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Review Article

Clean isn't always safe: zebrafish (*Danio rerio*) and the toxic truth of treated wastewater

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ABSTRACT. Wastewater discharges pose a significant risk to aquatic ecosystems due to the complex mixtures of contaminants they contain. Although the toxicity of individual contaminants is well documented, knowledge regarding the interactions among contaminants in wastewater mixtures remains limited. Zebrafish (*Danio rerio*) are a well-established biological model due to their genetic similarity to humans, transparent embryonic development, and sensitivity to pollutants, making them suitable for assessing wastewater toxicity. This systematic review followed PRISMA guidelines and analyzed studies published between 2015 and 2025 in Scopus and Scielo. A total of 615 articles were screened, and 52 met all inclusion criteria. Most studies focused on treated wastewater (n = 31), followed by raw wastewater (n = 17), and frequently evaluated the embryonic stage (n = 37). Industrial, domestic, and hospital wastewater were the most common sources. The predominant toxicological outcomes included acute toxicity and teratogenesis, although reproductive, genotoxic, oxidative, and behavioral effects were also reported. Pharmaceuticals and potentially toxic elements were the most frequently measured contaminants, whereas groups such as flame retardants, pesticides, and polycyclic aromatic hydrocarbons were rarely addressed. The reviewed studies indicate that exposure to some raw and treated wastewater samples can induce adverse effects on zebrafish development, particularly during early life stages. Major gaps include limited evaluation of larval and adult stages, underrepresentation of certain wastewater sources, and insufficient characterization of complex contaminant mixtures. Addressing these gaps is essential to strengthen environmental risk assessment and protect aquatic ecosystems.

Keywords: *Danio rerio*; ecotoxicology; wastewater; pollution; environmental toxicology; review

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INTRODUCTION

In Mexico, 54% of wastewater is untreated and discharged raw into natural water bodies. At the same time, the rest undergoes treatment consisting of mechanical separation of solid waste, sedimentation, biological oxidation of organic matter, and chemical disinfection with chlorine. Treated wastewater infiltrates the subsoil or is discharged into rivers, lakes, or oceans (Robledo-Zacarías et al. 2017). Raw wastewater is untreated sewage containing the original mix of contaminants from domestic, industrial, or urban sources. It has become a biological model due to its similar genome. In contrast, treated wastewater refers to the effluent discharged after physical, chemical, and/or biological treatment processes designed to reduce contaminant concentrations before discharge or reuse (Dotan et al. 2017).

Wastewater treatment plants (WWTPs) are facilities designed to remove physical, chemical, and biological contaminants from water used by human activities before it is returned to the environment. WWTPs were designed to treat wastewater from urbanization; however, they do not completely remove all contaminants (Papa et al. 2016). The efficiency of contaminant removal depends on the type of treatment, but in general, WWTPs are not fully equipped, resulting in low removal rates (Deblonde et al. 2011, Zhou et al. 2019).

The main sources of wastewater include domestic, agricultural, and livestock activities, industrial processes, and urban runoff. Treated wastewater contains two large groups of pollutants: traditional and emerging (Feng et al. 2022). Traditional pollutants are those whose toxic effects have been studied. This group includes potentially toxic elements such as chromium, mercury, nickel, cadmium, and arsenic (Zhou et al. 2019).

On the other hand, emerging pollutants are those whose presence has recently been studied in the environment and whose toxic effects are not fully known. Examples include pharmaceutical and personal care products, surfactants, pesticides, and microplastics (Von Sperling 2007, Ahmed et al. 2017, Zhou et al. 2019). These xenobiotics and their metabolites can cause acute and chronic toxicity in aquatic organisms and can even accumulate in their tissues, posing a threat to both ecosystems and humans (Brar et al. 2010, Babić et al. 2017).

Wastewater toxicity can be acute or chronic: acute toxicity refers to adverse effects that occur within a short period after exposure to a contaminant, commonly assessed by mortality in exposed organisms. On the other hand, chronic toxicity refers to effects that develop over time, such as alterations in growth, reproduction, and behavior, even at low pollutant concentrations (Gupta 2018).

In this context, zebrafish (*Danio rerio*) have become an important biological model because their genome is highly similar to that of humans. At least 70% of human genes have a homolog in zebrafish, and more than 82% of the genes that cause disease in humans are also found in zebrafish (Ka & Jin 2021). Its use in biomedicine extends to various fields, including the study of cancer, heart disease, and neurodegenerative diseases such as Alzheimer's, as well as drug development. It is also used as a model in research on infection and the innate antiviral immune response in fish (Cassar et al. 2020).

