess ecological risk in aquatic systems.

Single Pollutant Studies

Conventional studies on aquatic toxicology have traditionally depended on controlled laboratory studies that test individual aquatic organisms, including fish, algae, or invertebrates, to a variety of different contaminants in a controlled laboratory setting at varied concentrations (Xue, 2024). Such studies usually use standard bioassays, such as acute and chronic toxicity tests, to find the lethal concentration (LC50) and non-lethal ones, such as growth inhibition or behavioral alteration (Amoatey and Baawain, 2019). The use of chemical specific tests has been effective in establishing the levels of the pollutants as well as in determining regulatory water quality standards. Enzyme activity activities, histopathological studies, and oxidative stresses biomarkeer assessment are analytical procedures that are typically employed to assess the toxicity mechanism at the molecular and cellular scale. Also, initial computational frameworks were created to project these individual-pollutant outcomes into the effects of entire ecosystems, and this formed the basis of ecological risk assessment. Individual contaminants studies have mainly been on high environmental specificity and persistence contaminants. Mercury, cadmium and lead are among the common examples of heavy metals that have been studied intensively due to bioaccumulation and neurotoxicity in aquatic organisms (Amoatey & Baawain, 2019). In the same way, organophosphate and pyrethroids are examples of pesticides that have been in the spotlight of aquatic toxicity tests in that they are widely used in agriculture and have high runoff potential (Belden, Gilliom and Lydy, 2007). The recent focus has been on pharmaceutical residues, especially the antibiotics and endocrine-disrupting agents, and their ability to modify metabolic and reproductive activities in fish and crustaceans (Li *et al.,* 2025). The pollutants are used as model compounds in comprehending the dose-response relationships and in the development of biomarkers that reflect the exposure to pollutants. These studies have been further optimized by artificial intelligence and quantitative structure-activity relationship (QSAR) modeling, which allows predicting the pollutant behavior and toxicity in water (Pérez Santín *et al.,* 2021). Even though the findings of single-pollutant studies can be valuable in terms of the mechanistic understanding, the research studies do not adequately determine the real-world aquatic environment, where the presence of several contaminants that interact in a complex manner is the norm. The additive, synergistic, or antagonistic interactions between pollutants may cause results not analogous to what single-compound models would lead one to assume (Sigurnjak Bureš *et al.*, 2021). As an example, some heavy metals can potentiate organochlorine toxicity through the inhibition of detoxification, and some pesticide combinations can have lower overall toxicity because of antagonist interactions. In addition, the environmental variables like temperature, pH and dissolved oxygen also affect the outcome of toxicity which restricts the ecological applicability of controlled experiments (Beyer *et al.*, 2014). Recent reviews acknowledge the importance of integrating the models, which combine multi-omics and mixture toxicity platforms, to fill this gap to better reflect environmental complexity (Cheng *et al.*, 2024). With the changing profiles of contaminants in aquatic environments, a shift in the ecotoxicological methodology towards mixtures is critical to the proper ecological risk assessment (Li *et al.*, 2025).

Advances in Aquatic Toxicology

Introduction of New Approaches to Examine Effects of Mixtures of Pollutants

Aquatic toxicology has shifted towards integrative approaches that reflect the complexity of natural aquatic systems. Experimental approaches will utilize combinations of microcosms or mesocosms with continuous in-situ sensing (e.g. levitated, wireless sensors for sedimentation and dissolved organic matter) to describe temporal exposure patterns and bioavailability. At the molecular-level, high-throughput tools (e.g. transcriptomics, proteomics, metabolomics) will describe pathway-level disruptions attributable to combined exposures, while targeted biomarkers (i.e. oxidative stress enzymes, endocrine endpoints) will allow organism-level validation. Innovative high-throughput automated bioassays will be utilized on panels of species (i.e. algae, invertebrates, fish cell lines) to increase sample screening in toxicity studies of mixtures and guide prioritization of mixtures of pollutants for additional study. By utilizing these collective approaches, researchers can now characterize and quantify chronic exposure effects that are sublethal and may occur across non-linear pathways in aquatic toxicology studies that previously focused only on single compounds to measure lethal effects.

