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CYTOTOXIC EFFECTS OF EMERGING CHEMICAL CONTAMINANTS: AN IN VITRO TOXICOLOGICAL ASSESSMENT USING NEUTRAL RED RETENTION DATA

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ABSTRACT

The detection of emerging chemical contaminants is increasing in aquatic environments, and complex mixtures may result in biological effects which are not adequately captured by chemical monitoring alone. In this study, six environmental samples with publicly available chemical and NRB data were used to assess the cytotoxic effects of contaminant mixtures. Chemical profiles were defined by presence or absence of compounds, distribution of chemical classes and total concentration of contaminants, and cytotoxicity was determined at various REF levels. Differences among samples were examined using descriptive statistics, correlation analysis, non-parametric comparisons and simple regression to analyse the concentration-response patterns. In all, 457 target compounds were represented, the largest groups of pollutants being pharmaceuticals and pesticides. Samples H3–H6 had significantly higher chemical loads than H1 and H2 and higher Neutral Red cytotoxicity responses. Maximum cytotoxicity was obtained at 71% for H3, 74% for H4 and 62% for H6 with minimal effects in H1. In general, cytotoxicity increased with enrichment, and significant positive concentration-response relationships were found especially for H3 and H6. In addition, there were positive relationships between contaminant load and cytotoxicity under the highest exposure condition. The results indicate the utility of combining chemical characterisation and in vitro bioassays to assess the biological relevance of complex mixtures of environmental contaminants. The Neutral Red Retention stain was a helpful tool in determining cell injury and was used for sample differentiation to identify samples with different toxicological profiles.

Keywords: emerging contaminants; cytotoxicity; Neutral Red Retention; environmental mixtures; in vitro toxicology

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1. Introduction

Chemical contaminants are increasingly being found in other environmental compartments, including surface water, wastewater, sediments and are becoming an important environmental and toxicological issue. They are pharmaceuticals, pesticides, personal-care products, industrial chemicals, biocides, polymer additives and other chemicals that can be persistent in the environment or present as complex mixtures. They are constantly released and are not totally removed in traditional water treatment processes, leading to a chronic exposure of the aquatic organisms and possible impact on human health via environmental routes. Measuring the presence of chemicals may not fully capture the potential for biological risks due to the ability of chemicals to interact with each other and not necessarily causing a toxic effect when detected. Integrated methods integrating chemical characterisation and biological-effect assessment are thus becoming increasingly explored to assess contaminant-related hazards in aquatic systems (Grace et al., 2025).

The toxicological evaluation of chemical mixtures is especially noteworthy since it is possible for the effects of multiple chemicals to interact. Chemicals present in low concentrations, but many of them, may cause measurable cellular responses and the individual chemical measurements may not fully reflect the biological impact. Lastly, previous studies on the toxicity of environmental water mixtures have shown that it can have measurable cytotoxic effects in controlled laboratory conditions and therefore, assessing the toxicity of mixtures is important, not just that of a single chemical compound (Hakeem & Amare, 2022). Likewise, organic contaminant analysis with combination in vitro toxicity tests has demonstrated that bioassays can supplement data on the biological significance of chemical contamination (Albero et al., 2025). Safety evaluation is also being increasingly used in other chemical formulations and consumer exposures, which will require the evaluation of biological effects in addition to chemical composition (Manful et al., 2024).

Cytotoxicity assays in vitro are popular, as they can be controlled, reproducible and relatively quick for detecting adverse effects on cells. Cytotoxicity is a basic toxicological endpoint, as loss of cell integrity, metabolism or viability may be an early sign of biological damage after chemical exposure (Çetin, 2025). Cell viability and cytotoxicity assays assess various biochemical processes and cellular compartments and can be used to identify changes in cell membrane function, metabolic activity, lysosomal stability, and cell survival (Khalef et al., 2024). Thus, cytotoxicity testing can be regarded as a pivotal part of in vitro toxicity evaluation (Olegovna et al., 2025). New developments in experimental toxicology have also increased traditional 2D cultures to more complex biological models, thus enhancing their physiological relevance (Jităreanu et al., 2025).

The Neutral Red Uptake or Retention assay is a well-established method among various existing cytotoxicity assays to assess viability of cells by the function of the lysosomes. Neutral Red is a dye that is actively taken up by viable cells inside the lysosomes while cellular injury hampers the uptake of the dye, yielding a quantitative measure of cytotoxicity (Rodrigues et al., 2023). These assays have been successfully applied in the research of environmentally relevant chemicals and can be used to sensitively assess possible effects of exposure at cellular level (Sychrová et al., 2022). These experimental methods are increasingly being used together with high throughput and computational techniques to further refine the ecological hazard characterization (Nyffeler et al., 2025). The toxicological results may vary significantly depending on the factors like chemical structure, dose, particle properties, exposure time, and cellular model, and should be taken into account during the interpretation of the observed cytotoxicity (Nowak-Jary & Machnicka, 2025). In recent years the scope of cytotoxicity testing has further expanded beyond traditional in vitro testing to integrated in silico testing that enhances hazard assessment (Ziemba, 2025).

