

Ecotoxicological Assessment of Cigarette Filter Waste using *Daphnia*



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INTRODUCTION

Cigarettes are the most littered item in the world – 4.5 trillion annually. These contain filters which introduce toxic chemicals and plastics into waterbodies following their improper disposal. This disrupts food chains and affects the health of aquatic organisms and ecosystems. This study focused on the toxicity and sublethal effects from leachates of smoked and non-smoked filters, using the freshwater organism, *Daphnia*, as a bioindicator. Daphnids, commonly known as water fleas, are crustaceans frequently used for New Approach Methodologies (NAMs) in ecotoxicology due to their alignment with the 3Rs principles (Replacement, Reduction, Refinement) of animal testing.

METHODS

Toxicity was assessed with acute mortality following exposure to increasing concentrations of cigarette filter extracts. Survival analysis with Kaplan-Meier plots captured the long-term mortality, while growth was measured as the body length of daphnids over three weeks. Finally, the activities of key enzymes such as alkaline and acid phosphatase, β -galactosidase, lipase and peptidase were also quantified.

RESULTS AND DISCUSSION

Acute toxicity was observed only for the smoked cigarette filter extract, with mortality starting from 100 mL extract/L. Smoked filters had an EC_{50} of 424 mL extract/L after 24 hours, and 397 mL extract/L after 48 hours of exposure (Figure 1). In chronic exposures, non-smoked filters did not cause more than 50% mortality at any tested concentration, whereas extracts from smoked filters caused up to 100% mortality at 200 and 300 mL extract/L (Figure 2). These results indicate high levels of pollution from cigarette filters are fatal for daphnids and reveal potential sublethal effects at lower concentrations. Enzyme activity assays were conducted to confirm this and after 21 days of exposure, significant changes were observed (Figure 3). This indicated the disruption to processes such as transport of molecules, digestion, and detoxification. More visual effects such as changes in size were also observed. For the first week of exposure, significant differences in body size were observed across almost all concentrations, likely due to the younger daphnids' greater sensitivity to environmental stressors. After assessment at 14 and 21 days, most significant differences had diminished. This suggests that the daphnids may have acclimated to the pollutants over time (Figure 4). Overall, cigarette filters exhibited significant ecotoxicological effects on daphnids; however, these responses are not unique to daphnids since pathways of toxicity are shared even among distantly related species. This highlights their risk to freshwater ecosystems on a much broader scale.