Zebrafish are a reliable model for assessing wastewater toxicity because of their high sensitivity, transparent embryonic development, and well-characterized genetics (Nagel 2002, García-Cambero et al. 2019). From a practical perspective, zebrafish's high fertility rate and low laboratory maintenance costs make them a valuable tool; using this association can provide information on the potential risks to human health and the environment posed by wastewater (Porretti et al. 2022).

Two reviews have documented a sustained increase in pesticide concentrations, which are very common contaminants in wastewater, between 2012 and 2022, with hundreds of studies focusing on the toxic effects of herbicides, insecticides, and fungicides (Gonçalves et al. 2020, Cui et al. 2023). These affect various biological systems, including development, neurotoxicity, genotoxicity, immunotoxicity, and endocrine disruption, mainly in the early stages of development (embryos and larvae). In addition, associations have been identified between specific classes of pesticides, such as organophosphates, pyrethroids, and triazines, and mechanisms of action at the omics level, including transcriptomics, metabolomics, and microbiomics (Cui et al. 2023).

Although several studies examine the toxicity of individual compounds in wastewater, little is known about the effects of co-exposure to complex mixtures of contaminants that persist after treatment. Zebrafish have been useful for detecting adverse effects in early stages of development, but information on their exposure to wastewater in environmentally relevant situations is limited and scattered. Therefore, a review is needed to gather this evidence, identify patterns, and improve the assessment of environmental risk. This study examines the question: What are the toxic effects of raw and treated wastewater on zebrafish, as reported in studies published over the last 10 years?

Therefore, the objective of this study was to evaluate the toxic effects of exposure to raw and treated wastewater on zebrafish development through a systematic review of studies published in the last 10 years, to elucidate how these wastewater samples affect the biological processes of aquatic organisms, and to identify gaps in the scientific literature.

Literature search

The Scopus and Scielo databases were used for data mining, using the following search terms in English (Zebrafish or "*Danio rerio*" or "zebra fish") and (wastewater or sewage or effluent*) and (toxi* or effect* or mortal* or terato* or biomarker* or fecundity or endocrin*) for Scopus and in three languages for Scielo Spanish, Portuguese, and English ("Pez cebra" or peixe-zebra or zebrafish or "zebra fish" or "*Danio rerio*") and ("agua residual" or "agua residual tratada" or efluente* or "treated effluent" or "residual water" or sewage or effluent* or wastewater or sewage or effluent*) and (toxi* or effect* or mortal* or terato* or biomarker* or fecundity or endocrin* or effect* or fecundity or endocrine* or effect* or biomarker* or fecundity). The search covered the last ten years from 2015 to 2025.

Eligibility criteria

The PRISMA methodology was used (Kahale et al. 2022). Scientific articles in the areas of environmental sciences, biological sciences, and toxicology were considered, as were articles published in English, Spanish, and Portuguese. Reviews, articles in the fields of engineering, physics, energy, computing, and mathematics, as well as those published in Chinese, were excluded.

Study selection

Following an initial review of titles, abstracts, and keywords, 179 studies were selected, including those in which zebrafish were exposed to complex mixtures of raw and treated wastewater, as well as those reporting at least one toxicological parameter. All studies in which zebrafish were exposed to individual toxins, as well as those that discussed reducing toxicity through bioremediation, were excluded. After reading the full article, those that compared control groups and studies that discussed the possible environmental risks of raw or treated wastewater were included, while studies that were not conducted in a laboratory and those that did not report frequencies of effects and physicochemical parameters were excluded. Although 52 unique studies were included in the review, the analytical database comprised 59 records because some studies evaluated multiple wastewater types and/or different zebrafish developmental stages. Each unique combination of wastewater type and developmental stage was treated as a separate record for descriptive analyses.

Quality assessment

The methodological quality of the studies included in this review was evaluated using a checklist composed of 10 criteria: 1) consistency between the title and the article content, 2) clarity and structure of the abstract, 3) adequacy of the theoretical and methodological framework, 4) clear statement of the study objective and/or research question, 5) appropriateness and detail of the experimental design, 6) use of appropriate statistical analyses, 7) clarity in the presentation of results, 8) complementarity of figures and tables without unnecessary repetition, 9) consistency of the discussion and conclusions with the study objectives and results, and 10) overall clarity, coherence, and organization of the manuscript. Each criterion was scored as 1 (yes) or 0 (no), yielding a maximum score of 10. Studies with a score of 7 or higher were considered to have sufficient methodological quality and were included in the final analysis. Due to the substantial methodological heterogeneity among the included studies, a formal risk-of-bias assessment tool was not applied. Instead, methodological quality was evaluated using predefined criteria adapted to the objectives and heterogeneity of the reviewed studies.

