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Impact of tire particle leachates on microplankton communities in the Canary Islands

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ABSTRACT

Tire wear particles (TWP) are a major source of microplastics in the environment. Despite their prevalence, the effects of tire particle leachates on marine microplankton communities remains poorly understood. In this study, we assessed the acute impacts of tire particle leachates on the structure of coastal microplankton assemblages from the Canary Islands. Five laboratory experiments were conducted, exposing microplankton to a range of leachate dilutions over 72 h, with TWP leachates prepared from an initial concentration of 1 g L^{-1} .Our results revealed that the abundances of diatoms, most dinoflagellates, and ciliates were significantly reduced following exposure to leachates, with median effective concentrations (EC_{50}) ranging from 30 to 660 mg L^{-1} depending on the plankton community. Interestingly, *Ostreopsis cf. ovata,* a harmful algal bloom (HAB)-forming species, exhibited relatively high tolerance to tire particle leachates compared to other microplankton. Compared to other marine biota, ciliates appear to be most vulnerable plankton group to tire particle leachates ($EC_{50} = 30$ and 146 mg L^{-1}). The higher tolerance of *O. cf. ovata* to pollution compared to other phytoplankton species (resource competitors), in combination with other factors, may contribute to the rise of HABs in polluted coastal areas. Although field data on TWP are limited, the observed negative effects on microplankton occurred at environmentally relevant concentrations. Our results indicate that TWP pollution can significantly impact marine planktonic communities, highlighting the urgent need to reduce TWP emissions and develop less toxic tire rubber additives.

1. Introduction

Traffic-related emissions are a significant environmental and public health issue (Bai et al., 2022). Besides air pollution, an estimated six million tons of tire wear particles (TWP) are produced annually due to the friction between tire treads and road surfaces (Kole et al., 2017). Although TWPs have been identified as pollutants since the 1970s, these particles are now recognized as a major source of microplastic pollution (Sundt et al., 2014; Baensch-Baltruschat et al., 2021; Zhang et al., 2023). Our understanding of the environmental fate and impact of tire particles on marine ecosystems remains limited, especially when compared to conventional petroleum-based microplastics, which have garnered significant attention over the past few decades (Agamuthu et al., 2019; Avio et al., 2017; Delaeter et al., 2022; Mennekes and Nowack, 2022).

Tire wear particles (TWP) consist of synthetic rubber (such as petroleum-based butadiene rubber and styrene-butadiene rubber), natural rubber (polyisoprene), and chemical additives. Due to their small size, ranging from 10 nm to 1000 µm, typically less than 250 µm (Baensch-Baltruschat et al., 2021; Kreider et al., 2010), TWPs are easily transported into aquatic environments through surface runoff, stormwater, drainage systems, wastewater effluent, and atmospheric deposition (Parker-Jurd et al., 2021). Once in these systems, TWPs release a complex mixture of chemicals, referred to as leachates. Recent studies have shown that tire particle leachates contain dozens of potentially toxic substances, including benzothiazoles, polycyclic aromatic hydrocarbons (PAHs), flame retardants, antioxidants, and heavy metals (Capolupo et al., 2020; Müller et al., 2022; Page et al., 2022; Le Du-Carrée, 2024). Given the substantial contribution of TWPs to microplastic pollution (Boucher and Friot, 2017) and their high levels of toxic leachable additives, assessing their potential impacts on aquatic ecosystems is essential to evaluate the risk associated to these traffic-derived emissions.

Marine plankton plays a major role in most of the ecological processes (Valiela, 2015). Within this diverse group, its photoautotrophic component, known as marine phytoplankton, is particularly remarkable, providing more than 50 % of the oxygen in the Earth's atmosphere.

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Furthermore, phytoplankton play a key role in fixing dissolved inorganic carbon to produce organic matter (Fenchel, 1988; Field et al., 1998) and serve as the main prey for many marine organisms. Among phytoplankton, diatoms and dinoflagellates are the dominant groups of microplankton (20 – 200 µm) (Sieburth et al., 1978). Ciliates are also an important group of microplankton, and together with heterotrophic dinoflagellates, are major grazers of phytoplankton (Calbet, 2008). A recent study (Page et al., 2022) indicates that tire particle leachates are acutely toxic to three species of cultured phytoplankton. However, to our knowledge, there are no ecotoxicological studies on the effects of tire particle leachates on marine microplankton communities. The impacts of TWP pollution on key microplankton groups could lead to reduced primary production and the disruption of marine food webs, necessitating further attention.