Chemical and bioassay data from environmental samples have been taken from a publicly available data set from a low mountain river in Central Germany. The resource includes a large number of target-chemical measurements, as well as biological-effect information, such as Neutral Red (NR) cytotoxicity responses, which allows linking contaminant occurrence with in vitro toxicological results. This will provide a chance to examine the differences in chemical burden of various environmental samples, and to investigate concentration dependent cytotoxicity and if the loading of

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contaminant is related to increased cellular effects.

Objectives

- To characterize the occurrence and distribution of emerging chemical contaminants across the studied environmental samples.
- To evaluate concentration-dependent cytotoxicity using Neutral Red Retention measurements and compare responses among samples.
- To examine the relationship between chemical contaminant burden and observed in vitro cytotoxicity.

2. Materials and Methods

2.1 Study Design

A quantitative toxicological design that used secondary data was used to assess the cytotoxic effects of mixtures of emerging chemical contaminants in environmental water samples. Both target chemical analysis and in vitro Neutral Red cytotoxicity responses were incorporated into the analysis. Six environmental samples (H1 to H6) were studied, and the trip blank was kept largely for quality-control evaluation. The analytical strategy aimed to describe the frequency of contaminant occurrence, patterns of cytotoxicity, the effects of contaminant concentration on the responses, and the relationship between the contaminant burden and cellular toxicity.

2.2 Data Source and Study Material

Data were collected in a low mountain river in the middle of Germany for CEC monitoring with chemical and bioassay methods and were published on Zenodo (Weichert et al., 2024). Four data files were provided: LVSPE.csv, NR.csv, EBM.csv and FET.csv. LVSPE.csv was mainly employed for the characterization of chemical contaminants and NR.csv for the assessment of the Neutral Red cytotoxicity. There were 457 compounds observed in the chemical file across the six environmental samples compared to 189 observations in the Neutral Red file across H1–H6 and one trip blank.

2.3 Characterization of Emerging Chemical Contaminants

The LVSPE file was used to get chemical occurrence and concentration information. The file contained compound name, method detection limit, concentrations determined in H1–H6, quality-control data, chemical identifiers, and hierarchical classification of chemical-class data. The 457 compounds spanned several broad categories – such as pesticides, pharmaceuticals, biocides, intermediates, polymer additives, personal care and household chemicals, food and beverage chemicals, veterinary pharmaceuticals, additives, and per- and polyfluoroalkyl substances. For every environmental sample, the number of detected compounds, major chemical class of compounds detected, and the measured concentrations of the contaminants were summarized. If the original file structure had values where no chemical concentration was reported, they were considered non-detected or unavailable.

2.4 Neutral Red Cytotoxicity Assessment

The Neutral Red data set comprised three variables: sample identity, concentration (in as a relative enrichment factor (REF)), and cytotoxicity (%) of the sample. Cytotoxicity measurements were available for nine REF levels ranging from 0.15625 to 40.0. These concentrations were replicated and 27 observations were made for each concentration (H1, H2, H3, H4, H5, H6 and the trip blank). Neutral Red cytotoxicity percentage was assumed as the main biological response. Higher cytotoxicity values were considered indicative of more negative effects on cells caused by exposure to the environmental samples at the higher concentration.

2.5 Outcome and Exposure Variables

The main outcome was NR cytotoxicity (%). The major exposure factors were environmental sample

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ID and REF concentration. Chemical variables in the contaminant file comprised number of chemicals detected in each sample, distribution of compounds in the major chemical classes, and the total amount of contaminant measured. These variables were used to characterize the differences in chemical composition between H1-H6 and determine if there were any correlation with increase in the chemical composition of samples and increase in the cytotoxic response.

2.6 Data Processing

The two main files were screened for variable consistency, missing observations, duplicated observations, and chemical measurements that were not detected prior to the statistical analysis. Chemical compounds were classified based on classification present in the source file. For each environmental sample, detection counts and concentration summaries were done separately. Observations of neutral red were arranged by sample, and by the concentration of the REFs, and descriptive data were retained not averaged at the start of the analysis. The trip blank was reviewed as a quality-control reference only and not considered to be an environmental exposure site.

2.7 Statistical Analysis

Chemical contamination and cytotoxicity values were summarized using descriptive statistics. Data for categorical chemical classes were reported in frequencies and percentage. Approximately normally distributed continuous measurements were summarised as mean and standard deviations, skewed distributions were summarised as median and interquartile ranges. Where appropriate minimum and maximum values were also reported. Results for H1–H6 were summarised separately for the different REF concentrations tested for Neutral Red cytotoxicity. Concentration-response patterns were evaluated by the relationship between the concentration of REF and the cytotoxicity in each of the environmental samples. Pearson correlation was applied if the following assumptions were met approximate linearity and normality; if the assumptions were not met, Spearman rank correlation was applied. Where there was an approximately linear relationship between concentrations and changes in cytotoxicity, simple linear regression was used to estimate the direction and magnitude of the change. If distributional assumptions were not met an appropriate nonparametric comparison was used to test for differences in cytotoxicity among environmental samples. Correlations of sample level contaminant burden with cytotoxic response were also investigated. A two-sided p-value < 0.05 was used to define statistical significance.

