

DEVELOPMENTAL EFFECTS OF MICRO- AND NANOSCALE TIRE PARTICLES AND RECYCLED RUBBER ON ZEBRAFISH

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BACKGROUND

Many studies have evaluated the toxic effects of tire particles on aquatic environments by testing the leachate they give off in water. However, no studies to date have investigated the specific effects of micron and nanoscale tire particles themselves. With the increase of many toxic particulates being introduced into the ocean and other aquatic environments, there is serious concern as to how it could affect the life in these areas. Zebrafish (*Danio rerio*) were selected for these studies because they are vertebrates that share significant similarities to human development and can also be used as an ecotoxicity indicator for potential impacts on fish in aquatic systems. Previous studies reveal that tire particles can cause mortality and sub-lethal effects in multiple fish species, while recycled rubber does not show any overt toxicity but can release polycyclic aromatic hydrocarbons (PAHs), some of which are known toxicants.

INTRODUCTION

Plastics have become apart of almost every aspect of our life. Once products that contain these components are deemed obsolete, they are thrown away without a second thought. 368 million metric tons of plastic was produced in 2019 (Tiseo,2021). These plastic end up throughout the environment where they are slow to decompose. Over time they break down into mesoplastics (5-40 mm), microplastics (1-5000 μm), and nanoplastics (<0.1 μm). As the plastics degrade, they also leach toxic chemicals into the environment. Smaller plastics, <3 μm, can enter tissues other than the digestive tract. If that occurs these leaching particles can have catastrophic effects on the afflicted organism. Tires are made of many different plastics and account for 5-10% of global plastics that end up in the ocean. By assessing the development of embryonic zebrafish that are introduced to this contaminant, an easy observational assessment can be done using a microscope.

