Understanding the Impact of Car Tire Rubber Chemical Leachates on Marine Microalgae: Insights into Toxic Mechanisms and Ecosystem Implications

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INTRODUCTION

Micronized car tire rubber (CTR) particles contribute significantly to the presence of plastic particles in the environment, raising concerns about the leaching of chemical additives into the marine ecosystem. Some of these additives are highly toxic to aquatic organisms, posing a serious threat to microalgae, at the basis of the aquatic food web. As keystone organisms, any disturbances in microalgae communities can have far-reaching consequences for the entire aquatic ecosystem.

This study investigated the toxic effects of chemicals associated with CTR, released during 7 and 14 days of leaching, on four marine microalgal species: *Skeletonema pseudocostatum*, *Rhodomonas baltica*, *Isochrysis galbana*, and *Tetraselmis suecica*.

METHODOLOGY

The toxic effects of CTR-associated chemicals released after 7 and 14 days of leaching were initially studied. Multisizer coulter counter and flow cytometry were used as screening tools, where general toxicity was indicated by effects in growth rate, cell size and complexity and natural pigments content. Leachates were chemically analysed by ICP-MS and LC-ESI-qTOF-MS.

The most toxic leachate and the most sensitive species were selected to further analyse sub-lethal toxicity. A high-throughput methodology using flow cytometry was used, with several specific endpoints analysed (Fig. 1).

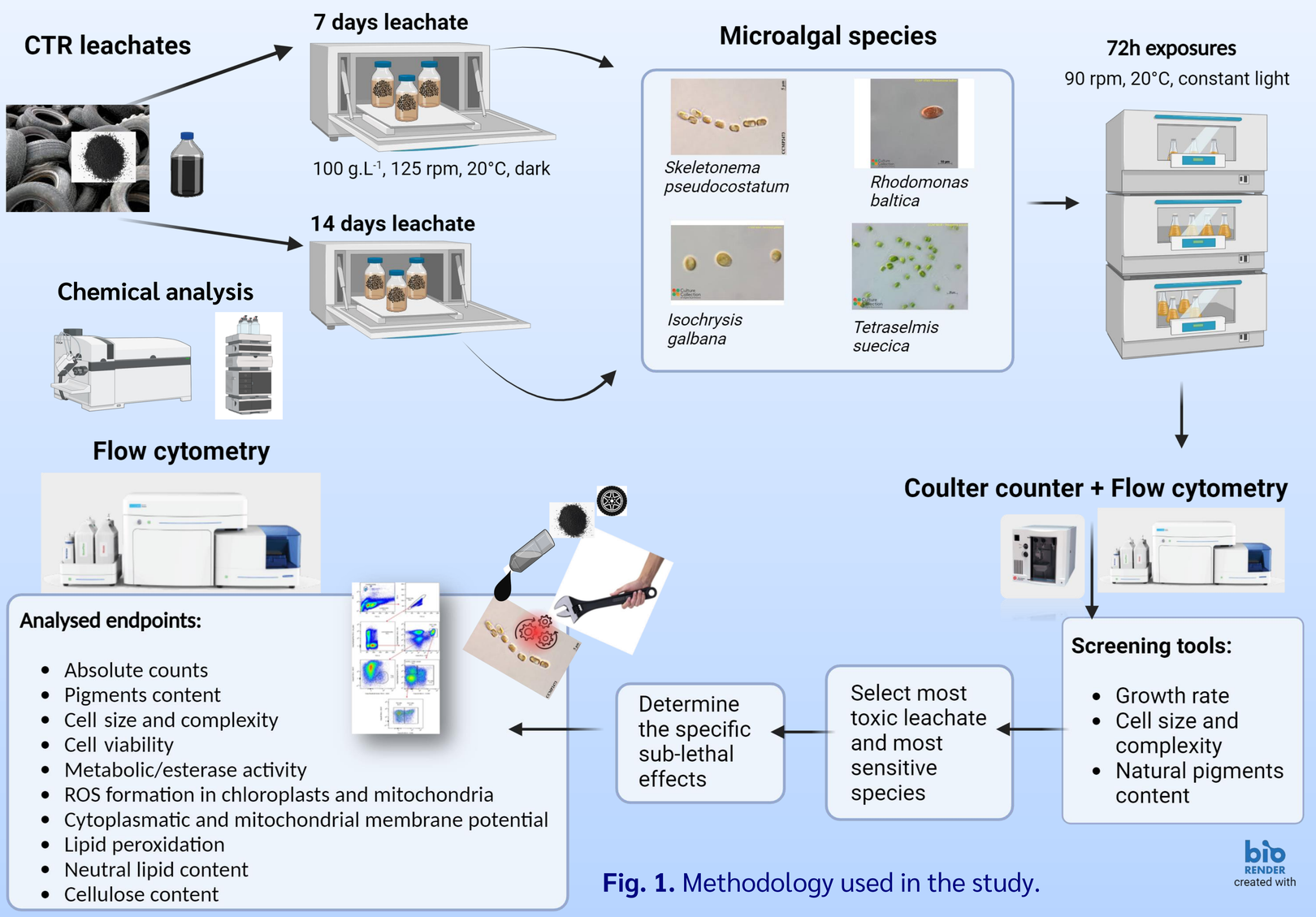

Fig. 1. Methodology used in the study.

Tab. 1. 72h EC_{50} values of the different microalgal species exposed to 7 and 14 days CTR leachates.