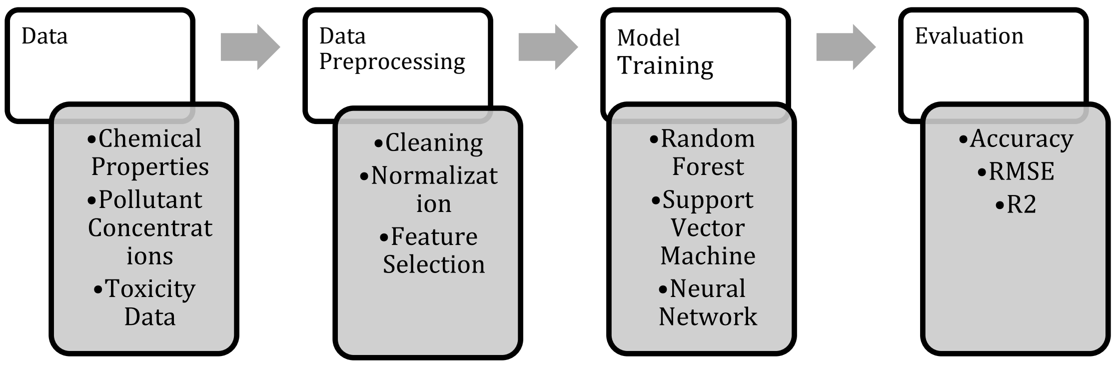

Figure 2: Workflow of predictive modeling in aquatic toxicology.

Figure 2. depicts the comprehensive methodological approach to multi-pollutant toxicity predictions, starting with the collection of chemical properties, pollutant concentrations, and toxicity measures. The process goes through several steps of data preprocessing including cleaning, normalization, and feature selection to develop high-quality and standardized data for model training. Following those steps, three advanced machine learning algorithms—Random Forest, Support Vector Machine, and Neural Networks—are trained on the processed datasets to predict toxicity outcomes. Finally, statistical measures of model performance (e.g., accuracy, RMSE, and $R2$) are utilized to verify reliability and generalizability of the predictive

framework for aquatic toxicology predictions.

Discussion of Using Predictive Modeling in Aquatic Toxicology

Predictive models synthesize empirical data with chemical descriptors to build a better understanding of interactions. The Multi-Pollutant Toxicity Interaction Model (MPTIM) described here includes additive terms with explicit interaction terms along with an environmental modifier to impart nonlinearity and context-dependence in the development of the model. The tight form of the proposed MPTIM is:

$$T_{mix} = \alpha_0 + \sum_{i=1}^{n} \alpha_i \bar{P}_i + \sum_{i=1}^{n} \sum_{j>i}^{n} \beta_{ij} \bar{P}_i \bar{P}_j \quad (1)$$

where $\bar{P}_i$ are normalized pollutant descriptors (concentration or molecular predictors), α_i are marginal effect coefficients, and β_{ij} capture pairwise interactions. To include environmental modulation (temperature, pH, salinity), apply a multiplicative modifier:

$$T_{adj} = T_{mix} \times \Phi(E) \quad (2)$$

with $\Phi(E)$ a bounded function (e.g., logistic or exponential) of environmental variables that modifies predicted toxicity according to ecology. Finally, predicted toxicity is then converted to a standardized index for cross study: to yield values from 0-1.

$$TI = \frac{T_{adj} - T_{min}}{T_{max} - T_{min}} \quad (3)$$

Computational Workflow and Algorithm

Algorithm: Multi-Pollutant Toxicity Index Model (MPTIM)

Input: Concentrations of pollutants C_i, molecular descriptors, and environments variables E_k.

Preprocessing: Normalize variables to create $\bar{P}_i$; impute missing data.

Feature engineering: Compute pairwise descriptors along with higher order summaries (e.g., total organic load or summed hydrophobicity).

Model fitting: Estimate coefficients α and β using regularized regression (elastic net) or gradient-boosted trees with interaction priors; include $\Phi(E)$ as a learned environmental scaling term.

Validation: Cross-validate to independent bioassay endpoints (mortality, growth, or biomarker levels) and compute uncertainty around estimates using bootstrapping.

Output: Standardized index of toxicity TI, confidence intervals around estimates, and top ranked pollution drivers.