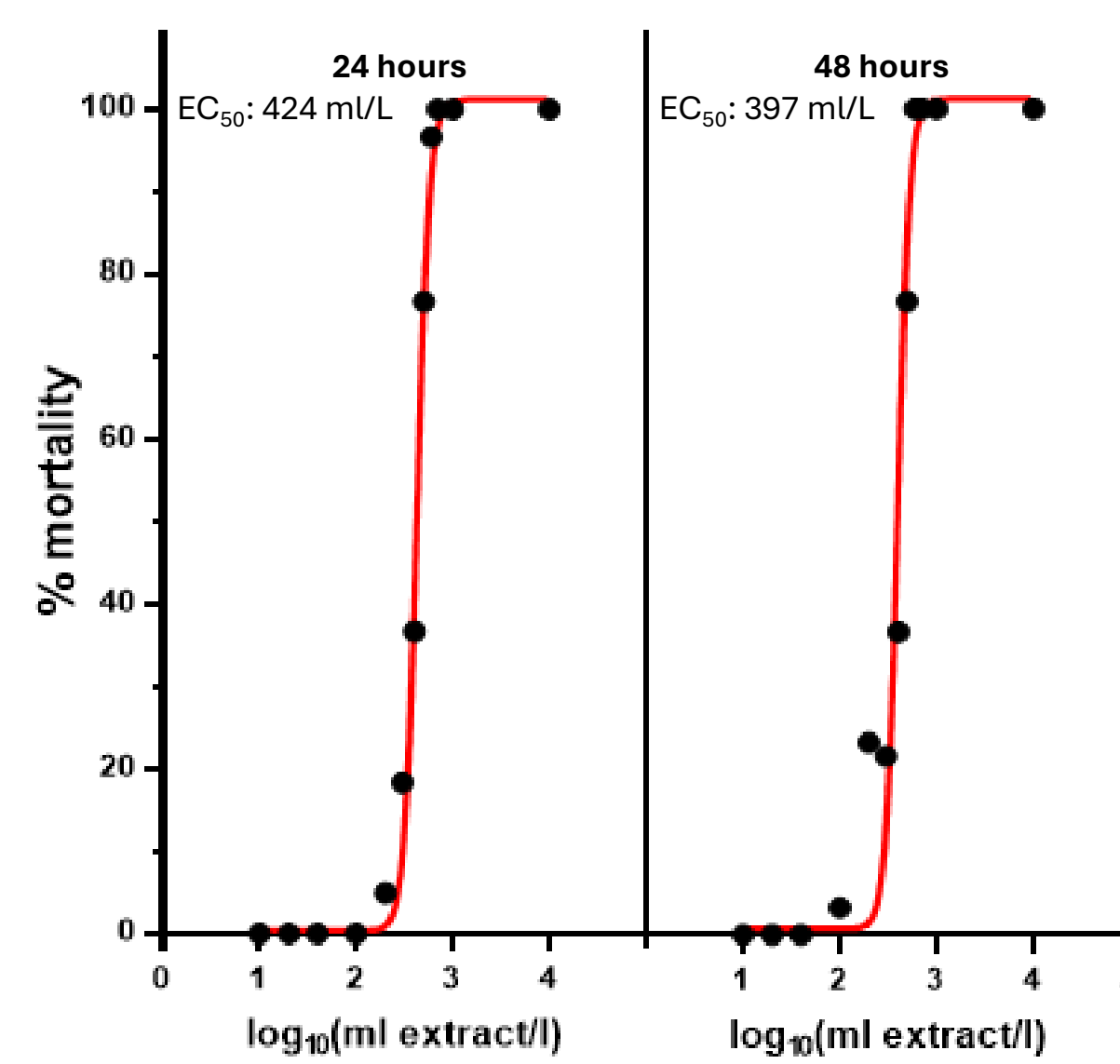


Figure 1. Acute toxicity of the smoked cigarette filter extract. Fifteen neonates were exposed for 24 and 48 hours per replicate and per concentration tested four replicates were scored. Points indicate the averages.