This study aimed to determine the acute toxicity of tire particle leachates on field-collected marine microplankton assemblages. Specifically, we investigated how exposure to different concentrations of tire particle leachates affects the abundance and composition of microplankton assemblages collected from coastal waters of Gran Canaria (Canary Islands, Spain). We hypothesize that exposure to leachates from tire particles negatively affects the growth of marine microplankton, and their effects can vary depending on the community composition. Our results provide the first data on the toxicity of tire rubber leachates on natural microplankton assemblages, offering valuable insights into the potential risk of TWP pollution on marine food webs.

2. Methodology

2.1. Collection of microplankton samples

The plankton samples were collected from coastal waters of Gran Canaria Island using a microplankton net with a mesh size of 20 µm. The samples were obtained from surface waters through multiple horizontal and vertical tows and collected from different sites on different dates (Table 1). For all cases, the water temperature and salinity during the collection were 20.3 ± 0.8 ºC and 36 ± 0.3 ‰., respectively. After collection, the samples were gently transferred into plastic containers and kept in a cool box until returning to the laboratory (< 2 h). While most samples were collected from the beach, samples from Station 3 were collected from offshore using a boat. In the laboratory, the samples were placed in glass beakers and maintained in a temperature-controlled room at 20ºC with cool-white, fluorescent lighting (80 $\mu mol \cdot m^{-2}\ s^{-1}$) and continuous aeration until starting the experiment (< 6 h from collection).

2.2. Leachate preparation

The tire particles were obtained from an unused car tire tread (Imperial 145/70–13 71T-Snowdragon) and micronized as described in Page et al. (2022). Tire particle leachates were prepared with autoclaved filtered seawater (A-FSW, salinity = 35 ± 2 ‰) using the procedure outlined by Almeda et al. (2023). The used seawater was collected nearshore (27º59’27’’ N, 15º22’01’’ W), filtered by 5 µm, UV sterilized and stored in a 7-meter-deep well at the Spanish Bank of Algae in Gran Canaria. Prior to leachate preparation, the seawater was triple-filtered using an activated carbon coconut shell filter (5 µm), PP melt-blown filter (1 µm) and PES membrane filter (0.1 µm) filter, and then autoclaved (1 atm, 120ºC, 20 min). A 1 g L^{-1} suspension of tire particles in A-FSW was prepared in a glass bottle filled and air-free closed with a polytetrafluoroethylene (PTFE) protected sea scree cap. This bottle was placed on a roller (15 rpm) in an incubator at 20°C in darkness for 72 hours. After the lixiviation time, the suspension was filtered over a pre-combusted (450ºC for 5 h) glass fiber filter (GF/F grade, 0.7 µm) with a vacuum pump system to remove the tire particles, resulting in a “stock leachate solution” (i.e., 100 % leachates). pH measurements of the leachate stock and A-FSW were taken in triplicate to ensure consistency; the pH of the leachates was 8.20 ± 0.02, similar to A-FSW (8.22 ± 0.01), so no pH adjustment was necessary. Leachates were generally used immediately after filtration, except for the third experiment, where the stock solution was stored at −20 ºC until use. The analysis of specific organic compounds and metals in the leachates was carried out according to the methods described by Rist et al. (2023). The concentrations of the detected PAHs, flame retardants, and metals are detailed in Rist et al. (2023) and are also available in Supplementary Information Table S1.

Table 1
Information on the sampling locations in the island of Gran Canaria where the microplankton samples were collected.

Sampling-Experiment #	Location	Date	Longitude	Latitude
E1	Tufia beach	09/05/22	15º23’45.9’’W	27º51’22.2’’N
E2	Arinaga beach	02/06/22	15º22’46’’ W	27º57’44’’N
E3	South coast	27/10/22	15º65’59.34’’ W	27º70’12.48’’N
E4	Arinaga beach	28/02/23	15º22’46’’ W	27º57’44’’N
E5	Arinaga beach	10/04/23	15º22’46’’ W	27º57’44’’N

2.3. Experimental setup and design

To initiate the bioassays, a microplankton community concentrate was prepared from the collected net samples. The samples were first filtered through a 100 µm mesh sieve to remove mesozooplankton. The remaining fraction (< 100 µm) was then concentrated using a 20 µm mesh-sieve and transferred to a glass beaker containing approximately 100 mL of A-FSW. The filtration was conducted gently, and the samples were kept submerged in A-FSW to prevent cell damage. Cell healthy appearance was checked in Sedgwick rafter counting chambers (1 mL) under an inverted microscope before starting each bioassay.