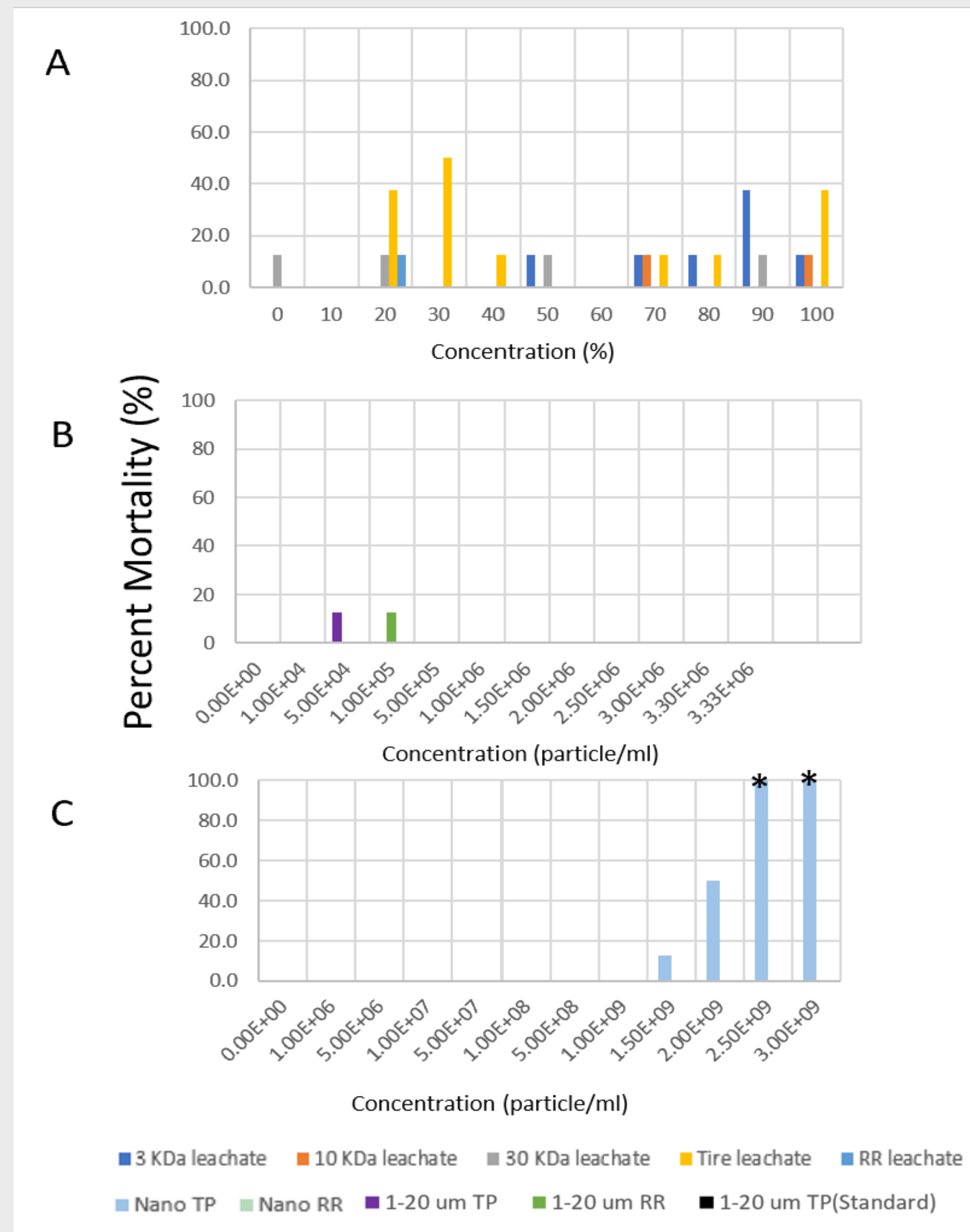


Figure [1]: Zebrafish mortality from 5-day exposure to (A) tire particle leachate, (B) 1-20 micrometer tire particles, and (C) nanoscale tire particle exposures.