Data extraction

The data were downloaded into an Excel database, and the following information was extracted from each article: country of study, wastewater type, wastewater source, toxicological parameters, zebrafish developmental stage, adverse effects observed, and contaminants evaluated. To provide an overview, we grouped data by wastewater type, source, zebrafish developmental stage evaluated, and effects of raw and treated water.

Of the 615 articles found in the databases, 52 met all relevant inclusion criteria (Fig. 1). The international journals that publish on the toxicity of raw and treated wastewater to zebrafish development are shown (Fig. 2a). Publications were concentrated on high-impact journals in the environmental and toxicological fields. The journal Science of the Total Environment stands out as the one that publishes the most studies, with 12 articles. This journal is notable for its interdisciplinary approach to environmental issues. It is followed by Chemosphere, with 7 articles, and Environmental Pollution, with 6 articles, both journals noted for their

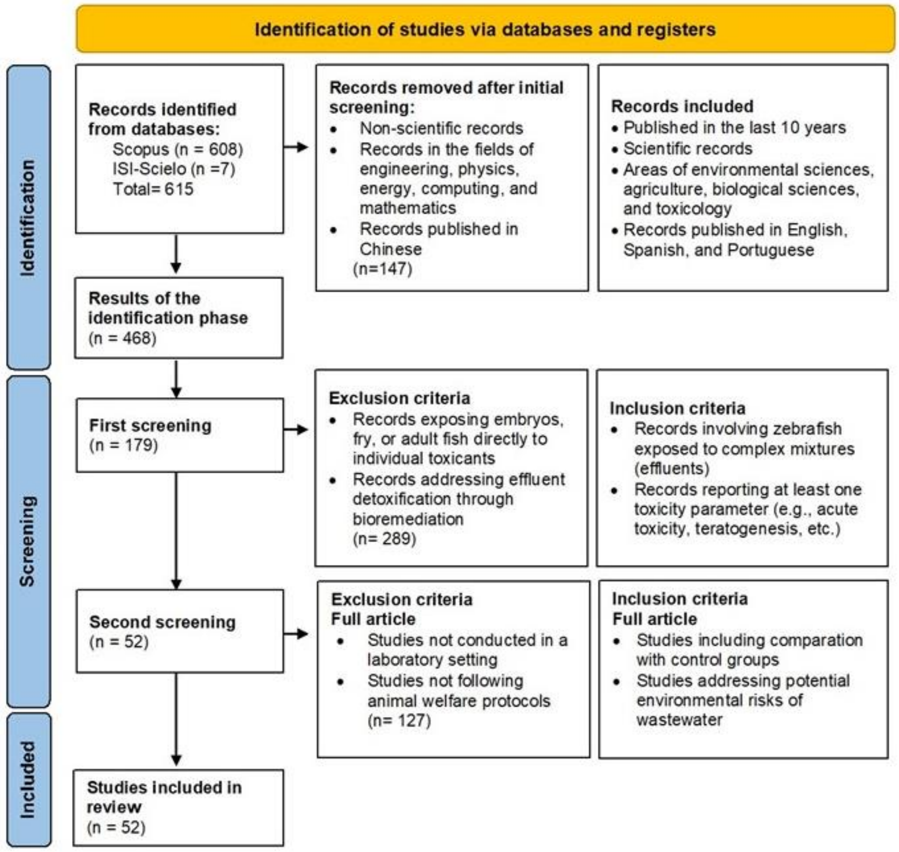

Figure 1. Flowchart showing the literature search process, including the selection and eligibility stages, developed according to PRISMA.

focus on environmental toxicology and environmental risk assessment. These results reflect that the topic of raw and treated wastewater toxicity in zebrafish development is multidisciplinary, encompassing diverse areas and approaches. They also show a concentration of publications in high-impact journals, indicating global and scientific interest in understanding and minimizing the effects of wastewater on aquatic ecosystems.

The annual (blue) and cumulative (red) evolution of publications on the toxicity of raw and treated wastewater to zebrafish development between 2015 and 2025 is shown (Fig. 2b). The overall trend reflects growing academic interest in this topic. Starting in 2016, the number of articles per year increased, peaking in 2017, then declined slightly and stabilized in subsequent years. In 2021, publications increased again and reached another peak between 2022 and 2023, which may be related to the return to laboratories after the COVID-19 pandemic and the rise in emerging pollutants associated with it. The red line represents the cumulative total, showing steady and progressive growth. This trend indicates that the topic has been prioritized, particularly given its relevance to environmental protection.