Each experiment involved exposing the microplankton assemblages to five different leachate dilutions in A-FSW (93 %, 50 %, 25 %, 12.5 %, and 6.25 %) and a negative control (only A-FSW) for 72 hours. The exposure leachate concentrations correspond to equivalent particle concentration of 0.93, 0.5, 0.25, 0.125, and 0.0625 g L^{-1}. Exposures were conducted in triplicate 34 mL glass bottles. An aliquot of the microplankton concentrate (2.5 mL) was added to each experimental bottle, followed by the appropriate volumes of A-FSW and stock leachate (100 %) to achieve the desired dilutions. Each bottle received 37.4 µL of B1 medium (Hansen, 1989) at a concentration of 1.1 mL L^{-1} to ensure nutrient availability during the exposure. Three additional bottles were prepared similarly than the experimental bottles, their content was fixed with 1 % of Lugol’s solution and used as “initials” to determine the initial concentration and composition of cells in each experiment as described above for the other samples (S.I. Table S2). The experimental bottles were closed with a PTFE-protected seal cap without headspace and placed on rollers at 15 rpm, 20ºC, and a 12:12 h day: night cycle using LED lights at an intensity of 72 $\mu mol \cdot m^{-2}\ s^{-1}$.

2.4. Sample analyses

After the exposure, samples were fixed with 1 % of Lugol’s solution and stored at 4 ºC in darkness. Microplankton abundance and composition were analysed using Uthermol (10–100 mL) or Sedgwick-Rafter (1 mL) counting chambers under a Leica DMi1 inverted microscope (x 200). The samples were mixed by hand rotation of the bottles (10 times) before being transferred to the counting chambers. Settling time was 12 hours (overnight) for Uthermol chambers and 5 min for Sedgwick rafter counting chambers. For counting and composition analyses, organisms were classified into 4 major categories: dinoflagellates, diatoms, ciliates and micrometazoans. For the category “ciliates” we distinguished between oligotrichs and tintinnids. Additionally, the dominant species/genus were counted separately and identified following the identification guides of Hallegraeff et al. (2003) and Ojeda Rodríguez (2011). Microscope images of representative species in each experiment were taken (Flexacam C1).

