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The Ecotoxicological Effects of Metformin under Global Warming Scenarios: *Daphnia magna* and *Danio rerio* Insights

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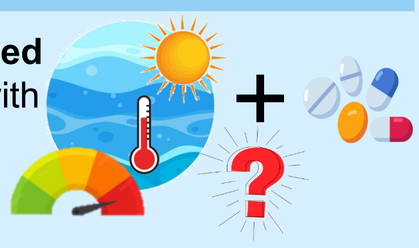
INTRODUCTION & AIM

Metformin (MET) is an antihyperglycemic drug prescribed to treat **type-II diabetes mellitus,** a disease **affecting over than 500 million people worldwide [1].**

Widely released into the **environment**, **endangering aquatic species** (e.g., **endocrine disruption, oxidative stress** and changes in **survival, growth,** and **reproduction**).[1]

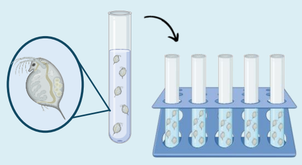
It is **essential** to **understand** how **climate-related pressures** (e.g. **global warming**), **combined** with **pharmaceutical residues**, are **threatening** the **resilience** of **freshwater ecosystems**.



This work aimed to **assess the ecotoxicological effects of metformin on *Daphnia magna* and *Danio rerio,* under climate change scenarios, focusing on the impacts of rising temperatures.**

METHODS

D. magna acute immobilization assay (OECD No. 202) [2]



0 to 120 mg MET/L

48 h of exposure

20 ºC OECD guideline

24 ºC High GHG emission IPCC scenario

24 h observations

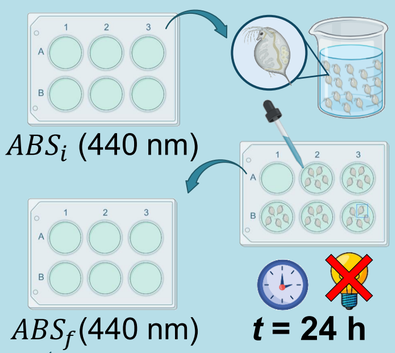
- Mortality
- Immobilization
- Irregular swimming behaviour
- Normal behaviour

D. magna feeding inhibition assay [3]

0 to 80 mg MET/L

20 ºC

24 ºC High GHG emission IPCC scenario



$$Feeding\ rate = \frac{\left(\left(V \times (ABS_i - ABS_f)\right)/t\right)}{n(=5)}$$

D. rerio embryo acute toxicity test (OECD No. 236) [4]

0 to 3000 mg MET/L

96 h of exposure

26 ºC OECD guideline

30 ºC High GHG emission IPCC scenario

24 h observations

- Mortality
- Hatchability
- Malformations

End of exposure

Size

Sub-individual endpoints

Oxidative homeostasis (CAT and GSTs activities)

Lipid Peroxidation (TBARS levels)

Neurotransmission (AChE activity)

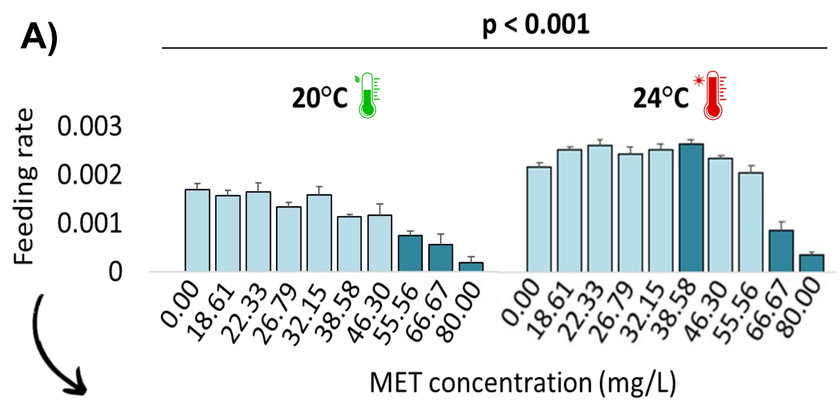
RESULTS & DISCUSSION

Acute toxicity of **MET** ↓ with ↑ **temperature.**

Table 1: LC_{50}, EC_{50} (mg MET/L) and respective confidence interval ($CI_{95\%}$) values obtained, after MET exposure, at 20 °C and 24 °C.