The countries that publish most on this topic are shown (Fig. 2c). Brazil leads the way in the Americas, which may be due to its large population and developed industrial sectors that generate large amounts of waste. It is also home to ecosystems such as the Amazon rainforest, which is highly vulnerable to pollutants, raising particular concerns about the impact of these waters on this ecosystem. The word cloud of the most frequently occurring words in the studies included in this review, with "toxicity" and "water pollutant" being the most prevalent, is shown (Fig. 2d).

The strategic diagrams are shown (Fig. 3). The upper left quadrant contains the topics "metabolism," "effluent treatment," and "oxidative stress," indicating that these are developed areas with a high degree of specialization. However, their low centrality suggests they are not very connected to or interlinked with other lines of research, indicating that these topics have been extensively studied but have generally been investigated

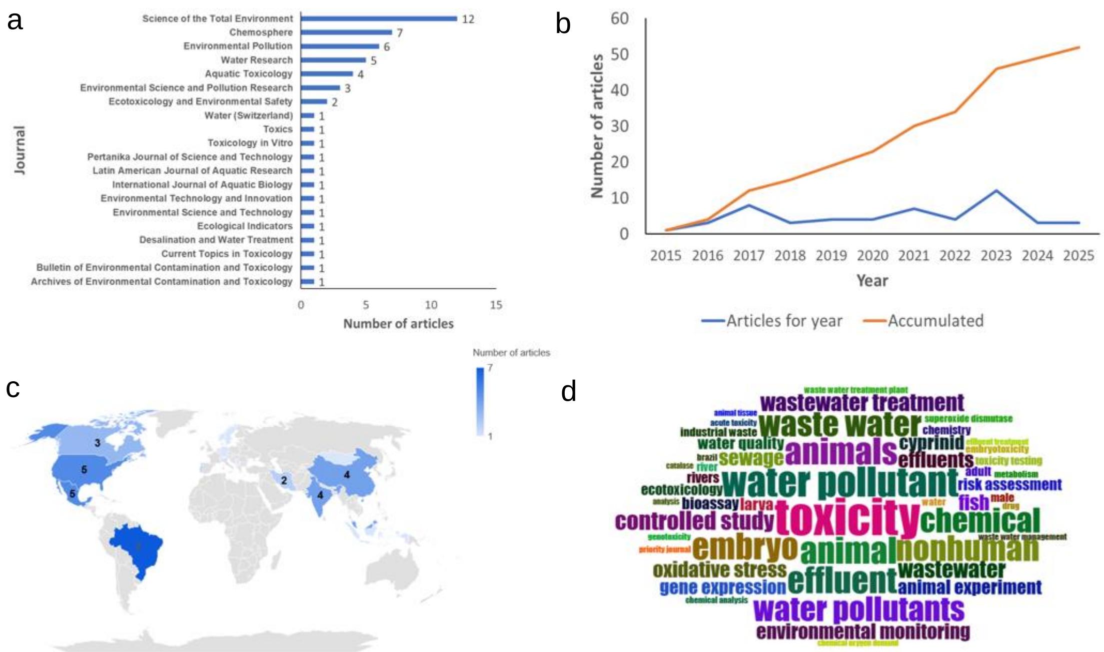

Figure 2. a) Number of articles and journals publishing on the toxicity of wastewater in zebrafish development, b) number of articles published in the last ten years on the toxicity of wastewater in zebrafish development and their cumulative total, c) countries that publish most on the toxicity of wastewater in zebrafish development, d) word cloud generated from the most frequent keywords in the studies included in the systematic review on the toxicity of wastewater in *Danio rerio*. The size of each term represents its relative frequency in the set of publications analyzed.

independently rather than within an integrated framework. On the other hand, the lower right quadrant contains the topics "non-human," "animal," and "animals," indicating that they have not been developed in depth. The absence of topics in the remaining quadrants suggests that research on the toxicity of raw and treated wastewater has not yet developed a consolidated thematic core or shown signs of evolving towards new areas. This situation could be interpreted as fragmentation of knowledge.

The distribution of the studies included in this review according to the source of the water, the type of wastewater (raw, treated, or not reported), and the stage of the fish evaluated is shown in Figure 4. Most studies focus on industrial wastewater (n = 26), followed by domestic wastewater (n = 11) and hospital wastewater (n = 10). Few studies addressed wastewater from mining or urban runoff, and other studies did not specify the source (n = 8). The 52 included studies generated 59 analytical records because several studies reported results for more than one wastewater type or developmental stage. Regarding wastewater type, 31 studies evaluated treated wastewater, 17 evaluated raw wastewater, and in 11 cases, the type was not reported.

