Linking Molecular and Phenotypic Endpoints in Zebrafish Larvae Exposed to Ciprofloxacin and Cadmium

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BACKGROUND

The EPIBOOST project aims at validating the epigenetic modifications caused by chemicals as biomarkers of relevance which can support more accurate environmental risk analysis. To do so, a multiparametric approach is pursued by looking at the effects of chemicals from the individual and biochemical to the transcriptomic and epigenetic level, with the aim to establish a link between phenotypic and molecular endpoints.

RESEARCH Aims

Using zebrafish eleutheroembryos we aim to evaluate developmental, behavioural, biochemical, transcriptomic and DNA methylation effects to later relate to molecular effects resulting from **cadmium** (Cd) and **ciprofloxacin** (CIP) exposures.

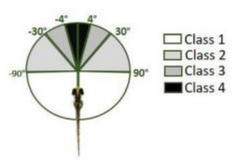
METHODS

Exposure: 96 hours post fertilization zebrafish *(Danio rerio)* eleutheroembryos exposed for 24 h to Cd (ranging from 0.24 to 24 µg/L) and CIP (10 to 50 mg/L). Exposures were performed in 6-well plates (10 individuals/well). At the end of the exposure 16 biological replicates (10 embryos/sample) per experimental group were collected and flash frozen.

Biochemical and molecular endpoints:

- 8 biological replicates/group were used for the determination of biotransformation enzymes activity by colorimetric assessment including: glutathione-s-transferase (GST), antioxidant enzymes catalase (CAT), glutathione reductase (GR) and superoxide dismutase (SOD), peroxidative damage by lipid peroxidase activity (LPO) and neurotoxicity markers as acetylcholinesterase (AChE).
- RNA/DNA extraction was performed from 8 biological replicates/group. Transcriptomics were assessed by a real-time microfluidic platform (total of 96 genes) and global DNA methylation by MethylFlash™ Global DNA Methylation (5-mC).

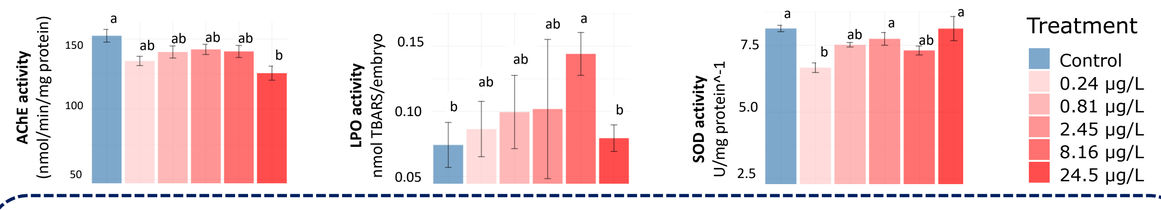
Behavioural endpoints: 24 organisms (individually exposed) per treatment.
Basal locomotor activity and swimming patterns assessed using the **Zebrabox** video tracking system.
Swimming patterns evaluated through the larvae path angles.