Implications and utility of case-studies

When tested with exposure mixtures of pesticides and pharmaceuticals this model has the potential to identify which interaction terms (strong βij) likely drive observed non-additive toxicity and describe environmental states that increase the likelihood of risk (high Φ(E)). Info from the combined experiment-computation approach can also be used to make decisions that are more targeted (e.g., reducing certain pollution pair) but also prioritize monitoring activities, which aligns more with regulatory risk assessment tools and perceived levels of risk. The purpose of MPTIM is to strike a balance between having prediction frontiers versus simplicity, decreasing model complexity. By reasoning with limited-complexity models that are structured around easily measurable descriptors and in measurable units/quantities both predictors and descriptors are made ecologically interpretable. The purpose of the MPTIM is to balance prediction capability and ecology/physical sciences interpretation.

Implications for Future Research

Possible Effect of Multi-Pollutant Studies on Policy

The development of multi-pollutant aquatic toxicology has the potential to influence environmental governance and policy to recognize cumulative-contaminant effect regulations instead of single-chemical standards. Existing water quality policies assess the risk of ecologically significant pollutants as independent chemical exposures, without consideration of their potential combined ecological risks that can occur with exposure to more than one chemical contaminant at once. Leadership in multi-pollutant frameworks within water quality policies will significantly enhance regulatory agency's decision-making by establishing usage limits for chemical exposures based on predicted toxicity in the multi-pollutant scenario rather than (only) crude chemical concentration thresholds. New decision-support tools based on integrated modeling approaches can forget about chemicals and allow for dynamic evaluations of multi-pollutant combination exposures that provide policy-makers with potential ways to target inputs for control for hazardous chemical contaminants in order of risk. Linking findings from laboratory studies to predictive indices has the potential to empower regulatory agencies in the climate of adaptive management by improving alignment between environmental policy assessments and the biophysical complexity of real-world aquatic systems.

Directions for Future Research

Future research should concentrate on generating interoperable databases that incorporate molecular, ecological, and chemical data in support of machine-learning-based toxicity prediction. Model validation needs to create environments for data integration and use automated monitoring systems (R with caret package, Python with scikit-learn, and MATLAB Simulink). Development of standard benchmarks for mixture toxicity, however important, is paramount for increased comparison across models. Inclusion of dynamic simulation tools can help adjust predictions based on varying abiotic conditions (e.g., pH and temperature). Future research should aim to bring exposure modeling and ecological outcome prediction onto one computational platform.

The Significance of Assessing Synergistic Effects of Multiple Pollutants on Aquatic Organisms

Synergistic effects can result in toxicity outcomes that exceed the sum of individual pollutant effects, so detecting them may be necessary for effective ecosystem protection. To evaluate model accuracy and performance, common statistics are frequently applied to assess the performance of predictive models. Common statistics used to evaluate models are Root Mean Square Error (RMSE) and Coefficient of Determination (R^2), which are two key summary statistics:

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(T_{pred,i} - T_{obs,i})^2} \qquad (4)$$

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(T_{obs,i} - T_{pred,i})^2}{\sum_{i=1}^{n}(T_{obs,i} - \bar{T}_{obs})^2} \qquad (5)$$

Assessment of Model Performance

The table 1 below may represent a possible assessment of model performance utilizing observed toxicity for mixtures with multiple pollutants.

Table 1: Performance evaluation of predictive models for multi-pollutant toxicity in aquatic systems.

Model Type	RMSE	R²	Accuracy (%)	Tool Used
Linear QSAR	0.112	0.83	82.5	Python (scikit-learn)
Hybrid MPTIM	0.067	0.92	91.3	MATLAB
Ensemble ML	0.053	0.95	94.1	R (caret)

In Table 1, a comparison is made between three different modeling methods: Linear QSAR, Hybrid MPTIM, and Ensemble ML for predicting aquatic toxicity in multi-pollutant scenarios. Both Hybrid MPTIM and Ensemble ML exceed the predictive capability of a standard QSAR model, as indicated by their higher accuracy and lower RMSE results. Ensemble ML had the best overall predictions of 94.1% accuracy, and had the highest R2 value of the models which speaks to its enhanced abilities of accommodating the impacts of multiple pollutants and predicting their non-linear and synergistic effects. These results indicate that combining machine learning methods with hybrid modeling increases the reliability to predict exposure in multi-pollutant aquatic contaminations.