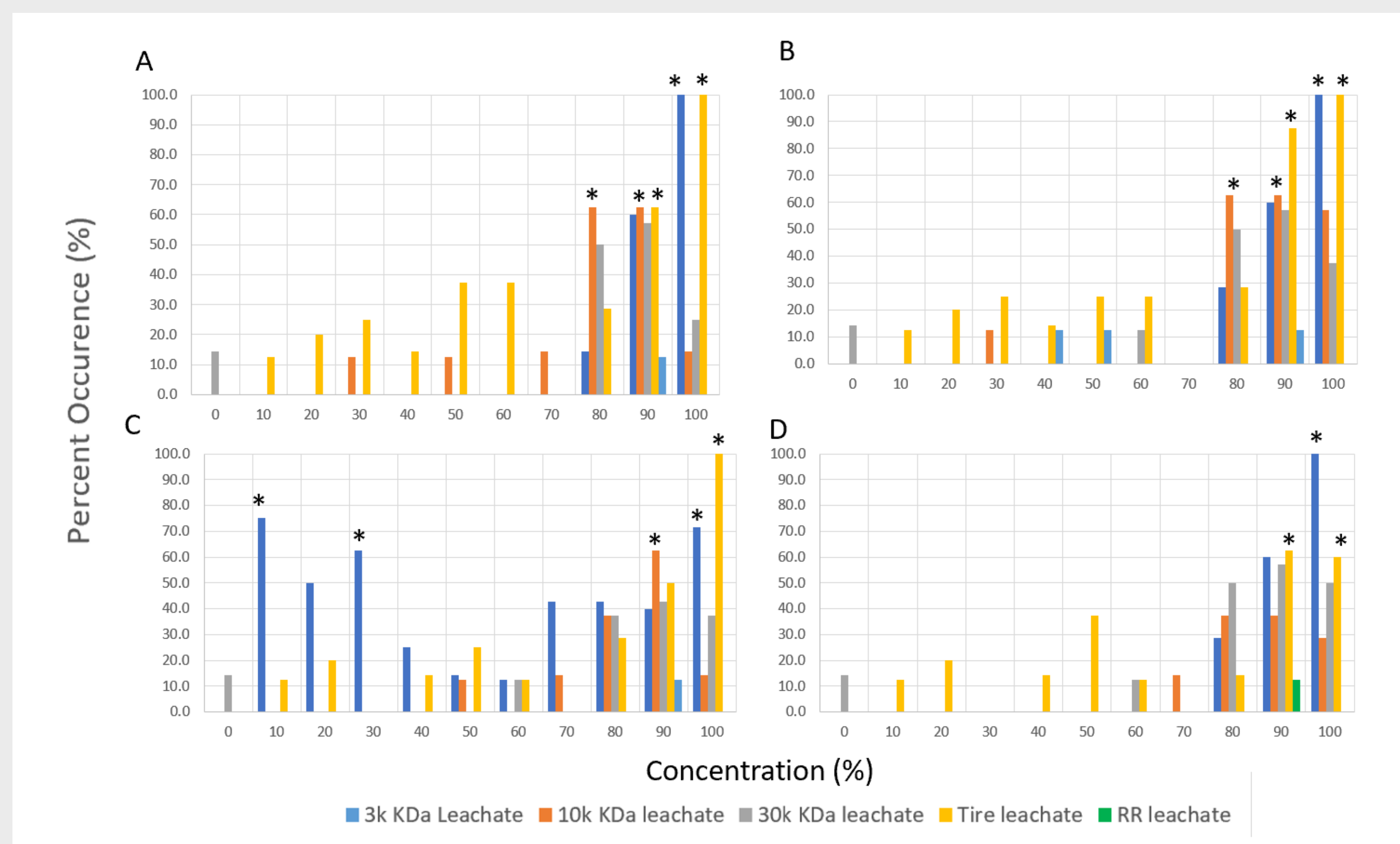


Figure [2]: Leachate exposures of mass fractionated leachate (3k, 10k, and 30k KDa), TP standard, and RR. (A) yolk sac edema, (B) pericardial edema, (C) touch response defects, (D) and jaw developmental defects are shown above.

RESULTS

- Exposure data was analyzed in sigma plot using the Fishers Exact test with an $\alpha=0.05$ and a sample size of 8.
- Nano-tire particles showed the only significant mortality at the two highest concentrations.
- Sublethal effects had varying significance with leachate showing the most development defects
- Nanoplastics, microplastics, and RR showed very few sublethal effects, most occurring from touch response. Nano RR showed significant occurrence at the higher concentrations for yolk sac edema. $>1.5 \cdot 10^9$ particles/ml
- All significant occurrence was found at higher concentrations so it can be inferred that tire particles and RR have a low toxicity.

DISCUSSION

Leachate found a general trend of 80% occurrence causing significance in developmental defects. 10k kDa mass fractionate showed the most significant effects for pericardial edema and yolk sack enema. 3k kDa leachate particles showed little effect except for touch response and at the highest concentrations. 30k kDa particles showed no significant effect on the zebrafish. It is likely the 10k kDa fractionate contains the most toxic particles that can be found in the tires. Other studies have found the

toxicity of acute exposures to be low. This agrees with the data we have found. Studies done with different organisms have found similar toxicity. Most of these studies have focused on leachate as it contains the most toxic components of tires. However, recent studies have found that a mixture of tire particles and leachate is more toxic. This is because of the particle's ability to sorb toxic to their surface for transport.