Classes of angles defined for the evaluation of larvae swimming pattern

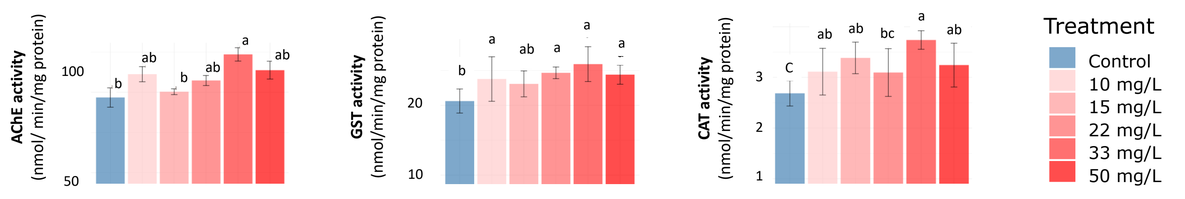
Biochemical results

Cadmium



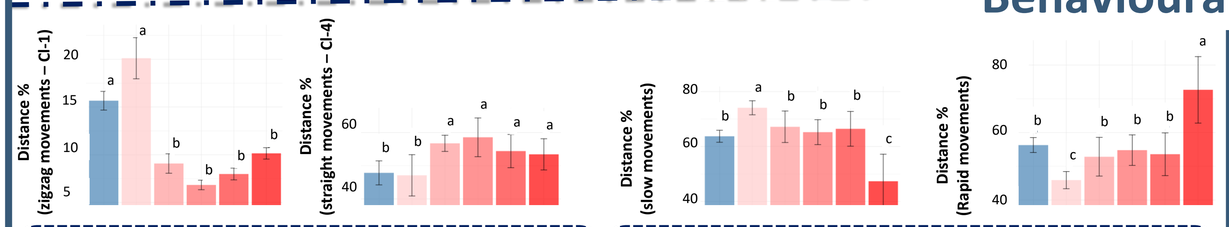
- **AChE activity was decreased at 24.5 µg/l suggesting neurotoxicity caused by Cd.**
- **Cd exposure increased LPO up to 8.16 µg/L, then decreased at the highest concentration (24.5 µg/L), indicating a non-linear oxidative stress response.**
- **The antioxidant system did not appear to be activated, as no effects were detected in GST, CAT, GPx, or GR activities. However, SOD activity was decreased at the lowest cadmium concentration (0.24 µg/L).**

Ciprofloxacin



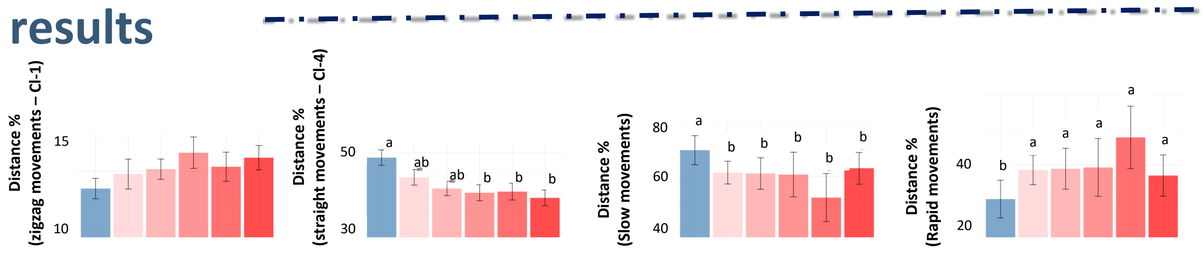
- **AChE activity was increased at 33 mg/L suggesting neurotoxicity caused by ciprofloxacin.**
- **The antioxidant system was activated with significant changes in GST and CAT activities.**
- **No lipid peroxidation was detected.**

Behavioural results



Changes in the proportions of zigzag movements & straight movements suggest effects in the fish swimming patterns.

Exposure to 0.24 and 24.5 ug/L of Cd provoked changes in the proportion of rapid/slow movements.