2.8 Ethical Considerations

This study consisted of secondary analysis of publicly available chemical and experimental bioassay data on the environment. No human subjects, identifying information, clinical data or personally identifiable biological data were involved. Thus, no further institutional ethics approval or informed consent was needed for the present secondary analysis. The original data source and publication was cited to allow transparency and proper attribution.

3. Results

3.1 Profile of Emerging Chemical Contaminants

In total 457 target compounds were analysed, 231 of which were found in at least one of the six environmental samples. Pharmaceuticals were the biggest class found, followed by intermediates, pesticides, personal-care and household products, and polymer additives. Food and beverage-related compounds, dyes, personal-care chemicals, household chemicals and pharmaceuticals were also found to have high detection proportions. Table 1 provides a summary of the distribution of monitored and detected chemicals by major compound classes and Figure 1 displays the distribution of monitored and detected chemicals by major compound classes.

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Table 1. Distribution of monitored and detected emerging chemical contaminants by compound class

Compound class	Monitored compounds, n	Detected compounds, n	Detection proportion (%)
Pharmaceutical	143	95	66.4
Pesticide	143	56	39.2
Intermediate	38	17	44.7
Personal care and household	24	16	66.7
Polymer additive	29	15	51.7
Biocide	38	13	34.2
Food and beverage	13	8	61.5
PFAS	7	4	57.1
Additive	8	4	50.0
Dye	4	3	75.0
Veterinary pharmaceutical	8	0	0.0
Natural compound	1	0	0.0
Solvent	1	0	0.0

As shown in Figure 1, pharmaceutical compounds dominated the detected chemical profile, whereas pesticides represented the second-largest group. Other compound classes occurred less frequently but contributed to the overall complexity of the contaminant mixture.

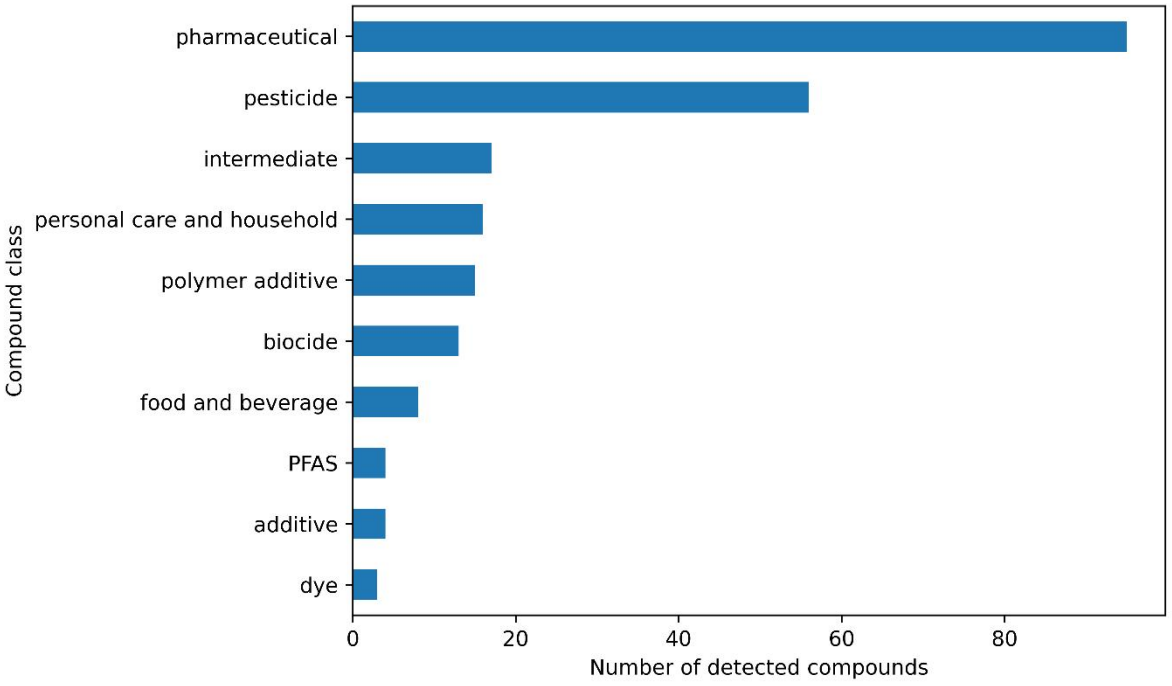

Figure 1. Detected Emerging Contaminants by Chemical Class

3.2 Chemical Burden Across Environmental Samples

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H1–H6 showed a significant amount of variation in chemical contamination. The highest summated concentration of all the compounds detected was in H6 while H1 had the lowest number of compounds detected. The chemical burdens of H3, H4, H5 and H6 were significantly higher than that of H1 and H2. H6 also had the highest sum of measured concentration with H3 having a concentration that was also very close. The contamination was significantly different from one location to the next, as shown by the gradient in Table 2 and in the number of contaminants detected, as shown in Figure 2.

Table 2. Chemical contaminant burden across environmental samples

Sample	Detected compounds, n	Summed concentration	Mean concentration	Median concentration	Maximum concentration
H1	14	141.2	10.09	5.25	44.0
H2	64	5,126.3	80.10	4.45	1,697.8
H3	189	65,104.9	344.47	16.90	7,569.3
H4	169	50,636.3	299.62	11.90	6,932.7
H5	161	47,594.2	295.62	12.60	8,038.2
H6	196	66,789.0	340.76	19.90	11,448.9

The distribution shown in Figure 2 confirmed a clear increase in chemical complexity from H1 and H2 toward H3–H6, with H6 containing almost 200 detected compounds.