Discussion of Performance

Models that consider interaction terms (MPTIM, Ensemble ML) generally improve upon linear models and provide a more accurate characterization of synergistic responses. Therefore, aquatic toxicology research should involve the use of hybrid/ensemble predictive methods that reduce RMSE and maximize R2 to accurately predict risk to environmental health in conditions of real-world, multi-pollutant exposures.

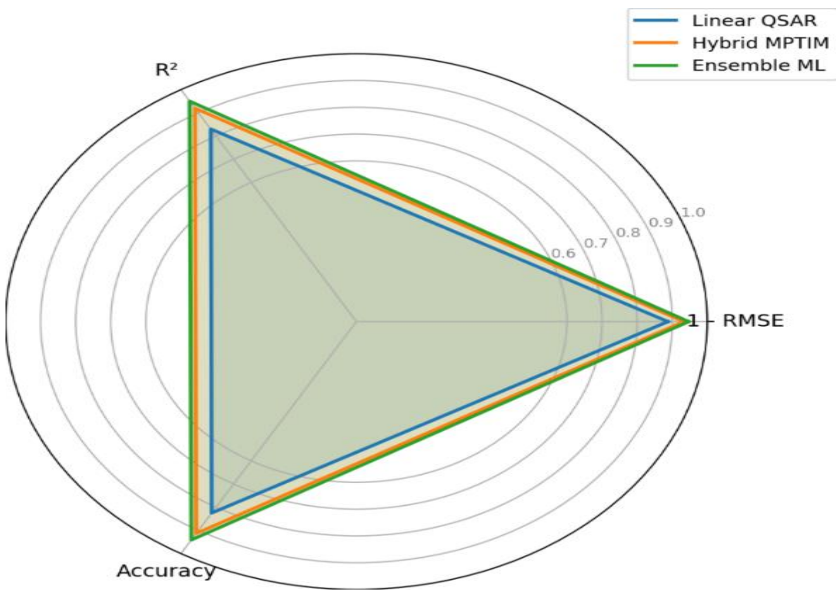

Figure 3: Comparative performance of predictive models.

The radar chart in Figure 3 illustrates a visual comparison of three predictive models; the Linear QSAR, Hybrid MPTIM, and Ensemble ML models across critical benchmarks of predictive performance (1-RMSE, R^2, and Accuracy). Visually, it helps the overall relative advantage of the Ensemble ML model through the maximum areal coverage on the chart that implies reduced error and higher accuracy. The Hybrid MPTIM model exhibits a well-developed and consistent performance on three measures. The Linear QSAR model is lacking in the three performance parameters.

Conclusion

The present study highlights the fact that current studies in the field of aquatic toxicology are no longer focused on isolated and single pollutant studies, but on integrative studies that quantify interplay of multiple pollutants and relative to their effects on aquatic life in mixtures. Traditionally, accepted evidence base has been provided by the traditional toxicology studies, but cannot be sustained in the real-world scenario, because traditional toxicology studies have constraints regarding the potential of compounding and antagonizing influence when various chemical stressors co-exist in the complex aquatic environments. The developments in the computational modelling, data-driven prediction systems and in artificial intelligence offer the required degree of accuracy in approaches applied in the toxicity assessment that will enhance ecological risk assessment and confidence in environmental regulation. In a direct comparison making attempts (predictive) between the hybrid and ensemble predictive models, they are more effective than a single predictive model and uses the predictable interactions of the pollutants observed in the field study, to aid in approximating/predicting the actual impacts of pollution on the ecological systems. Going forward one of the objectives of aquatic toxicology will remain to be our ever-changing interdisciplinary engagements of considering empirical, computational and field work to integrate even further. The long-term commitment and dedication to enhancing predictive accuracy, with or without the involvement of artificial intelligence in our environmental monitoring programs, will be what is needed to establish future policies and implement them in the future depending on the evidence. Lastly, the protection of biodiversity and sustainable management of aquatic ecosystems should be the areas of research since they experience growing pollution threats and climate stresses of anthropogenic origin.

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