2.5. Data analyses/ statistics

Data were tested for normality and homogeneity of variances using

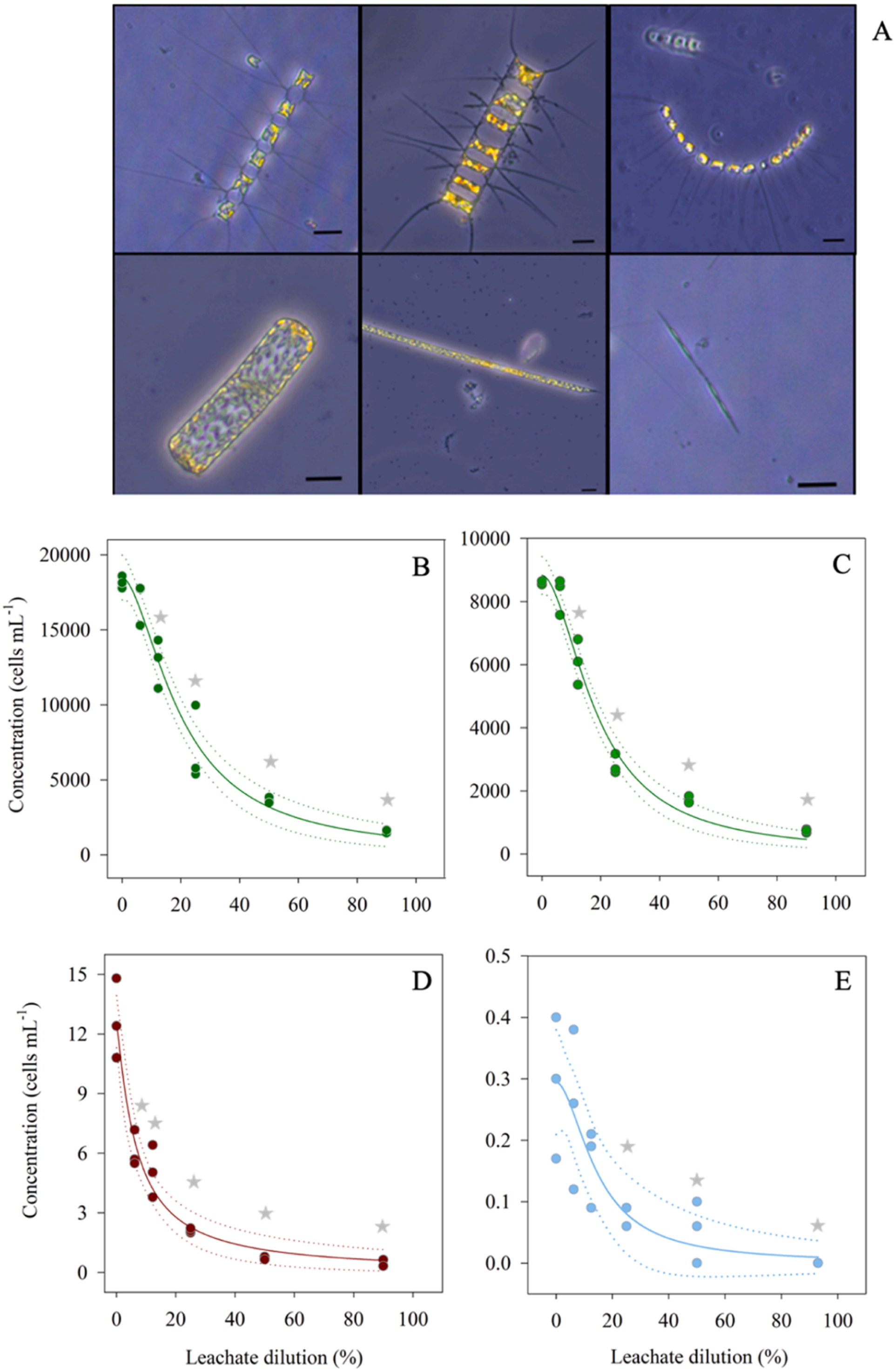

Fig. 1. A: Microscope images of representative species from the microplankton assemblage in E1 (Table 1), displayed from left to right and top to bottom: *C. decipiens*, *C. lorencianus*, *C. curvisetus*, *Lauderia* sp., *Proboscia alata*, and *Pseudo-nitzschia* spp. Abundance of total diatoms (B), *Chaetoceros* spp. (C), total dinoflagellates (D) and total ciliates (E) after 72 h of exposure to the various leachate dilutions. The solid lines represent the fitted curves based on Eq. 1., with the dotted lines showing the 95 % confidence bands. The estimated model parameters are presented in Table 2. The stars denote statistically significant differences in cell concentration compared to the control ($p < 0.05$).

the Shapiro-Wilks and Levene Tests, respectively. Parametric data were analysed using one-way ANOVA followed by Dunnett's post hoc test to identify significant differences between control and treatment groups ($p < 0.05$). Non-parametric Kruskal-Wallis tests with pairwise comparisons were applied when data did not meet parametric test assumptions ($p < 0.05$). Based on the pairwise comparison results, we obtained the "Lowest Observed Effect Concentration (LOEC)" as the lowest leachate dilution that cause an effect significantly different than the control. Data analyses were performed using IBM SPSS Statistics 21.

Graphing was done with Sigmaplot software. Cell concentration data after 72 hours of exposure to the different leachate dilutions were fitted to a logistic sigmoid model (Eq. 1), where C is the cell concentration (cells mL^{-1}), C_0 is the cell concentration in the absence of leachates (cells mL^{-1}), D is the leachate dilution (%), ED_{50} is the median effective dilution (i.e., the leachate dilution required to reduce the cell population by half compared to cell concentration in the absence of leachates, C_0), and b is the slope.

$$C= C_0 / (1 + (D/ ED_{50}))^{b} \qquad (1)$$

The EC50 ($g\ L^{-1}$) was estimated from the ED_{50} (%) considering the concentration of particles in stock solution (100 % = 1 $g\ L^{-1}$).