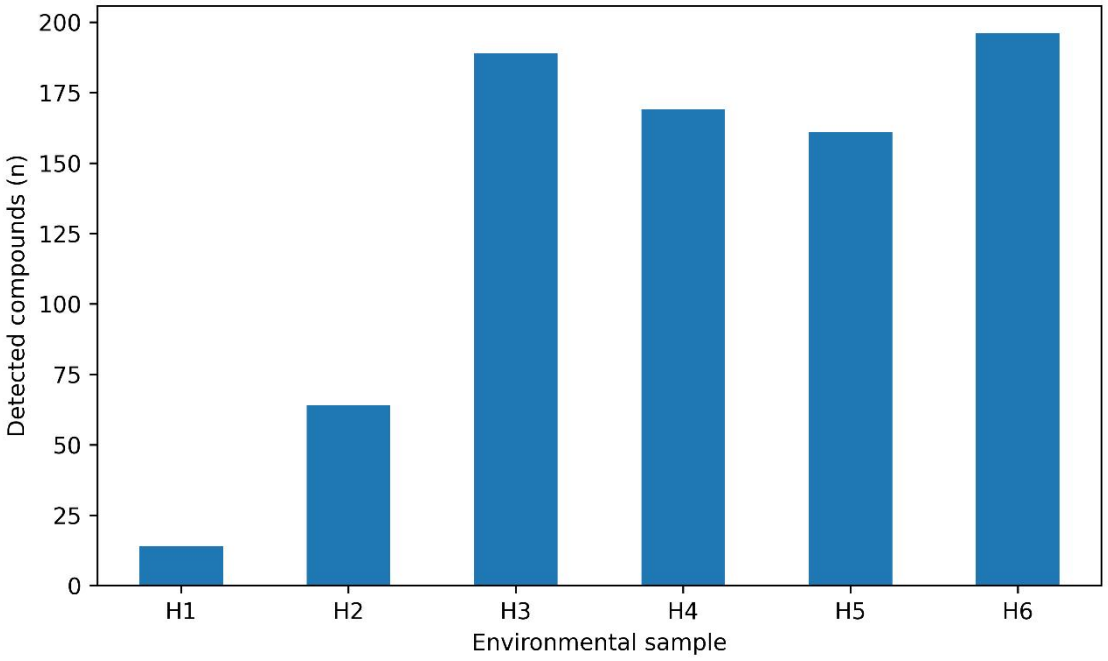

Figure 2. Detected Chemical Contaminants Across Environmental Samples

3.3 Neutral Red Cytotoxicity Profiles

The range of Neutral Red responses was great among samples. The minimal cytotoxicity was obtained in H1, which was found to be 0.19% overall mean of tested exposure range. H4, H6, and H3 had higher overall mean cytotoxicity, respectively, than did the others. The highest individual cytotoxicity of 74% was obtained with H4, 71% with H3 and 62% with H6. The distributions of toxicity data were very skewed, with many values at the lower concentrations showing little or no

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response, as evidenced in Table 3.

Table 3. Descriptive statistics of Neutral Red cytotoxicity across environmental samples

Sample	n	Mean ± SD (%)	Median (%)	IQR (%)	Minimum (%)	Maximum (%)
H1	27	0.19 ± 0.79	0.0	0.0	0	4
H2	27	4.93 ± 7.11	0.0	10.5	0	29
H3	27	6.22 ± 15.37	0.0	4.0	0	71
H4	27	10.26 ± 16.27	1.0	16.0	0	74
H5	27	5.04 ± 9.47	0.0	5.0	0	30
H6	27	8.81 ± 13.85	5.0	11.5	0	62

Overall cytotoxicity distributions differed significantly among the six environmental samples when all tested concentrations were considered (Kruskal–Wallis H = 18.63, p = 0.002). Figure 3 further demonstrates the wider cytotoxicity distributions in H3, H4, H5, and H6 compared with H1.