72h EC_{50} (%)	7 days	14 days
Skeletonema pseudocostatum	14.2	3.3
Rhodomonas baltica	33.7	6.4
Isochrysis galbana	32.9	5.6
Tetraselmis suecica	33.2	8.3

RESULTS AND DISCUSSION

The 14 days leachate was the most toxic for all species, with *S. pseudocostatum* being the most sensitive (Tab. 1).

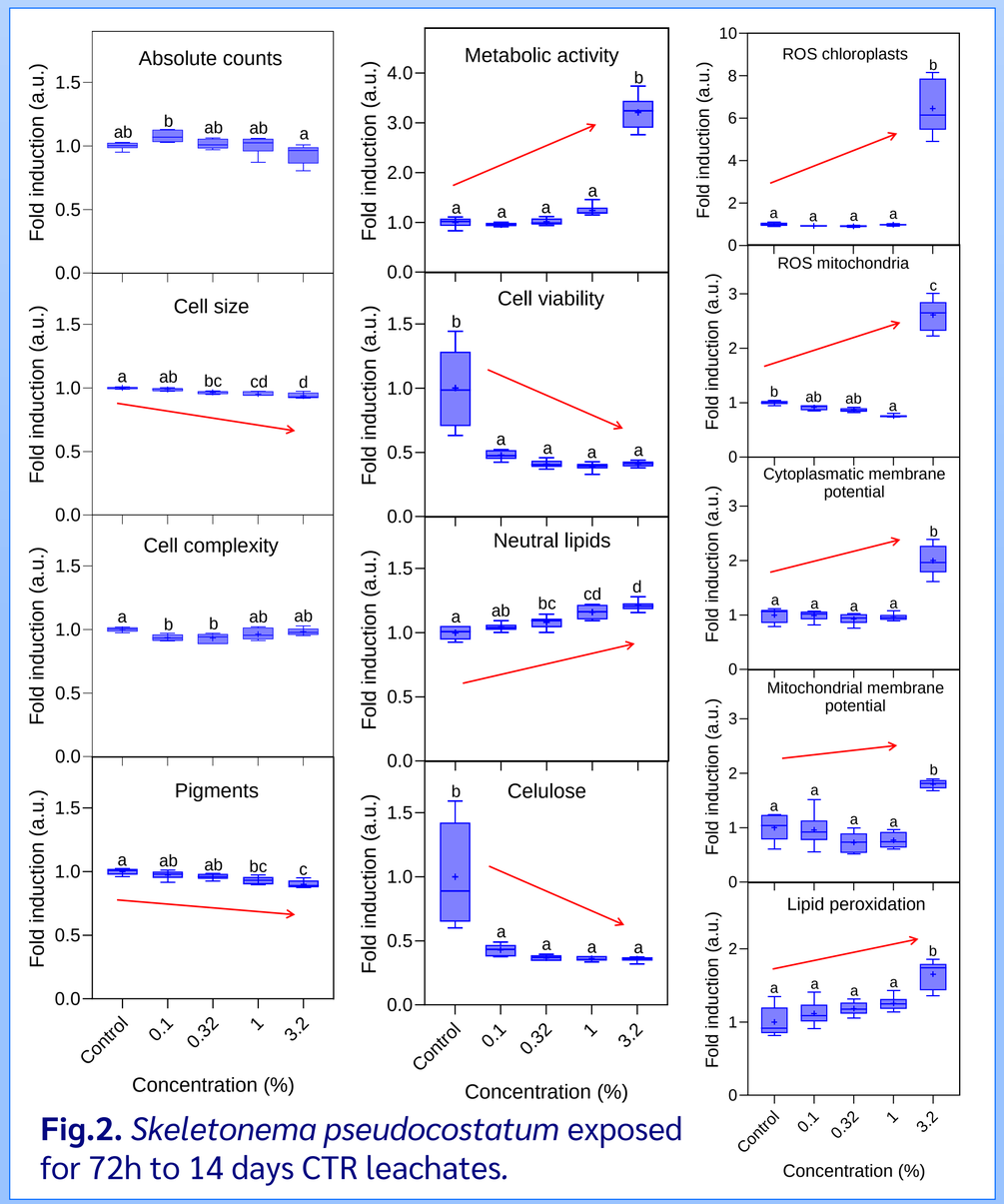

Fig.2. *Skeletonema pseudocostatum* exposed for 72h to 14 days CTR leachates.

Chemical analysis of leachates showed high concentrations of Zn (1.0 to 1.5 mg.L^{-1}), as well as the presence of benzothiazole and 2-hydroxybenzothiazole, hexamethoxymethylmelamine, dicyclohexylurea and diphenylamine.

Sublethal concentrations were chosen to further investigate the specific toxicity of the 14 days leachate to *Skeletonema pseudocostatum* (Fig. 2):

- ✓ Ultrastructural differences, membrane hyperpolarization, alterations in membrane permeability and effects on photosynthesis were observed;
- ✓ The specific toxic mechanisms were revealed by the oxidative stress endpoints:
  - ➢ ROS formation in chloroplasts and mitochondria and increase in LPO;
  - ➢ Damage on the integrity of cell and mitochondrial membranes.

Fig. 3. illustration on the specific toxicity of the 14 days CTR leachate to *Skeletonema pseudocostatum.*

CONCLUSIONS

Specific toxic mechanisms of CTR leachates to crucial components of microalgae cells was revealed: ROS formation and related oxidative stress was the most sensitive endpoint (Fig. 3).

This study highlights the importance of comprehensively understanding the potential impacts of plastic-associated chemicals to the marine ecosystem.

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