The specific growth rate (SGR, d^{-1}) was calculated:

$$SGR= \ln (C_f/C_i) / t \qquad (2)$$

where C_f and C_i are, respectively, the final and the initial averaged cell concentrations of each treatment, and t is the incubation time (days).

3. Results

In the first experiment (E1), representative species of the microplankton assemblages were diatoms of the genus *Chaetoceros* (*C. decipiens, C. lorencianus, C. curvisetus), Lauderia spp., Proboscia alata,* and *Pseudo-nitzschia spp* (Fig. 1A) and different species of dinoflagellates (*Prorocentrum* spp) at lower concentrations. Exposure to tire particle leachates caused a notable decrease in cell concentration for total diatoms, *Chaetoceros spp.*, and total dinoflagellates (Fig. 1B-D). Significant differences between the control and leachate dilutions were observed at concentrations higher than 12.5 % for total diatoms (Fig. 1B) and *Chaetoceros spp.* (Fig. 1C). Dinoflagellates showed a significant reduction in cell concentration compared to the control in all leachate dilutions, with a LOEC of 0.06 $g\ L^{-1}$ (Fig. 1D, Table 2). The sigmoidal model relating cell concentration to leachate dilutions fits well across all groups ($R^2 = 0.88–0.98$, Table 3). The estimated EC_{50} values for total diatoms, *Chaetoceros spp.,* and total dinoflagellates were 0.20, 0.21 and 0.10 $g\ L^{-1}$, respectively (Table 2). Micrometazoans (mostly copepod nauplii) were observed at very low concentrations in some dilutions and no clear trend in their abundance relative to leachate concentrations was detected (S.I., Table 2).

In the second experiment, conducted with the sample collected in Arinaga in June 2022 (E2, Table 1), we observed that the community was dominated by the HAB-forming dinoflagellate *Ostreopsis cf. ovata*, alongside other dinoflagellates and diatoms such as *C. pentagonum, Amphora spp.*, *Actinocyclus octonarius, Guinardia striata,* and *Licmophora spp*. (Fig. 2A). The abundance of *Ostreopsis* was slightly affected by the exposure to tire particles (Fig. 2D), whereas cell concentrations of diatoms and other dinoflagellates showed a sharp decline (Fig. 2B, C). The sigmoidal model showed a strong fit for both diatoms ($r^2 = 0.90$, Table 2) and dinoflagellates ($r^2 = 0.91$, Table 3), but not for *O. cf. ovata*. The estimated EC_{50} values for diatoms and dinoflagellates were nearly identical, at 0.2 $g\ L^{-1}$ (Table 2). Micrometazoans (e.g., copepod nauplii, meroplankton) were observed at low concentrations and their abundance decreased with increasing leachate concentration (S.I., Table S4).

In the third experiment (E3), conducted with a sample collected offshore to the south of Gran Canaria (Table 1), the microplankton community was dominated by various species of diatoms (*Grammatophora spp.* and *Cylindrotheca spp.;* Fig. 3A), small dinoflagellates (*Gymnodinium spp.*, *Scrippsiella* spp, *Prorocentrum spp.;* Fig. 3A) and ciliates (e.g., tintinnid *Ascampbelliella*; Fig. 3A). All studied groups exhibited a decline in cell concentration with increasing leachate concentration (Fig. 3B-D). The LOEC for diatoms, dinoflagellates, and ciliates was 0.062 $g\ L^{-1}$. The sigmoidal model fits the data well ($R^2 = 0.90–0.98$), with estimated EC_{50} values of 0.05 for diatoms, 0.08 for dinoflagellates, and 0.03 $g\ L^{-1}$ for ciliates (Table 2).