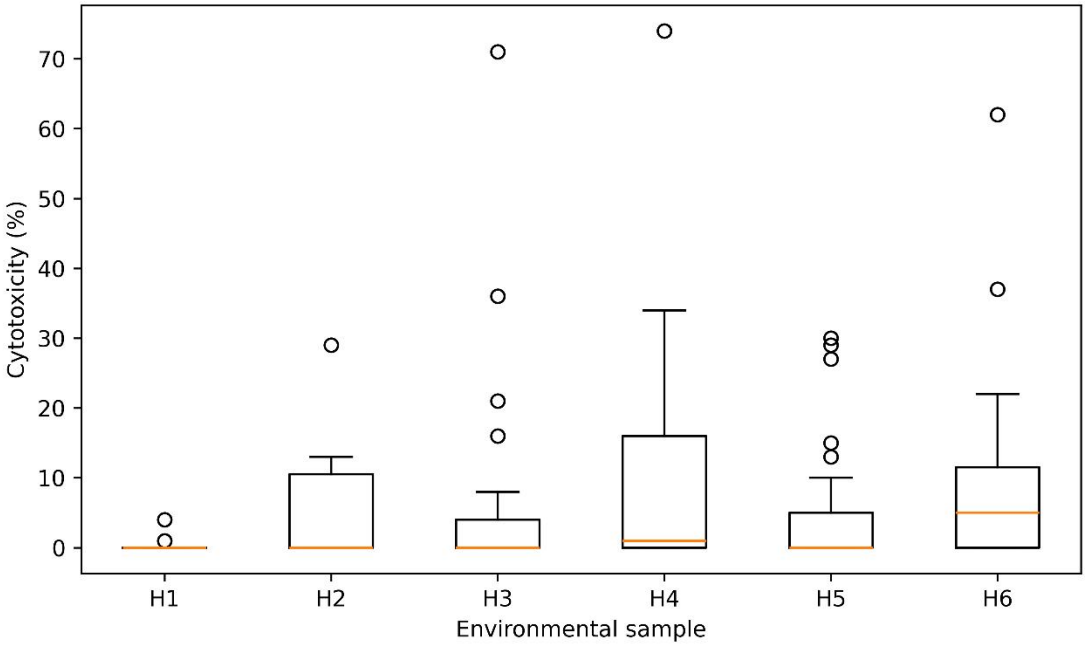

Figure 3. Distribution of Neutral Red Cytotoxicity by Environmental Sample

3.4 Concentration-Dependent Cytotoxicity

Generally, the cytotoxicity increased as the concentration of the REF increased, but the trend varied with the samples. The concentration-response relationship was best defined for H3 and H6. Mean cytotoxicity at 40 REF reached approximately 35.7% in H3, 36.0% in H4, and 36.7% in H6, compared with only 1.7% in H1. H5 also exhibited a positive trend at the highest exposure level and H2 exhibited a less consistent exposure trend. The complete concentration-response profiles are shown in Figure 4.

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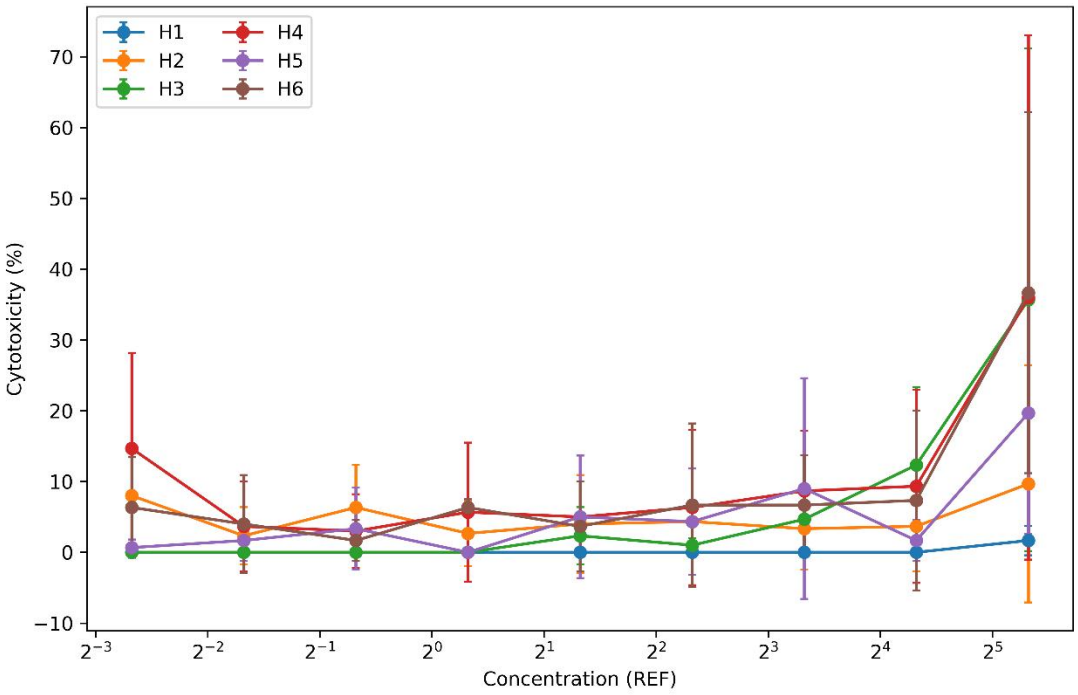

Figure 4. Neutral Red Cytotoxicity Across REF Concentrations

Correlation and regression results further characterized these concentration-response patterns (Table 4). H3 showed a very strong positive monotonic association between REF and mean cytotoxicity, while H6 also demonstrated a strong significant association. Linear regression indicated significant increasing trends for H1, H3, H4, H5, and H6, whereas H2 showed no significant linear relationship.