Regarding zebrafish developmental stages, the embryonic stage was the most studied, with 37 studies, followed by the adult stage with 16, and finally the larval stage with 6. Of the 52 articles included in this review, 69 toxic effects were identified and grouped into 12 toxicological parameters (Fig. 5). This visualization allows us to identify the main categories of toxicity evaluated in the included studies and the frequency with which they are reported by wastewater type. The most commonly evaluated toxic effects were acute toxicity and teratogenesis, present in both raw and treated wastewater studies. Other effects were also identified, including reproductive, genotoxic, oxidative, phenotypic, and histopathological effects. To a lesser extent, behavioral alterations were reported, along with cardiotoxic, cytotoxic, and neurotoxic effects. A significant proportion of studies did not specify the type of wastewater used, limiting the ability to make accurate comparisons between raw and treated wastewater. Nevertheless, these findings indicate that some raw and treated wastewater samples were associated with adverse effects at different levels of biological organization in zebrafish. Those effects are described in Table 1.

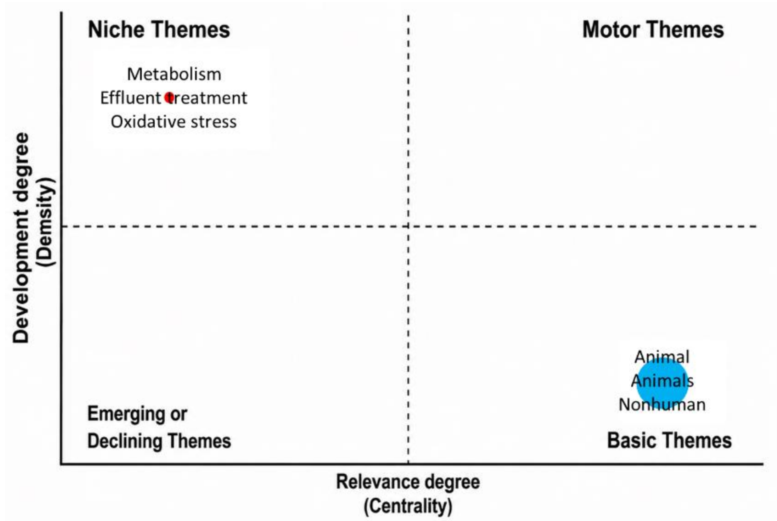

Figure 3. Strategic diagram of the topics identified in studies on wastewater toxicity in zebrafish. The two-dimensional representation is based on two metrics: centrality (X-axis), which indicates a topic's connections to others, and density (Y-axis), which reflects a topic's internal development.

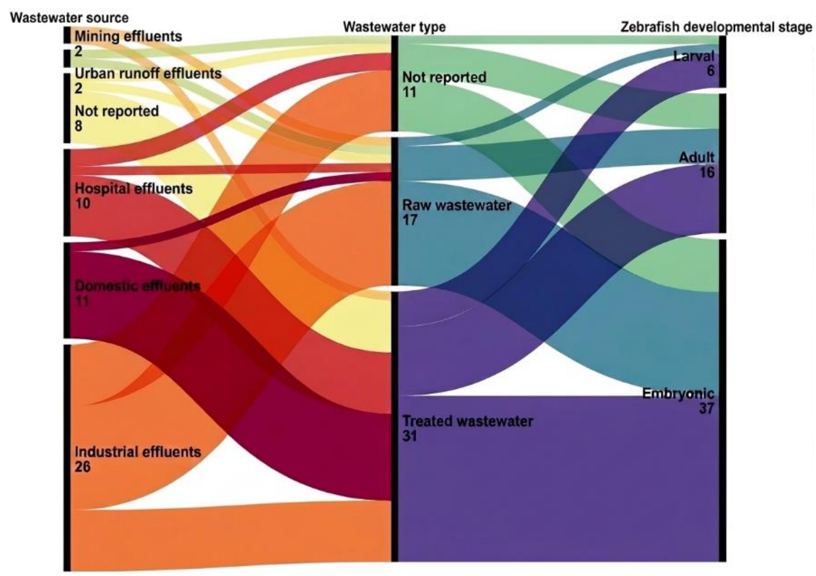

Figure 4. Wastewater source and type used in toxicity analyses, as well as the stage of zebrafish development most frequently evaluated in wastewater toxicity studies.

