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Summary of Dissertation

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DEGREE OF DOCTOR OF PHILOSOPHY

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Energetic Mechanisms Underlying Naphthenic Acid Fraction Compound Toxicity in Rainbow Trout Mitochondria and Hepatocytes

The Canadian oil sands represent one of the largest reserves of unconventional crude oil globally, with bitumen extraction generating substantial volumes of oil sands process-affected water (OSPW). This wastewater contains complex mixtures of inorganic and organic contaminants, among which are naphthenic acid fraction compounds (NAFCs), widely recognized as major contributors to aquatic toxicity. Based on structural similarities to other toxic carboxylic acids and prior evidence of mitochondrial vulnerability, this thesis tested the overarching hypothesis that NAFC toxicity is mediated through disruption of mitochondrial energetics.

A multi-level experimental approach was employed using isolated liver mitochondria and primary hepatocytes from rainbow trout (Oncorhynchus mykiss). Environmentally relevant NAFC mixtures were extracted and purified from aged oil sands tailings water, while a pure NAFC, 3,5-dimethyladamantane-1-acetic acid, was used to assess mechanistic responses. High-resolution respirometry was applied to quantify mitochondrial oxygen consumption, membrane potential, and hydrogen peroxide emission.

In isolated mitochondria, exposure revealed pronounced mitochondrial impairment characterized by inhibition of electron transport system activity, partial uncoupling of oxidative phosphorylation, and stimulation of mitochondrial reactive oxygen species (ROS) emission. Relative inhibitory potency of electron transport chain complexes followed the order CIV ≥ CI > CIII > CII. Specific ROS production was localized to site IIF within complex II, indicating targeted interference rather than nonspecific oxidative stress.

In hepatocytes, mitochondrial disturbances translated into impaired energy metabolism, elevated intracellular ROS, reduced ATP levels, loss of viability, and predominance of necrotic cell death at higher concentrations. These findings demonstrate that NAFC toxicity arises from mitochondrial dysfunction linking altered bioenergetics and redox imbalance to cellular injury.

Publications

1- Kalvani, Z., Kamunde, C., Stevens, D., & van den Heuvel, M.R. (2024). A model naphthenic acid decouples oxidative phosphorylation through selective inhibition of mitochondrial complex activity. Environmental Toxicology and Pharmacology, 107, 104386.

2- Kalvani, Z., Tetteh, P., Kamunde, C., Stevens, D., & van den Heuvel, M.R. (2025).
Naphthenic Acid-Induced ROS Emissions by Rainbow Trout Mitochondria. Toxics, 13, 1015.

Presentations

1. Kalvani Z, Kamunde C, Stevens D, Van den Heuvel R. 2023. Effects of Naphthenic acid on mitochondrial reactive oxygen species. Canadian Ecotoxicology Workshop 49th Annual Conference, October 02-05, Ottawa, Ontario. (oral presentation)

2. Kalvani Z, Kamunde C, Stevens D, Van den Heuvel R. 2024. Naphthenic acids contribute to oxidative stress by eliciting the emission of reactive oxygen species in mitochondria. Canadian Society of Zoologists 63rd Annual Conference, May 06-10, Moncton, NB. (poster presentation)

3. Kalvani Z, Kamunde C, Stevens D, Van den Heuvel R. 2024. Naphthenic acids influence site-specific H2O2 emission differently in rainbow trout liver mitochondria. Aquaculture Association of Canada 40th Annual Conference, June 16-19, Charlottetown, PEI. (oral presentation)

4. Kalvani Z, Tetteh PA, Kamunde C, Stevens D, Van den Heuvel R. 2024. The impact of naphthenic acid on Rainbow trout liver mitochondrial reactive oxygen species metabolism. Society of Environmental Toxicology and Chemistry 45th Annual Meeting, October 20-24, Fort Worth, Texas. (oral presentation)

5. Kalvani Z, Kamunde C, Stevens D, Van den Heuvel R. 2024. A model naphthenic acid reduces oxidative phosphorylation through selective impacts on complex activity. Canadian Ecotoxicology Workshop 50th Annual Conference, October 06-09, Kitchener, Waterloo. (oral presentation)

Biographical

Born in Iran