Table 4. Concentration-response relationships for Neutral Red cytotoxicity

Sample	Spearman ρ	p-value	Regression slope	R^2	Regression p-value
H1	0.548	0.127	0.036	0.765	0.002
H2	0.150	0.700	0.099	0.273	0.149
H3	0.940	<0.001	0.866	0.968	<0.001
H4	0.500	0.170	0.667	0.750	0.003
H5	0.653	0.057	0.382	0.693	0.005
H6	0.773	0.015	0.723	0.827	<0.001

3.5 Integrated Chemical Burden and Cytotoxicity

A possible correlation between chemical contamination and cytotoxicity was indicated by an integrated comparison. H1 exhibited the lowest contamination levels, the lowest sum of concentrations and the lowest cytotoxic response (40 REF), while H3–H6 had significantly higher levels of contamination and higher cytotoxic responses. Based on the area under the concentration-response curve, H4 had the greatest measure of total cytotoxic response over the concentration range tested, followed by H3 and H6 (Table 5).

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Table 5. Integrated contaminant burden and Neutral Red cytotoxicity metrics

Sample	Detected compounds, n	Summed concentration	Cytotoxicity at 40 REF, mean (%)	Cytotoxicity AUC
H1	14	141.2	1.67	16.67
H2	64	5,126.3	9.67	207.06
H3	189	65,104.9	35.67	584.79
H4	169	50,636.3	36.00	606.85
H5	161	47,594.2	19.67	316.80
H6	196	66,789.0	36.67	566.69

A strong positive correlation existed between the number of contaminants detected and mean cytotoxicity at 40 REF ($\rho = 0.943$, $p = 0.005$). A comparable correlation was found between the sum of the chemical concentrations and the cytotoxicity at 40 REF (Spearman $\rho = 0.943$, $p = 0.005$). Given the number of environmental samples (n=6) used in these sample-level analyses, associations should be considered exploratory.

The results indicated that the level of chemical contaminants varied significantly between the six environmental samples, as did the NR cytotoxicity. The contaminant groups that were found most often were pharmaceuticals and pesticides, and H3–H6 had the highest overall chemical loads. Cytotoxicity generally increased with higher REF concentrations and H3, H4 and H6 had the highest responses. In total, there was a positive correlation between contaminant burden and cytotoxicity, suggesting a possible link between combination of contaminants and cytological responses.

4. Discussion

The present study showed significant variations in chemical contamination in the six environmental samples as well as in the Neutral Red cytotoxicity. H3-H6 had significantly more chemical compounds and higher summed levels of chemicals detected than H1 and H2, and H3-H6 also had increased cytotoxic responses, particularly at higher REF levels. H3, H4 and H6 exhibited the highest toxicity in cells at 40 REF, while H1 were consistently seen to have little effect on cells. This trend indicates that in general, higher level of chemical complexity and higher concentration may lead to higher adverse cellular effects. The high concentration cytotoxicity was also significantly associated with contaminant burden, which further indicates the potential toxicological significance of complex mixtures in the environment, although the limited number of sample locations must be interpreted with care.

The increase in cytotoxicity at higher concentrations observed in this study is in line with earlier findings that cell response is more intense when a chemical concentration is higher. A series of combined chemical and biological testing studies have also shown that cytotoxicity can give information which cannot be obtained from chemical concentrations alone (Feng et al., 2023). Similar studies on multifunctional chemical formulations have also concluded that chemical composition and biological compatibility need to be considered concurrently in the evaluation of toxicity (Ballal et al., 2025). Thus, the differences between the H1 – H6 responses could be due to both the total amount of chemicals present as well as the type and interactions between the chemicals. The extent and way samples with comparable burden can lead to differing cellular responses may be attributed to molecular features (Calé et al., 2025).

The notable responses seen in the H3, H4 and H6 are also consistent with those reported in studies on individual emerging contaminants. For instance, 6PPD-quinone has been found to disrupt essential

cellular processes in vitro by inducing mitochondrial dysfunction, highlighting the potential for recently identified environmental contaminants to cause such effects (Mahoney et al., 2022). The toxicity of related contaminants can be altered through structural changes, underscoring the need to consider transformation products and metabolites in environmental assessments (Nair et al., 2025). Similarly, rapid exposure to quaternium-15 has been linked to detectable cellular and physiological changes, highlighting the sensitivity of cell-based assays to exposure to contaminants (Multisanti et al., 2023). Exposure to benzisothiazolinone has been shown to cause similar cellular changes, signaling that emerging chemicals can impact biological function even at controlled short-term exposure (Impellitteri et al., 2025).

The results are also applicable to other classes of contaminants besides traditional dissolved chemicals. The toxicity of emerging pollutants like micro- and nanoplastics is complex, depending on the type of polymer as well as the size and type of particle (Dou et al., 2025). Chemical loaded microplastics will also have an added biological effect as a carrier of other contaminants (Li et al., 2023). Similarly, environmental nanomaterials have been found to exhibit toxicological effects that vary with their composition and exposure profiles (Rusconi et al., 2024), and engineered nanostructures can also exhibit variable toxicological effects based on material properties and biological context (Vihodceva et al., 2025). The observations further support the necessity to consider mixture toxicity as a result of chemical concentration, composition and physicochemical properties.

Methodologically, the Neutral Red response proved to be a good overall measure of cell damage. Cell-based aquatic models have been found to be useful for assessing contaminant-related cytotoxicity and provide a convenient method for environmental hazard assessment (de Souza et al., 2023). Applied cytotoxicity testing has also proven to be valuable in differentiating between materials and different formulations with varying biological safety profiles (Kandarova et al., 2025). The use of cellular systems also for screening potentially harmful chemical exposures is further supported by the in vitro testing of biocides and antifungal compounds (Liao et al., 2024). Additional ecotoxicological approaches based on mussel cells and hemocyte responses also show the usefulness of cellular biomarkers for detecting biologically relevant environmental stress (Udayan et al., 2024).

