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ANTIBIOTIC STRESS RESPONSES IN AQUATIC BIOINDICATOR SPECIES

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Introduction

The increased detection of antibiotics in freshwater ecosystems has raised concerns about their ecotoxicological impact, particularly on sensitive bioindicator species. Among the most studied compounds, tetracycline is frequently found in surface waters due to their widespread use in human and veterinary medicine and their incomplete removal during wastewater treatment procedures.

Exposure to tetracycline impairs key biological functions in the freshwater cladoceran *Daphnia magna*. Sub-lethal concentrations reduce fecundity, disrupt molting and trigger oxidative stress responses due to altered enzymatic activity, or changes in the expression of stress-related genes [1].

Chronic exposure to environmentally relevant concentrations of tetracycline (e.g., 0.1 mg/L) has been shown to reduce the population growth rate of aquatic organisms, with cumulative and even transgenerational effects that may compromise long-term population sustainability [2].

In green microalgae, even low concentrations of tetracycline like 280 µg/L can inhibit photosynthesis, reduce chlorophyll content, and suppress cell division. Furthermore, antibiotic exposure may destabilize the microbial communities within the algal phycosphere, the microenvironment surrounding algal cells, thereby indirectly impairing nutrient acquisition and overall algal health [3].

The aim of study was acute toxicity evaluation of tetracycline on aquatic organisms.

Materials and methods

Freshwater bioindicators *Daphnia magna* and *Pseudokirchneriella subcapitata* were used to assess the toxicity of tetracycline hydrochloride (≥98%, Sigma-Aldrich). Tetracycline solutions (6.25–100 mg/L) were freshly prepared in test media.

Acute immobilization tests with *D. magna* followed ISO 6341, exposing neonates for 24 and 48 h at 20 ± 2 °C in darkness. Immobilization and mortality were observed microscopically.

Algal growth inhibition tests followed ISO 8692. *P. subcapitata* was exposed for 72 h under continuous light (6,000–10,000 lux) at 21 ± 2 °C. Growth was monitored via OD_{680} at 24, 48, and 72 h to calculate growth rates and inhibition.

Results and discutions

The EC_{50} at 72h value was determined to be below than 6.25 mg/L, indicating high toxicity of tetracycline to algal growth within the tested concentration range (6.25–100 mg/L), where growth inhibition reached 95–100%. These preliminary results suggest that elevated toxicity is likely due to the photodegradation of tetracycline under test conditions (light exposure and 23°C), resulting in more toxic byproducts such as anhydrotetracycline and epitetracycline hydrochloride. These degradation products not only contribute to increased oxidative stress in algal cells but also produce a brown coloration that interferes with spectrophotometric chlorophyll measurements, potentially compounding observed growth inhibition.

In comparison, *Daphnia magna* showed a lower sensitivity than algae, with a 48-hour EC_{50} of approximately 57 mg/L. While this suggested that cladocerans were less vulnerable than algae in the short term, exposure nonetheless resulted in measurable adverse effects.

Concentration (mg/L)	Mortality Effect %
100mg/L	95%
50mg/L	55%
25 mg/L	25%
12.5 mg/L	5%
6.25 mg/L	0%

Given the ecological importance of *Daphnia magna* as a keystone species in freshwater food webs, disruptions in survival, reproduction, and feeding could have cascading consequences for higher trophic levels.

These results reinforce the need for stringent monitoring of antibiotic residues in aquatic systems and the development of advanced wastewater treatment technologies to mitigate their release.

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