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Ecotoxicological relevance of some pollutants and antibiotics administered to zebrafish, in combination with antioxidants

PHD THESIS ABSTRACT

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Abstract

This doctoral research investigates the ecotoxicological relevance of various emerging pollutants such as heavy metals, pesticides, and antibiotics on zebrafish (*Danio rerio*), a widely accepted model in aquatic toxicology. Due to their small size, transparent embryos, short life cycle, and physiological similarity to higher vertebrates, zebrafish are suitable for assessing both behavioral and biochemical effects of environmental contaminants. The study evaluates the impact of individual and combined exposures to pollutants, as well as the potential protective role of natural antioxidants including α-lipoic acid, quercetin, and N-acetyl-L-cysteine. Behavioral tests, such as the mirror test, swimming performance and sociability assessments, were combined with biochemical analysis of oxidative stress markers like superoxide dismutase, catalase, glutathione peroxidase, and malondialdehyde. The results indicate significant alterations in locomotor and social behaviors following pollutant exposure, with partial improvements observed after antioxidant treatment. These findings support the use of zebrafish as a relevant ecotoxicological model and contribute to the understanding of the impact of pollutants on aquatic ecosystem health, while highlighting the potential of antioxidants in mitigating toxic effects.

To achieve the proposed goal, the research pursued the following main objectives:

Objective 1: To evaluate the effect of individual administration of different pollutants and potentially protective substances on zebrafish behavior, in order to clarify the behavioral mechanisms induced by chemical exposure.

Objective 2: To investigate the interactions between toxic substances by evaluating the effects of binary and tertiary mixtures on zebrafish behavior.

Objective 3: To elucidate the fundamental ecotoxicological mechanisms involved in chemical toxicity by correlating biological, physiological and behavioral changes.

Objective 4: To contribute to the development of behavioral ecotoxicology by performing a detailed analysis of the effects of toxic substances on the behavior of aquatic organisms, in order to support effective environmental protection measures.

Chapter 1 discusses the current scientific understanding of how various pollutants and protective agents affect zebrafish. It begins by exploring the behavioral effects of pollutants, including deltamethrin, arsenic, mercury, and SiO_2 nanoparticles, which are known for their toxicity and persistence in the environment.

The chapter highlights the main sources of environmental contaminants, resulting mainly from industrial processes, agriculture, and improper waste disposal. Special attention is paid to antibiotics such as ampicillin and amoxicillin, which are widely used in both human and veterinary medicine. Their release into the environment contributes to the spread of antibiotic resistance and poses a threat to aquatic ecosystems.

In contrast, natural and synthetic antioxidants, such as alpha-lipoic acid, quercetin, and N-acetyl-L-cysteine, are discussed for their ability to reduce oxidative stress and cellular damage, offering potential protective effects against pollutant-induced toxicity.

Zebrafish are presented as a reliable model organism in ecotoxicological studies due to their small size, transparent embryos, genetic similarity to higher vertebrates, and complex behavioral patterns. The chapter reviews their locomotor activity, social interactions, anxiety-like responses, and aggression as key indicators of neurobehavioral alterations following exposure to chemicals.

Overall, Chapter 1 provides an integrated perspective on the effects of pollutants on aquatic life, highlighting the relevance of zebrafish for behavioral ecotoxicology and the potential role of antioxidants in attenuating toxic effects.

Chapter 2 describes the materials and methods used in the study. Adult zebrafish, aged 6 to 7 months, were obtained from several suppliers to ensure genetic diversity. The fish were acclimated for one month under controlled laboratory conditions that followed OECD guidelines for water temperature, pH, hardness, and oxygen levels. They were kept in tanks with regular water changes and a light-dark cycle of 10 hours light and 14 hours dark. The fish were fed twice daily with tropical fish flakes.

Several behavioral tests were performed, including swimming performance in a T-maze, sociability using a cross-maze with other fish as stimuli, color preference test, and mirror bite test to assess aggression. Behavior was recorded with infrared cameras and analyzed using EthoVision XT software. Each test lasted 4 minutes after a 30-second acclimation period.

After behavioral testing, fish were euthanized and brain and body tissues were collected. These samples were analyzed to measure the activity of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, and catalase.

In Chapter three, the results and discussion of the study are presented. This section focuses on how arsenic exposure affects the behavior of zebrafish. The data clearly show that arsenic causes a significant reduction in the locomotor activity of the fish, with higher doses producing stronger effects. Zebrafish exposed to arsenic swam slower and covered a shorter distance compared to the control group, indicating that arsenic impairs their motor functions.

Furthermore, arsenic exposure influenced social behavior. Treated fish showed less interest in interacting with other zebrafish and tended to avoid them, which may reflect increased anxiety or stress caused by the toxin. These behavioral changes suggest that arsenic not only affects physical movement, but also alters neurological pathways related to social interaction.

Chapter 3 presents the results of several experimental studies focusing on the behavioral and biochemical impacts of various emerging aquatic contaminants on zebrafish. In general, exposure to substances such as arsenic, deltamethrin, methylmercury chloride, antibiotics like ampicillin and amoxicillin, and silica nanoparticles resulted in significant alterations in locomotor activity, including decreased swimming distance and speed, as well as disruptions in social behavior. These effects suggest neurotoxic or anxiogenic responses and reflect damage to motor control systems or impairment of social perception. In several cases, combined exposure to multiple pollutants resulted in more severe behavioral impairments compared to the individual substances, indicating possible additive or synergistic toxic effects. Antioxidant compounds such as quercetin, N-acetylcysteine, and alpha-lipoic acid were also evaluated for their protective role. Although these agents showed moderate improvements in behavior and markers of oxidative stress, they did not fully restore normal function, indicating the limitations of antioxidant-based interventions under conditions of complex toxic exposure. The findings confirm that zebrafish represent a sensitive and reliable model for detecting neurobehavioral toxicity and support the importance of monitoring emerging contaminants and exploring new strategies for environmental and public health protection.

Studies highlight the significant impact of emerging contaminants, such as antibiotics, pesticides, heavy metals, and SiO2 nanoparticles, on the anxiety-related locomotor and social behavior of zebrafish, a model species widely used in ecotoxicology. Exposure to these pollutants resulted in notable alterations in motor activity, characterized by decreased swimming distance and speed, as well as changes in social behavior, marked by a pronounced avoidance of social stimuli, indicating potential anxiogenic or neurotoxic effects. Specifically, SiO2 nanoparticles caused hyperactive locomotor responses and changes in color preference, suggesting sensory system disruption or a neurochemical imbalance. Furthermore, combined exposure to pollutants, such as arsenic with deltamethrin or ampicillin, produced more severe effects than the individual substances, revealing synergistic or cumulative interactions between the compounds.

Administration of antioxidants such as quercetin, α-lipoic acid, and N-acetylcysteine partially improved locomotor performance and social behavior, indicating a moderate protective effect. However, despite this partial protection, normal behavior was not fully restored, highlighting the limitations of these compounds under combined toxic stress conditions. The results support the use of zebrafish as a sensitive ecotoxicological model for assessing the behavioral effects of aquatic pollutants and emphasize the need for enhanced monitoring of emerging contaminants in natural environments, along with the development of effective strategies to mitigate toxic effects using antioxidant compounds.