The study has several implications. Combined with an in vitro bioassay, chemical monitoring could give a better picture of environmental hazard than monitoring chemicals alone. However, secondary data were used, just six environmental samples were compared, and the effects of the cytotoxicity were mixture-level responses, not necessarily attributable to individual compounds. Thus, it is not possible to create causal relationships. Future research should involve more sampling locations, individual testing of priority contaminants and in defined mixtures, additional cellular endpoints and testing of the reproducibility of the responses observed using Neutral Red testing across multiple cell systems and exposure periods.

5. Conclusion

The present study revealed that in vitro cytotoxicity was significantly different among the environmental samples assessed and associated with the mixture of emerging chemicals. Chemical profiling revealed a variety of contaminants, including pharmaceuticals and pesticides, as the most common types of contaminants found. Chemical burdens of the samples H3–H6 were significantly higher than that of samples H1 and H2 and correlated with the Neutral Red cytotoxicity response, especially at higher REFs. H3, H4 and H6 were the most cytotoxic, while H1 was always least toxic to cells. The concentration-response trends observed suggest that cytotoxicity tended to increase with increasing sample enrichment, with varying degrees and consistency of response between samples. The positive association between contaminant burden and cytotoxicity at the highest exposure also indicates that the more complex and concentrated the chemicals, the more adverse cellular effects may result. The results demonstrate that combining chemical exposure data with in vitro bioassays can be useful because the impact of complex environmental mixtures may not be fully captured by the chemical concentration data alone. Neutral Red Retention was a good indicator of cellular injury and helped to discriminate samples based on toxicological profile. But the small number of

environmental samples and the fact that the exposure occurs at the mixture level, make it difficult to attribute any toxicity to individual contaminants. To improve environmental risk assessment, future research must involve more sampling locations, defined mixture experiments, individual compound tests, and other cellular endpoints.

References

1. Albero, B., Sánchez-Argüello, P., Martín-Esteban, A., Tampio, E., Laaksonen, I., & Pérez, R. A. (2025). Analysis of Organic Contaminants and In Vitro Cytotoxicity to Test the Suitability of External Organic Matter Processing. ACS Environmental Au, 5(4), 376-386.
2. Ballal, N. V., Narkedamalli, R., Shenoy, P. A., Das, S., Balasubramanian, S. K., Varghese, J., ... & Tay, F. R. (2025). Biological and chemical properties of new multi-functional root canal irrigants. Journal of Dentistry, 153, 105551.
3. Calé, A., Elblová, P., Andělová, H., Lunova, M., & Lunov, O. (2025). Analyzing molecular determinants of nanodrugs' cytotoxic effects. International Journal of Molecular Sciences, 26(14), 6687.
4. Çetin, Y. (2025). Cytotoxicity: A Crucial Toxicity for In Vitro Experiments. In Cytotoxicity-A Crucial Toxicity Test for In Vitro Experiments. IntechOpen.
5. de Souza, I. R., Sivek, T. W., de Oliveira, J. B. V., Canavez, A. D. P. M., de Albuquerque Vita, N., Schuck, D. C., ... & Leme, D. M. (2023). Cytotoxicity assays with zebrafish cell lines. Journal of Visualized Experiments (JoVE), (191), e64860.
6. Dou, J. Y., Liu, S., Wang, C. Y., Dai, X., Lian, L. H., Cui, Z. Y., ... & Wu, Y. L. (2025). Microplastics and nanoplastics, emerging pollutants, increased the risk of pulmonary fibrosis in vivo and in vitro: a comparative evaluation of their potential toxicity effects with different polymers and size. Toxicology, 154304.
7. Feng, D., Li, X., Fan, X., Guo, Y., Zhang, J., Yuan, H., ... & Han, T. (2023). Cytotoxicity, endocrine disrupting activity, and chemical analysis of 42 food contact silicone rubber products. Science of the Total Environment, 872, 162298.
8. Grace, D. N., Rorie, A., & Prasse, C. (2025). In chemico toxicity approaches to assess, identify and prioritize contaminants in water. Nature Water, 3(8), 854-866.
9. Hakeem, I. J., & Amare, N. (2022). In Vitro Evaluation of the Cytotoxic Potential of Environmental Contaminant Mixtures Present in Water for Human Use. Adsorption Science & Technology, 2022, 3102007.
10. Impellitteri, F., Riolo, K., Zicarelli, G., Porretti, M., Multisanti, C. R., Piccione, G., ... & Faggio, C. (2025). Evaluation of cellular and physiological alterations of cells from Mytilus galloprovincialis exposed to benzisothiazolinone. Ecotoxicology and Environmental Safety, 290, 117631.
11. Jităreanu, A., Agoroaei, L., Caba, I. C., Cojocaru, F. D., Vereștiuc, L., Vieriu, M., & Mârțu, I. (2025). The evolution of in vitro toxicity assessment methods for oral cavity tissues—from 2D cell cultures to organ-on-a-chip. Toxics, 13(3), 195.
12. Kandarova, H., Pôbiš, P., Sáková, O., & Jakubovská, A. (2025). Assessment of cytotoxicity, skin and oral irritation of medical devices with hydrating properties: A case study. Interdisciplinary Toxicology, 15(1), 19-24.
13. Khalef, L., Lydia, R., Filicia, K., & Moussa, B. (2024). Cell viability and cytotoxicity assays: Biochemical elements and cellular compartments. Cell biochemistry and function, 42(3), e4007.
14. Li, J., Jong, M. C., Gin, K. Y. H., & He, Y. (2023). Size-dominated biotoxicity of microplastics laden with benzophenone-3 and ciprofloxacin: Enhanced integrated biomarker evaluation on mussels. Environmental Pollution, 333, 122018.
15. Liao, Z., Laurent, N., Hirt-Burri, N., Scaletta, C., Abdel-Sayed, P., Raffoul, W., ... & Applegate, L. A. (2024). Sustainable primary cell banking for topical compound cytotoxicity assays: Protocol validation on novel biocides and antifungals for optimized burn wound care. European burn journal, 5(3), 249-270.