In the studies analyzed, the most evaluated contaminants in treated wastewater were pharmaceuticals and potentially toxic elements (Fig. 6), reflecting concern about their presence in the environment and their effects on aquatic organisms. On the other hand, in raw wastewater, there was a greater emphasis on volatile organic compounds and potentially toxic elements, with a high frequency of unreported contaminants. Generally, articles that do not report the type of wastewater do not measure contaminants in the water. Certain groups of contaminants, such as flame retardants, perfluoroalkyl substances, sweeteners, polycyclic aromatic hydrocarbons, and pesticides, have been poorly evaluated, highlighting research opportunities to comprehensively characterize the complex mixtures in wastewater. The higher frequency of studies using treated wastewater suggests an interest in evaluating treatment effectiveness, but this has shifted attention away from the risks associated with raw water, which can be even more toxic

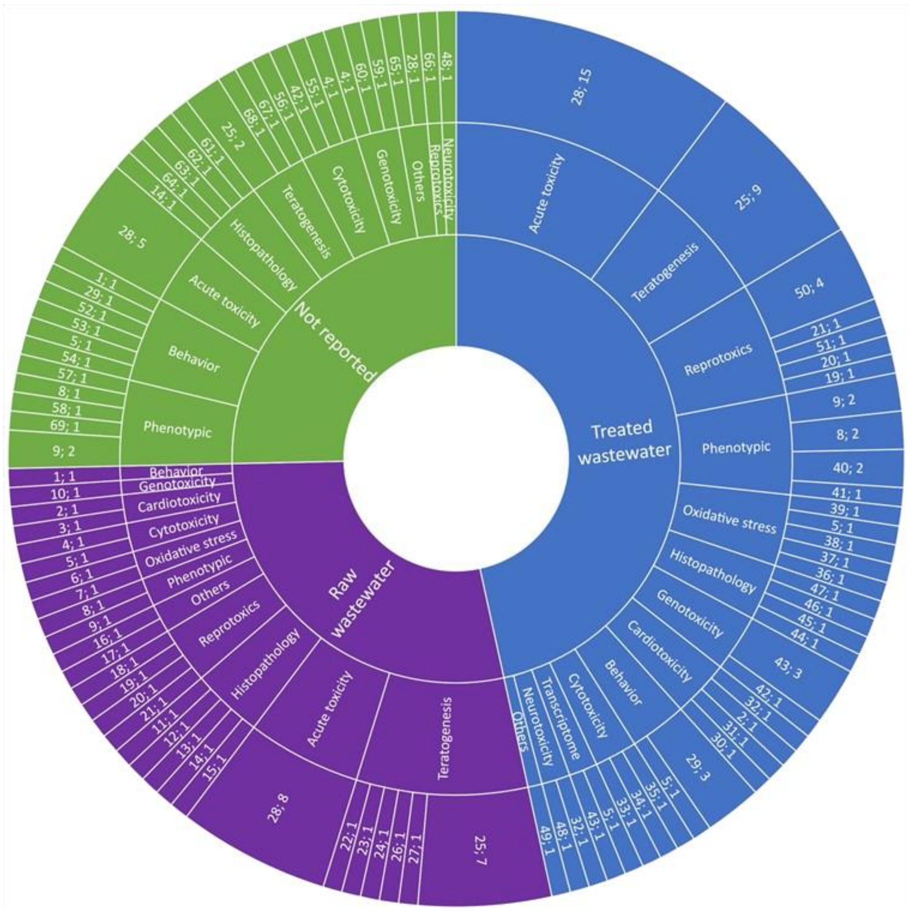

Figure 5. Main toxic effects of raw and treated wastewater on zebrafish development. The inner ring indicates the type of wastewater evaluated (raw, treated, or not reported), and the middle ring shows the categories of toxic effects. In the outer ring, labels are presented in the format effect ID; number of articles reporting that effect, where the effect ID corresponds to the numbering used in Table 1 to describe each specific toxic effect.

than treated water due to the high loads of contaminants and organic matter it carries. The high frequency of studies focusing on the embryonic stage of zebrafish leaves unexplored the possible chronic or cumulative effects at later stages, such as the larval or adult stages. This limits our understanding of the long-term impact of wastewater and is consistent with review studies conducted with glyphosate (Ames et al. 2022), plant products (Falcão et al. 2018), ketamine (Huang et al. 2022), and nanomaterials (Mishra et al. 2023), where they show that the most commonly used stage of zebrafish is the embryonic stage due to its ease of manipulation. On the other hand, less-studied wastewater sources (mining and urban runoff) represent opportunities for future research.