	20 °C	24 °C
LC_{50} (48h)	70.64 (65.56 - 75.72)	76.09 (72.66-79.53)
EC_{50} (48h)	53.67 (50.00 - 57.33)	61.38 (58.17-64.59)

D. magna feeding inhibition assay



EC_{50} (20 °C) = 54.18 (46.8−61.6) mg/L

EC_{50} (24 °C) = 63.62 (61.38−65.86) mg/L

MET toxicity ↓ with ↑ **temperature.**

Significant interaction between **temperature** and **MET concentrations.**

D. rerio embryo acute toxicity test

At 26 ºC, no significant effects were detected on **hatching time (100%), malformations (25%),** or **mortality (0%) levels.**

Mortality levels increased sharply, reaching **30%** at **1500 mg/L** and **100%** at **3000 mg/L MET, at 30 ºC.**

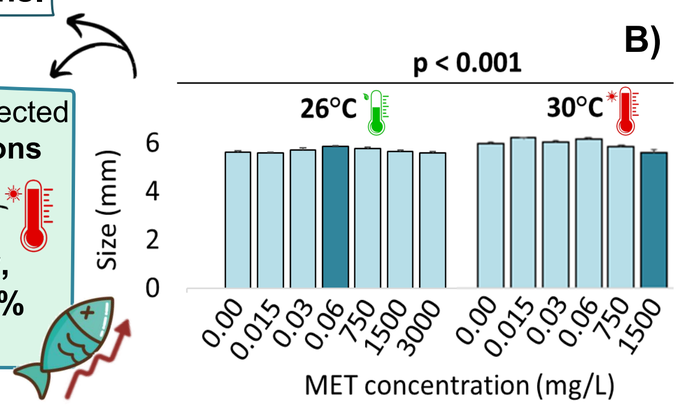


Figure 1: A) Feeding rates of D. magna after acute MET exposure at 20 °C and 24 °C. B) Larvae size of D. rerio after 96 h of MET exposure at 26 °C and 30 °C. Data are expressed as mean ± standard error. Significant interactions between MET and temperature are shown. Dark blue columns indicate significant differences between the control group and MET treatments (Dunnett's test; p < 0.05).

Table 2: Summary of sub-individual responses after MET exposure under normal and elevated temperature conditions.

	CAT		GSTs		TBARS		AChE	
Acute immobilization	-----		-----		↗	↘	↘	↗
Feeding inhibition	↗		↗	↗	↗	↗	↘	
Fish embryo acute toxicity	↗	↘	↘	↘		↘	↘	

Sub-individual endpoints

All sub-individual responses reveal a **significant interaction** between **temperature** and **MET concentrations.**

Oxidative damage and **neurotoxicity** were observed at **both species.**

Significant Effect No Effect

CONCLUSIONS

- **MET** was **toxic** to **both species**, with ***D. magna*** showing **greater sensitivity**.
- **Increased temperature** influenced **MET toxicity**, **altering individual** and **sub-individual responses** in both species.
- In *D. magna*, some **compensatory mechanisms** were detected, suggesting a **short-term adaptive potential**.
- A **decline in both species populations** can **compromise** the **resilience** of **freshwater ecosystems**, altering **water quality** and **trophic dynamics**.
- **Further research** should consider **interactions with multiple stressors** (e.g., pollutant mixtures and abiotic factors alterations) and **evaluate long-term effects** under a **climatic change scenario.**

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