In the fourth experiment (E4), which involved the sampling at the Arinaga station in February 2023 (Table 1), only the dominant species *Ostreopsis cf. ovata* was analyzed (Fig. 4A). the cell concentration decreased with increasing leachate concentration (Fig. 4B), but the reduction was lower compared to the other species (Fig. 4). The LOEC for *Ostreopsis cf. ovata* in E4 was 0.5 $g\ L^{-1}$ and the estimated EC_{50} value was 0.66 L^{-1} (Table 2). In the fifth experiment (E5), conducted with a sample from Arinaga in April 2023 (Table 1), the community included as dominant species the dinoflalleglate *O. cf. ovata* and the diatoms *Pleurosigma elongatum, Actinocyclus octonarius, Licmophora spp., Trachyneis spp.,* and *Cylindrotheca spp* (Fig. 4A). All groups were negatively affected by leachate exposure but the decline in cell concentration was more pronounced for total diatoms and other dinoflagellates (Fig. 4C, D) than for *O. cf. ovata* (Fig. 4E). LOEC values were 0.13, 0.06 and 0.5 $g\ L^{-1}$ for total diatoms, other dinoflagellates, and *Ostreopsis*, respectively. The sigmoidal model fits the data well ($R^2 = 0.86–0.96$), with estimated EC_{50}

Table 2

The lowest observed effect concentration (LOEC) across the tested concentrations ($g\ L^{-1}$) and the parameters obtained from logistic sigmoid model (Eq. 1) used to describe the relationships between cell concentrations (cells mL^{-1}) and tire particle dilutions (%) after 72 h of exposure. #: experiment number as indicated in Table 1, C_0: cell concentration (cells mL^{-1}) in the absence of leachates; b: slope; ED_{50}: median effect dilution (%); SE: standard error; r^2: coefficient of determination; t and p are, respectively, the t-statistic and significance values for the estimated ED_{50} (%); EC_{50}: estimated median effect concentration ($g\ L^{-1}$).

#	Microplankton group/species	LOEC ($g\ L^{-1}$)	NOEC ($g\ L^{-1}$)	C_0 ± SE	b ± SE	ED_{50} ± SE	r^2	t	p	EC_{50} ± SE ($g\ L^{-1}$)
E1	Total Diatoms	0.125	0.063	18507 ± 710	1.7 ± 0.2	20 ± 2	0.97	11.2	< 0.0001	0.20 ± 0.02
	Chaetoceros spp.	0.125	0.063	8835 ± 285	1.8 ± 0.2	19 ± 1	0.98	14.0	< 0.0001	0.21 ± 0.01
	Total Dinoflagellates	0.063	< 0.063	13 ± 1	1.1 ± 0.3	6.7 ± 1.7	0.88	3.9	0.0015	0.10 ± 0.02
	Total Ciliates	0.250	0.125	0.3 ± 0.04	1.8 ± 0.7	14.6 ± 4.2	0.72	3.5	0.0035	0.15 ± 0.04
E2	Total diatoms	0.125	0.063	77 ± 4	0.8 ± 0.1	24 ± 5	0.90	5.3	< 0.0001	0.23 ± 0.05
	Other dinoflagellates	0.125	0.063	5.9 ± 0.3	1.1 ± 0.2	15 ± 3	0.91	5.6	< 0.0001	0.20 ± 0.03
	O. cf. ovata	0.063	< 0.063	50 % reduction no observed (EC_{50} >1 $g\ L^{-1}$)						
E3	Total diatoms	0.063	< 0.063	30 ± 2	0.7 ± 0.1	4.7 ± 1.5	0.93	3.2	0.0066	0.05 ± 0.02
	Total dinoflagellates	0.063	< 0.063	61 ± 2	0.8 ± 0.1	7.9 ± 1.0	0.98	7.9	< 0.0001	0.08 ± 0.01
	Total ciliates	0.063	< 0.063	2.8 ± 0.2	0.6 ± 0.2	3.0 ± 1.7	0.90	1.8	0.0978	0.03 ± 0.02
E4	*O. cf. ovata*	0.500	0.250	25 ± 1	0.9 ± 0.2	65 ± 12	0.87	5.6	< 0.0001	0.70 ± 0.1
E5	*O. cf. ovata*	0.500	0.250	66 ± 2	2.9 ± 0.6	69 ± 5.8	0.88	11.8	< 0.0001	0.66 ± 0.06
	Total diatoms	0.125	0.063	90 ± 3	1.2 ± 0.1	39 ± 4	0.96	9.7	< 0.0001	0.38 ± 0.04
	Other dinoflagellates	0.063	< 0.063	11 ± 1	0.8 ± 0.2	12 ± 3	0.86	3.6	0.0027	0.12 ± 0.03

Table 3
Summary of toxicity of tire particle leachates on marine plankton from previous studies.