The fact that the most frequently evaluated effects are death and malformations at the embryonic stage suggests that exposure to contaminants at this stage could have severe impacts on the ecosystem and indicates the great danger posed by certain contaminants. There is a lower proportion of effects at the level of neurotoxicity, transcriptome, cardiotoxicity, and oxidative stress. There are some studies on pesticides in which these toxic effects are most commonly studied (Gonçalves et al. 2020, Cui et al. 2023), which may suggest that many of these xenobiotics target the nervous system and heart (Ruíz-Arias et al. 2023).

The fact that potentially toxic elements and pharmaceuticals are the most closely monitored indicates that these pollutants are perceived as the most dangerous due to their potential to cause immediate or long-term negative effects on aquatic ecosystems and human health. Traditional pollutants pose the greatest risk in treatment plants and should therefore be prioritized (Zhou et al. 2019). The lack of study of other

Table 1. Adverse effects on zebrafish development caused by exposure to raw and treated wastewater. MDA: malondialdehyde, AST: aspartate aminotransferase, LDH: lactate dehydrogenase, ALP: alkaline phosphatase, ALT: alanine aminotransferase, CAT: catalase, GGT: gamma-glutamyl transferase.

ID	Associated effect	ID	Associated effect	ID	Associated effect
1	Anxiety	24	Embryo coagulation	47	Delayed eye development
2	Decreased heart rate	25	Malformations in embryonic development	48	Acetylcholinesterase inhibition
3	Reduced metabolic respiration rate	26	Unabsorbed yolk sac	49	Changes in microbiota
4	Increased apoptosis	27	Absence of protruding mouth	50	Induction of vitellogenin in males
5	Not reported	28	Mortality	51	Increased reproductive success
6	Increased expression of genes involved in biotransformation	29	Reduced active swimming	52	Hyperactivity
7	Lipid peroxidation	30	Increased heart rate	53	Erratic swimming
8	Decreased growth	31	Increased respiratory rate	54	Loss of equilibrium
9	Decreased hatching success	32	None	55	Increased reactive oxygen species (ROS)
10	Increased MDA, AST, LDH, ALP, ALT, and CAT, with decreased GGT	33	Altered lipid metabolism pathways	56	Decreased cell viability
11	Increase in apoptotic hepatocytes	34	Altered energy metabolism	57	Color change
12	Vessel dilation	35	Reduced aminoacids levels	58	Intestinal bloating
13	Hyperemia	36	Elevated biomarkers of oxidative damage	59	Altered expression of genes related to detoxification
14	Necrosis	37	Lipid and protein damage	60	Altered expression of genes related to the antioxidant response
15	Vacuolization	38	Increased antioxidant activity	61	Congestion
16	Elevated erythropoietin levels	39	Reduction in antioxidant response	62	Hemorrhage
17	Reduced aerobic capacity	40	Lipid accumulation	63	Hyperplasia
18	Decreased fertility	41	Significant weight loss	64	Hypertrophy
19	Impaired mitochondrial function	42	DNA damage	65	Reduced movement of the operculum
20	Reduced integrity and viability of sperm membranes	43	Inhibition of gene expression	66	Reduced lactate content in testes
21	Reduced sperm motility	44	Disruption of muscle organization	67	Yolk sac edema
22	Absence of pectoral fins	45	Notochord malformations	68	Pericardial edema
23	Absence of swim bladder inflation	46	Delayed brain development	69	Delayed development

types of pollutants indicates that they exceed our analytical capacity (Richardson & Ternes 2022, Medina & Farmer 2024). These authors highlight limitations in current analytical techniques and therefore recommend developing new techniques to address these challenges effectively.

The toxic effects reported in zebrafish exposed to raw and treated wastewater are likely the result of the combined action of multiple contaminants that affect interconnected molecular and physiological pathways. Pharmaceuticals have been associated with developmental abnormalities, behavioral alterations, endocrine disruption, and changes in gene expression (Porretti et al. 2022). Volatile organic compounds may induce oxidative stress, neurotoxicity, and genotoxic effects (Lu et al. 2025). One of the most frequently reported mechanisms is oxidative stress, characterized by the overproduction of reactive oxygen species and alterations in antioxidant defenses, leading to lipid peroxidation, DNA damage, mitochondrial dysfunction, and apoptosis (Derikvandy et al. 2020). Endocrine disruption is another relevant mechanism, particularly for pharmaceuticals and pesticides that interfere with hormonal signaling and reproductive processes (Biswas et al. 2021). Sensitivity to toxic effects varies among developmental stages, and early life stages are particularly useful for evaluating developmental toxicity in zebrafish (Hill et al. 2005). Overall, the available evidence suggests that wastewater toxicity results from the interactions of multiple contaminants, acting through diverse mechanisms that connect molecular changes to effects at cellular, physiological, and organismal levels.