16. Mahoney, H., da Silva Junior, F. C., Roberts, C., Schultz, M., Ji, X., Alcaraz, A. J., ... & Brinkmann, M. (2022). Exposure to the tire rubber-derived contaminant 6PPD-quinone causes mitochondrial dysfunction in vitro. Environmental Science & Technology Letters, 9(9), 765-771.
17. Manful, M. E., Ahmed, L., & Barry-Ryan, C. (2024). Cosmetic formulations from natural sources: safety considerations and legislative frameworks in the European Union. Cosmetics, 11(3), 72.
18. Multisanti, C. R., Riolo, K., Impellitteri, F., Chebbi, I., Faggio, C., & Giannetto, A. (2023). Short-term in vitro exposure of Pinctada imbricata's haemocytes to quaternium-15: exploring physiological and cellular responses. Environmental Toxicology and Pharmacology, 101, 104198.
19. Nair, P., Barrett, H., Tanoto, K., Xie, L., Sun, J., Yang, D., ... & Peng, H. (2025). Structure and toxicity characterization of alkyl hydroxylated metabolites of 6PPD-Q. Environmental Science & Technology, 59(15), 7474-7484.
20. Nowak-Jary, J., & Machnicka, B. (2025). Toxicity of magnetic nanoparticles in medicine: contributing factors and modern assessment methods. International Journal of Molecular Sciences, 26(17), 8586.
21. Nyffeler, J., Harris, F. R., Willis, C., Byrd, G., Blackwell, B., Escher, B. I., ... & Harrill, J. A. (2025). A combination of high-throughput in vitro and in silico new approach methods for ecotoxicology hazard assessment for fish. Environmental Toxicology and Chemistry, 44(9), 2599-2621.
22. Olegovna, T. E., Sattarovna, K. Z., Rustamovna, U. M., Arziyevna, T. N., & Bakhrievna, A. O. (2025). Cytotoxicity is the key test for in vitro toxicity. Cytotoxicity Crucial Toxicity Test In vitro Exp, 71-71.
23. Rodrigues, R. M., Stinckens, M., Ates, G., & Vanhaecke, T. (2023). Neutral red uptake assay to assess cytotoxicity in vitro. In Cell Viability Assays: Methods and Protocols (pp. 237-245). New York, NY: Springer US.
24. Rusconi, T., Riva, L., Punta, C., Solé, M., & Corsi, I. (2024). Environmental safety of nanocellulose: an acute in vivo study with marine mussels Mytilus galloprovincialis. Environmental Science: Nano, 11(1), 61-77.
25. Sychrová, E., Yawer, A., Laboha, P., Basu, A., Dydowiczova, A., Virmani, I., ... & Sovadinova, I. (2022). In vitro testicular toxicity of environmentally relevant endocrine-disrupting chemicals: 2D vs. 3D models of prepubertal Leydig TM3 cells. Environmental Toxicology and Pharmacology, 93, 103869.
26. Udayan, G., Giordano, M. E., Pagliara, P., & Lionetto, M. G. (2024). A Toxicological and Ecotoxicological Assay Based on Mussel (Mytilus galloprovincialis) Hemocytes Motility. JoVE (Journal of Visualized Experiments), (214), e67285.
27. Vihodceva, S., Šutka, A., Iesalnieks, M., Orlova, L., Pludonis, A., Otsus, M., ... & Kasemets, K. (2025). Emerging investigator series: CeO2/CuO nanostructured composite with enhanced antimicrobial properties and low cytotoxicity to human keratinocytes in vitro. Environmental Science: Nano, 12(1), 276-291.
28. Weichert, F. G., Brack, W., Brauns, M., Fink, P., Johann, S., Krauss, M., & Hollert, H. (2024). Chemical analysis dataset for contaminants of emerging concern and bioanalytical data for samples from a low mountain stream in Central Germany [Data set]. Zenodo. https://doi.org/10.5281/zenodo.10892039
29. Ziemba, B. (2025). Advances in cytotoxicity testing: from in vitro assays to in silico models. International Journal of Molecular Sciences, 26(22), 11202.