Ecological Group	Species	Leachate conc. (g L^{-1})	Incubation time (h)	EC$_{50}$/LC$_{50}$ (g L^{-1})	Ref.
Phytoplankton	*Skeletonema costatum*	80	72	15.2 ± 0.96 (EC$_{50}$)	(Capolupo et al., 2020)
	Rhodomonas salina	1	24	0.39 (EC$_{50}$)	(Page et al., 2022)
	Rhodomonas salina	1	48	0.55 (EC$_{50}$)	(Page et al., 2022)
	Rhodomonas salina	1	72	0.64 (EC$_{50}$)	(Page et al., 2022)
	Thalassiosira weissflogii	1	24	0.60 (EC$_{50}$)	(Page et al., 2022)
	Thalassiosira weissflogii	1	48	0.65 (EC$_{50}$)	(Page et al., 2022)
	Thalassiosira weissflogii	1	72	0.73 (EC$_{50}$)	(Page et al., 2022)
	Heterocapsa steinii	1	24	0.11 (EC$_{50}$)	(Page et al., 2022)
	Heterocapsa steinii	1	48	0.11 (EC$_{50}$)	(Page et al., 2022)
	Heterocapsa steinii	1	72	0.23 (EC$_{50}$)	(Page et al., 2022)
Zooplankton (copepods)	*Acartia longiremis*	5–35	48	< 5 (LC$_{50}$)	(Halsband et al., 2020)
	Calanus sp.	5–35	48	35 (LC$_{50}$)	(Halsband et al., 2020)
	Tigriopus japonicus	10	96	5.34 (LC$_{50}$)	(Yang et al., 2022)
	Acartia tonsa	5	24	1.85 ± 0.17 (LC$_{50}$)	(Bournaka et al., 2023)
	Acartia tonsa	5	48	0.54 ± 0.07 (LC$_{50}$)	(Bournaka et al., 2023)
	Acartia tonsa	5	72	0.22 ± 0.06 (LC$_{50}$)	(Bournaka et al., 2023)
	Temora longicornis	5	24	4.43 ± 4.6 (LC$_{50}$)	(Bournaka et al., 2023)
	Temora longicornis	5	48	0.68 ± 0.09 (LC$_{50}$)	(Bournaka et al., 2023)
	Temora longicornis	5	72	3.80 ± 0.07 (LC$_{50}$)	(Bournaka et al., 2023)
	Centropages hamatus	5	24	3.00 ± 0.18 (LC$_{50}$)	(Bournaka et al., 2023)
	Centropages hamatus	5	48	2.06 ± 0.22 (LC$_{50}$)	(Bournaka et al., 2023)
	Centropages hamatus	5	72	1.18 ± 0.24 (LC$_{50}$)	(Bournaka et al., 2023)
	Oithona davisae	5	24	3.03 ± 0.09 (LC$_{50}$)	(Bournaka et al., 2023)
	Oithona davisae	5	48	1.79 ± 0.07 (LC$_{50}$)	(Bournaka et al., 2023)
	Oithona davisae	5	72	1.47 ± 0.12 (LC$_{50}$)	(Bournaka et al., 2023)
	Amonardia normanni	5	24	4.99 ± 1.22 (LC$_{50}$)	(Bournaka et al., 2023)
	Amonardia normanni	5	48	4.12 ± 0.18 (LC$_{50}$)	(Bournaka et al., 2023)
	Amonardia normanni	5	72	3.43 ± 0.21 (LC$_{50}$)	(Bournaka et al., 2023)
	Acartia tonsa (Nauplii)	1	48	0.401 ± 0.021 – 0.505 ± 0.022 (LC$_{50}$)	(Moreira et al., 2024)
	Acartia tonsa (Copepodite)	1	48	0.486 ± 0.022 (LC$_{50}$)	(Moreira et al., 2024)
	Acartia tonsa (Adult)	1	48	0.591 ± 0.035 0.599 ± 0.048 (LC$_{50}$)	(Moreira et al., 2024)
Meroplankton (larvae/embryos)	*Paracentrotus lividus*	1	72	0.158 (EC$_{50}$)	(Rist et al., 2023)
	Arbacia lixula	1	72	0.345 (EC$_{50}$)	(Rist et al., 2023)
	Diadema africanum	1	72	0.456 (LC$_{50}$)	(Rist et al., 2023)
	Mytilus galloprovincialis	80	48	1.78 (EC$_{50}$)	(Capolupo et al., 2020)

values of 0.38 g L^{-1} for total diatoms, 0.12 g L^{-1} for other dinoflagellates, and 0.66 g L^{-1} for *Ostreopsis* cf. *ovata* (Table 2).