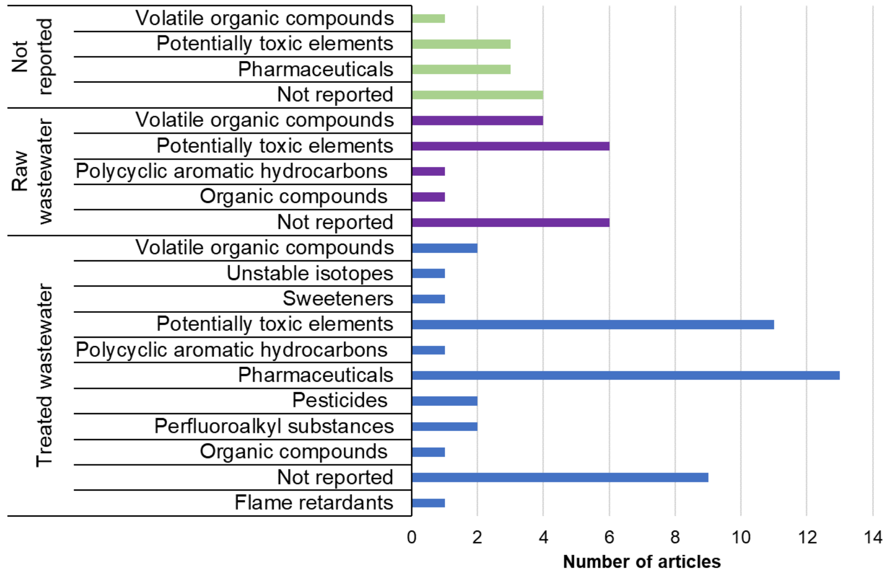

Figure 6. Frequency of the main contaminants measured in toxicity studies of raw and treated wastewater on zebrafish development.

The contaminants identified in the reviewed studies were grouped into broad categories, including pharmaceuticals, potentially toxic elements, volatile organic compounds, and pesticides, to facilitate comparison among studies. However, many studies did not chemically characterize the wastewater samples, and those that did generally analyze only a limited number of contaminants. As a result, the available information did not allow for a comprehensive characterization of wastewater mixtures or the identification of dominant compounds, toxic equivalency factors, or priority contaminants. Therefore, the present review provides a qualitative overview of the principal contaminant groups associated with toxic effects in zebrafish rather than a compound-specific prioritization.

Additional limitations of this review include a high proportion of unreported data and an imbalance in the toxic effects evaluated. Information gaps include underrepresentation of certain wastewater sources, limited assessment of larval and adult stages, and poorly evaluated contaminants. These findings highlight the need for further studies that consider the complexity of raw and treated wastewater and address key gaps to strengthen environmental risk assessment.

CONCLUSION

This review indicates that exposure to raw and treated wastewater samples can adversely affect zebrafish development, with acute toxicity and teratogenesis being the most frequently reported outcomes during embryogenesis. Pharmaceuticals and potentially toxic elements were the most commonly studied contaminants, although many pollutant groups remain underexplored. However, limited reporting of contaminant concentrations and wastewater treatment technologies across studies restricts direct comparisons and broad generalizations about the toxicity of treated wastewater and its associated environmental risks. These findings underscore the need to broaden research beyond early life stages and to expand the evaluation of diverse wastewater sources and complex contaminant mixtures. Strengthening this knowledge is essential for improving environmental risk assessment and supporting strategies to safeguard aquatic ecosystems and human health.

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Credit author contribution

K. Ponce-Palomera: conceptualization, methodology, investigation, data curation, writing-original draft preparation, and visualization; Z. Aguirre-García: investigation, data curation, writing-original draft preparation, and visualization; D. Badillo-Zapata: validation, formal analysis, writing-review and editing, and supervision; F. Vega-Villasante: validation, formal analysis, writing-review and editing, and supervision; A. Rojas-García: validation, formal analysis, writing-review and editing, and supervision; S. Guerrero-Galván: conceptualization, methodology, validation, formal analysis, writing-review and editing, and supervision.

Conflicts of interests statement

The authors declare no conflict of interest.

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