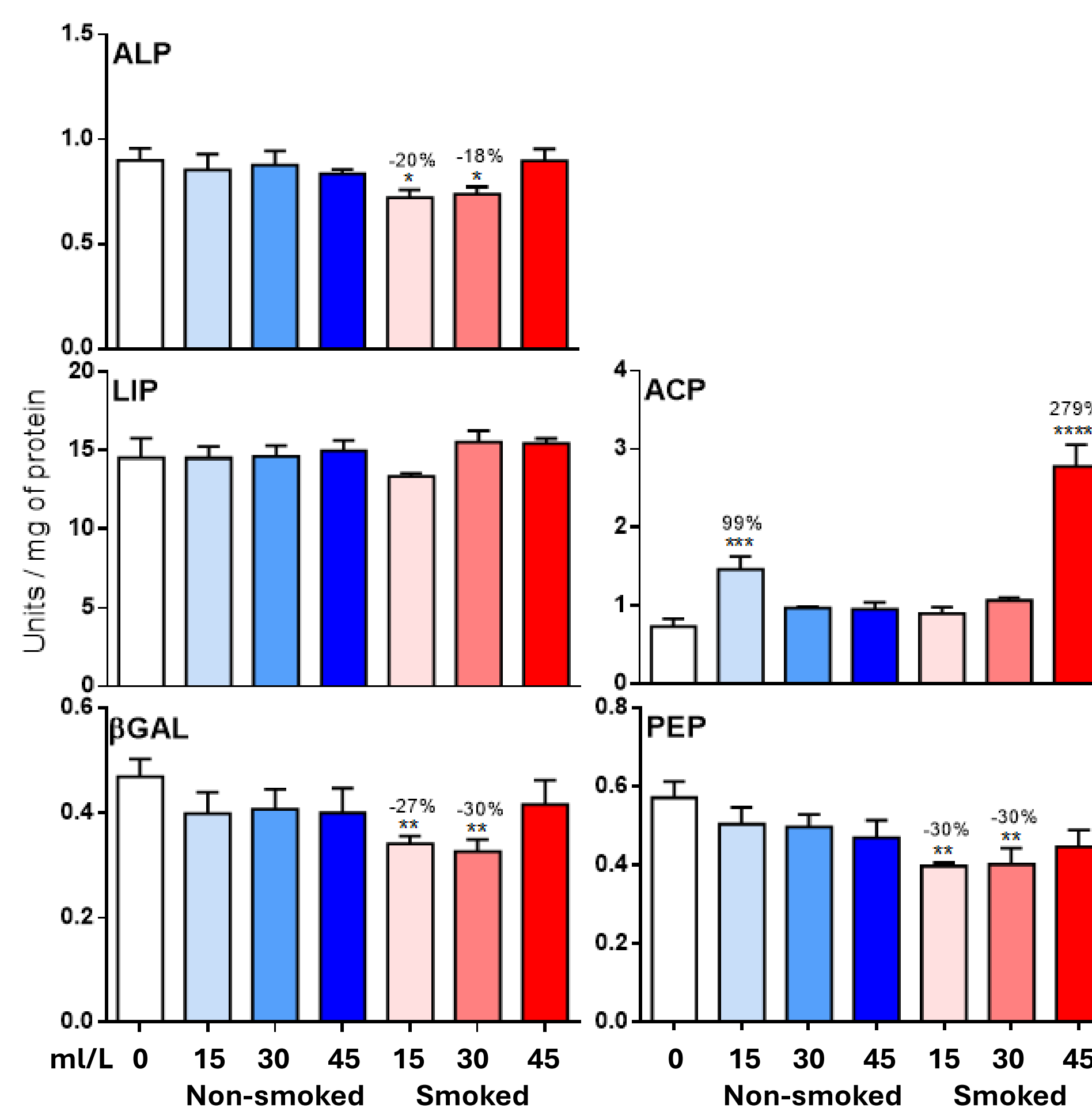


Figure 3. The impact of cigarette filter extracts on enzyme activity after 21 days. Data represent average \pm SD (N = 3 replicates for each condition). Statistically significant differences were identified with two-way ANOVA, followed by comparisons with the unexposed control. Asterisks indicate statistical significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.

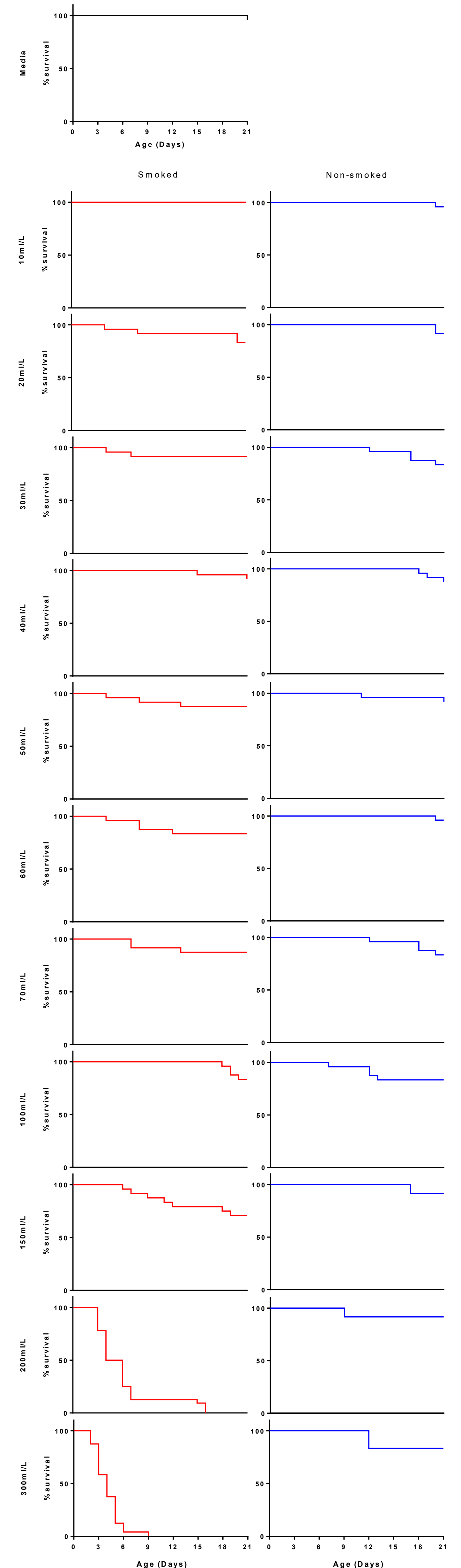


Figure 2. Kaplan Meier survival analysis for daphnids exposed to filter extracts for 21 days. Data represent averages from three independent replicates per concentration.