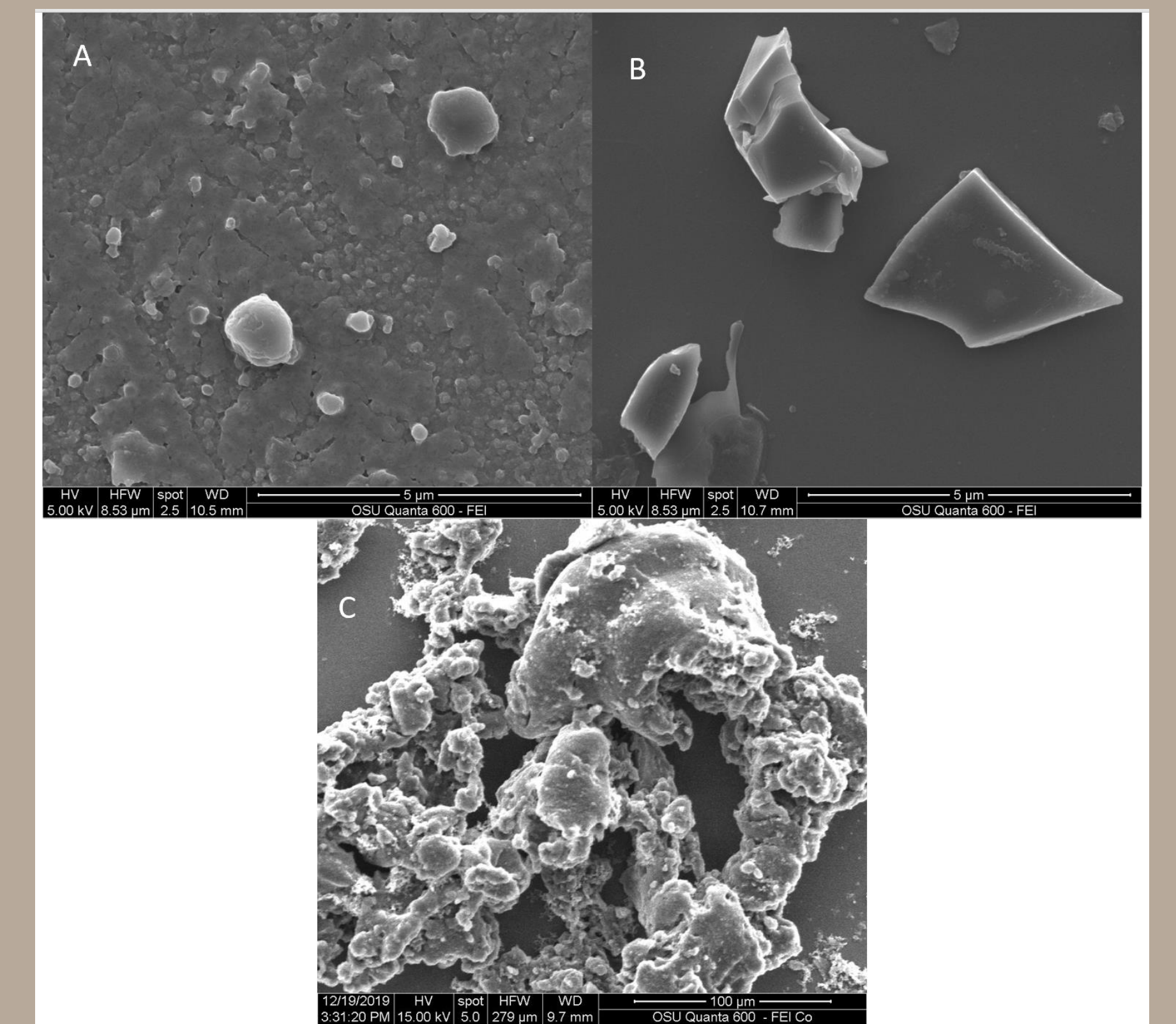


Figure [3]: SEM Images of (A) Nano tire sized less than 1 μm, (B) micro tire sized at 1-20 μm, (C) Recycle rubber that appears to have aggregated together. Nano tire particles are mostly spherical while the micro size tire particles are jagged chunks. The recycle rubber was sized at 1-20 μm but looks to have a rounder shape before aggregating.

CONCLUSION

- Toxicity of tire particles and recycled rubber are low.
- Tire microparticles and recycled rubber had very little effect on the zebrafish so further studies should focus on leachate and nanoparticles.
- Tire nanoparticles appear to be the most lethal particle as they can travel through any tissues.
- Leachate cause the most sublethal effects which can have lasting effects on the contaminated organism.
- Further research should be to continue testing tire particles on zebrafish to increase plausibility of our hypothesis.
- Additional research can investigate the long-term effects tire particles have on growth, potentially looking at the fertility of zebrafish and other organism.

ACKNOWLEDGEMENTS

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