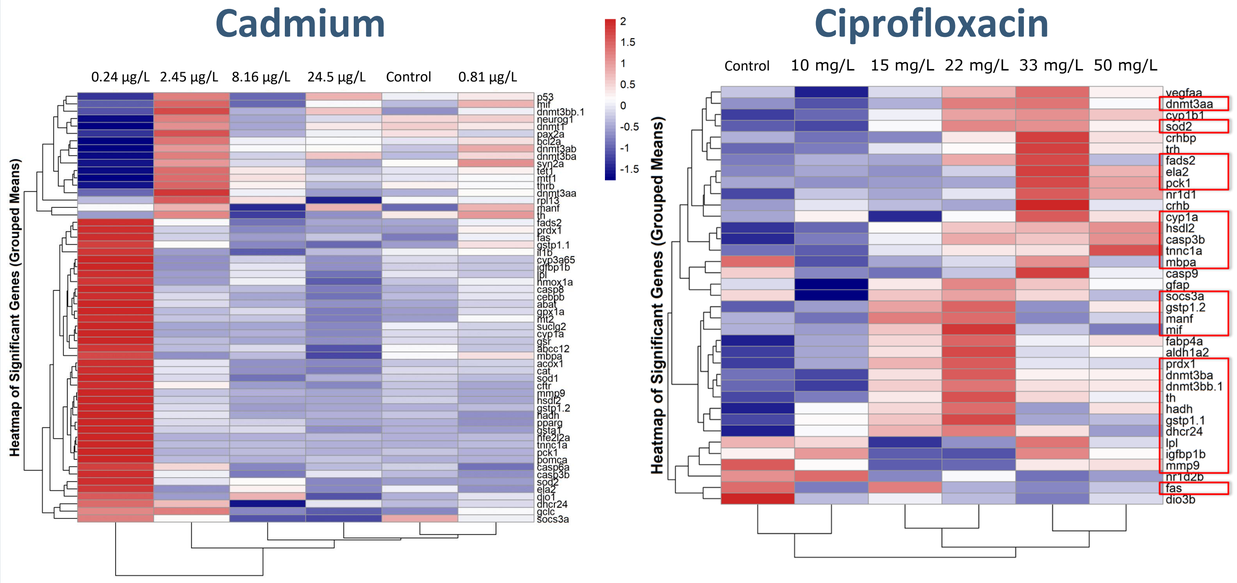


Exposure to ciprofloxacin decreased the frequency of straight movements.

The proportion of rapid/slow movements were affected in all tested concentrations when comparing to control.

Transcriptomics & Epigenetics results

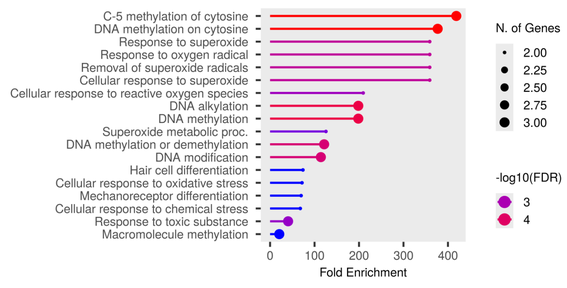
mRNA abundance



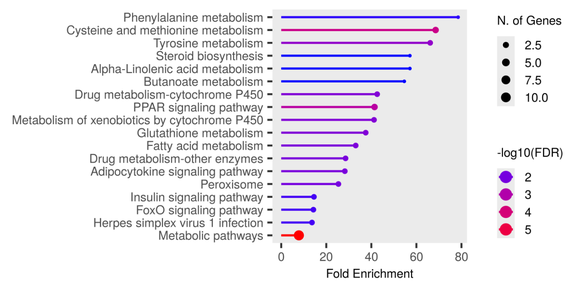
(58 affected genes)

(37 affected genes)

Biological Processes



KEGG Pathways



The 25 genes commonly affected by Cd and Ciprofloxacin are involved in key metabolic processes, including amino acid, lipid, and xenobiotic metabolism, as well as oxidative stress responses and DNA methylation. Results suggest that both contaminants trigger overlapping disruptions in metabolic and stress-regulatory pathways.

Global DNA methylation results

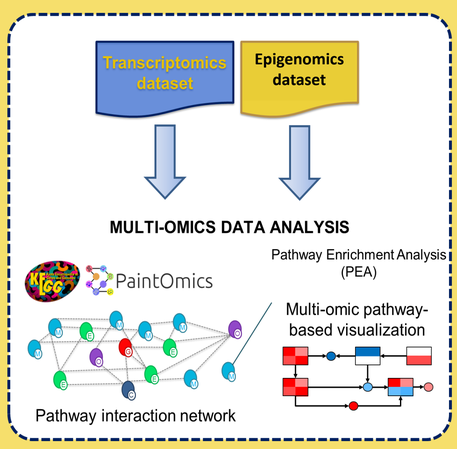
No effects were observed in Global DNA methylation for both cadmium nor ciprofloxacin.

TAKE-HOME MESSAGE

This study highlights that cadmium and ciprofloxacin, though chemically distinct, induce overlapping molecular and phenotypic effects in zebrafish larvae. Both exposures affected neurotoxicity markers, swimming behaviour, and selectively modulated oxidative stress responses. Importantly, 25 genes were commonly regulated across treatments, associated with core metabolic, oxidative stress, and epigenetic pathways, suggesting potential shared molecular signatures of chemical-induced stress in aquatic organisms.

FURTHER STUDIES

To conduct a multi-omic approach consisting of Transcriptomic (RNA-seq) and Epigenetic (EM-seq) analyses to better elucidate the mode of action of cadmium and ciprofloxacin at a genome-wide level. This integrative strategy will help uncover novel biomarkers and mechanistic insights, improving environmental risk assessment frameworks for aquatic toxicants.



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