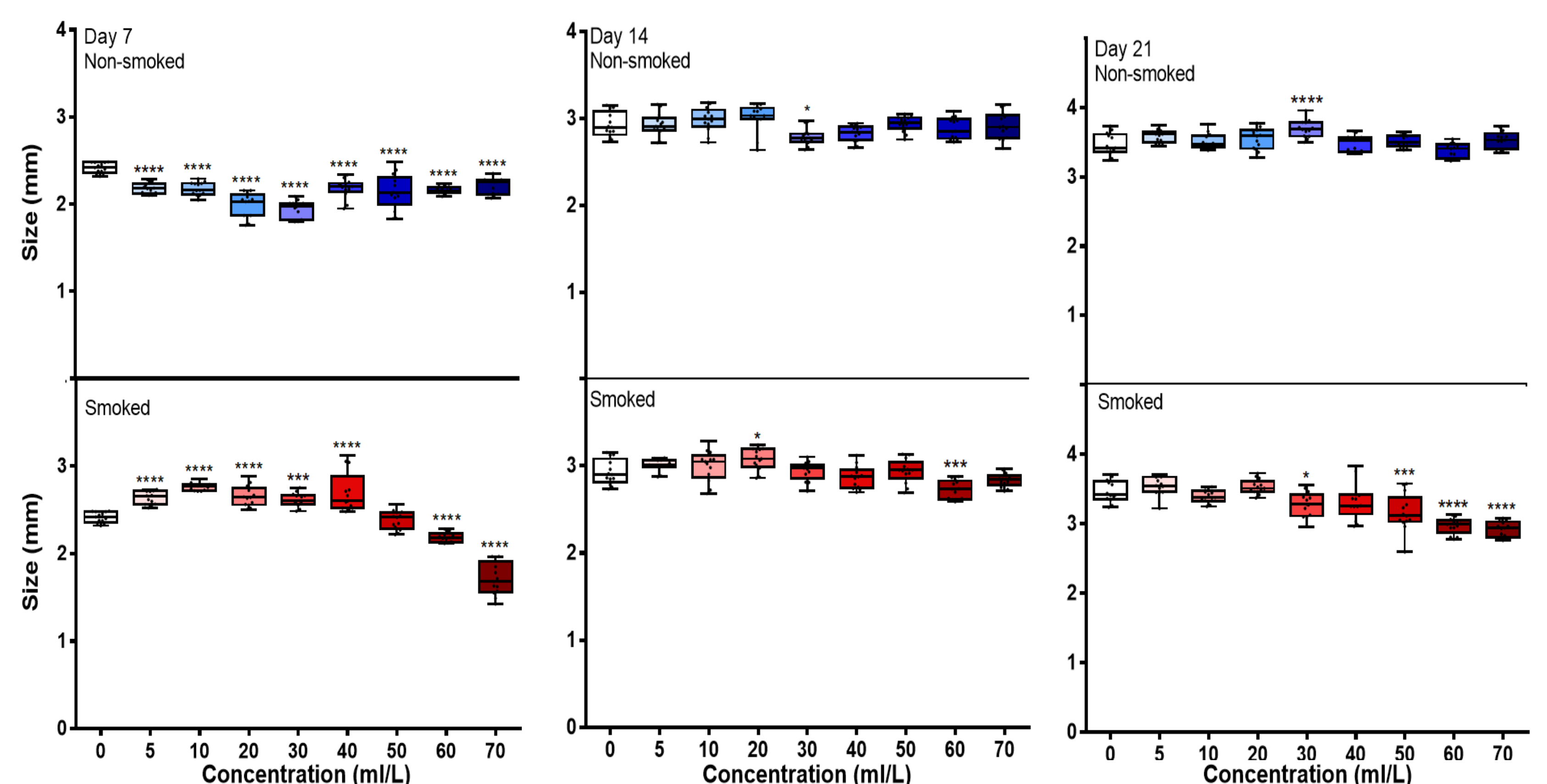


Figure 4. The impact of filter extracts on the size of daphnids. Data represent average \pm SD (N = 13 replicates of individual animals per concentration). Statistically significant changes were identified with one-way ANOVA by comparisons with the unexposed control. Asterisks indicate statistical significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.