Overall, growth rates for all studied microplankton groups were diminished relative to controls and declined with higher leachate concentrations, with varying degrees of sensitivity across different taxonomic groups (Fig. 5), supporting and validating our main hypothesis.

4. Discussion

4.1. Sensitivity of phytoplankton and other microplankton to tire particle leachates

Previous research on tire particle leachates' effects on marine phytoplankton is limited and primarily focused on a few cultured species (Capolupo et al., 2020; Page et al., 2022). Page et al. (2022) employed the same tire particles and leaching protocol as in this study to assess toxicity in monocultures of the flagellate *Rhodomonas salina*, the dinoflagellate *Heterocapsa steinii,* and the diatom *Thalassiosira weissflogii*, reporting EC$_{50}$ (72 h) values of 0.64, 0.23, and 0.73 g L^{-1}, respectively. In comparison, our study generally found lower EC$_{50}$ values for field-collected phytoplankton (EC$_{50}$ = 0.05 – 0.38 g L^{-1} for diatoms; EC$_{50}$= 0.08 – 0.20 g L^{-1} for "total/other dinoflagellates", Table 2), except for *O. cf. ovata,* which exhibited higher tolerance to leachates, with an EC$_{50}$ value of 0.66 g L^{-1} (E4, E5) or showing no significant effect (E2, EC$_{50}$ >1 g/L, Table 2). Capolupo et al. (2020) reported an EC$_{50}$ of 19.0 % (equivalent to 15.2 g L^{-1}) for *Skeletonema costatum* using a different methodology with a higher solid-to-liquid ratio (80 g L^{-1}), a value much higher than those observed in our study for diatoms (0.05 – 0.38 g L^{-1}; Table 3). The differences between our results and theirs likely stem from methodological variations, particularly the solid-to-liquid ratio. Our findings show that the sensitivity (EC$_{50}$) to tire particle leachates within diatoms and dinoflagellates can vary up to one of magnitude depending on the species/community (Table 2) and that, generally field-collected phytoplankton species appear more vulnerable to tire particle leachates compared to cultured species used in laboratory studies (Table 3). To our knowledge, no prior research has investigated the toxicity of tire particle leachates on marine ciliates. Our results revealed that ciliates appear to be the most sensitive microplankton group to these leachates, aligning with previous studies that have demonstrated their high vulnerability to pollution (Almeda et al., 2014, 2018). When compared to studies on planktonic metazoans, our data indicate that eukaryotic microplankton is generally more sensitive to tire leachates than copepods and meroplankton, except for *O. cf. ovata* (Tables 2 and 3).

4.2. Comparison of the toxicity of tire particle leachates with other plastic leachates

Growing evidence suggests that tire wear particles are more toxic to marine plankton than most conventional microplastics. For example, Capolupo et al. (2020) found that leachates from tire particles and polypropylene (PP) were the most toxic to the marine *diatom Skeletonema costatum*, with EC$_{50}$ values of 15.2 g L^{-1} and 14.4 g L^{-1}, respectively. In contrast, leachates from polyvinyl chloride (PVC) were less toxic (EC$_{50}$ = 28 g L^{-1}), while polystyrene (PS) and polyethylene terephthalate (PET) showed either much lower toxicity (EC$_{50}$ > 80 g L^{-1} for PS) or no effect in the case of PET. Similarly, Chae et al. (2020) tested expanded polystyrene (EPS) leachates on four species of microphytoplankton, finding no significant damage to photosynthesis and, in some cases, even enhanced growth compared to the controls. Schiavo et al. (2021) reported moderate toxicity from PP, PS, and polyethylene (PE) pellets, with PP being the most toxic (EC$_{50}$ = 8.5 g L^{-1}), followed by

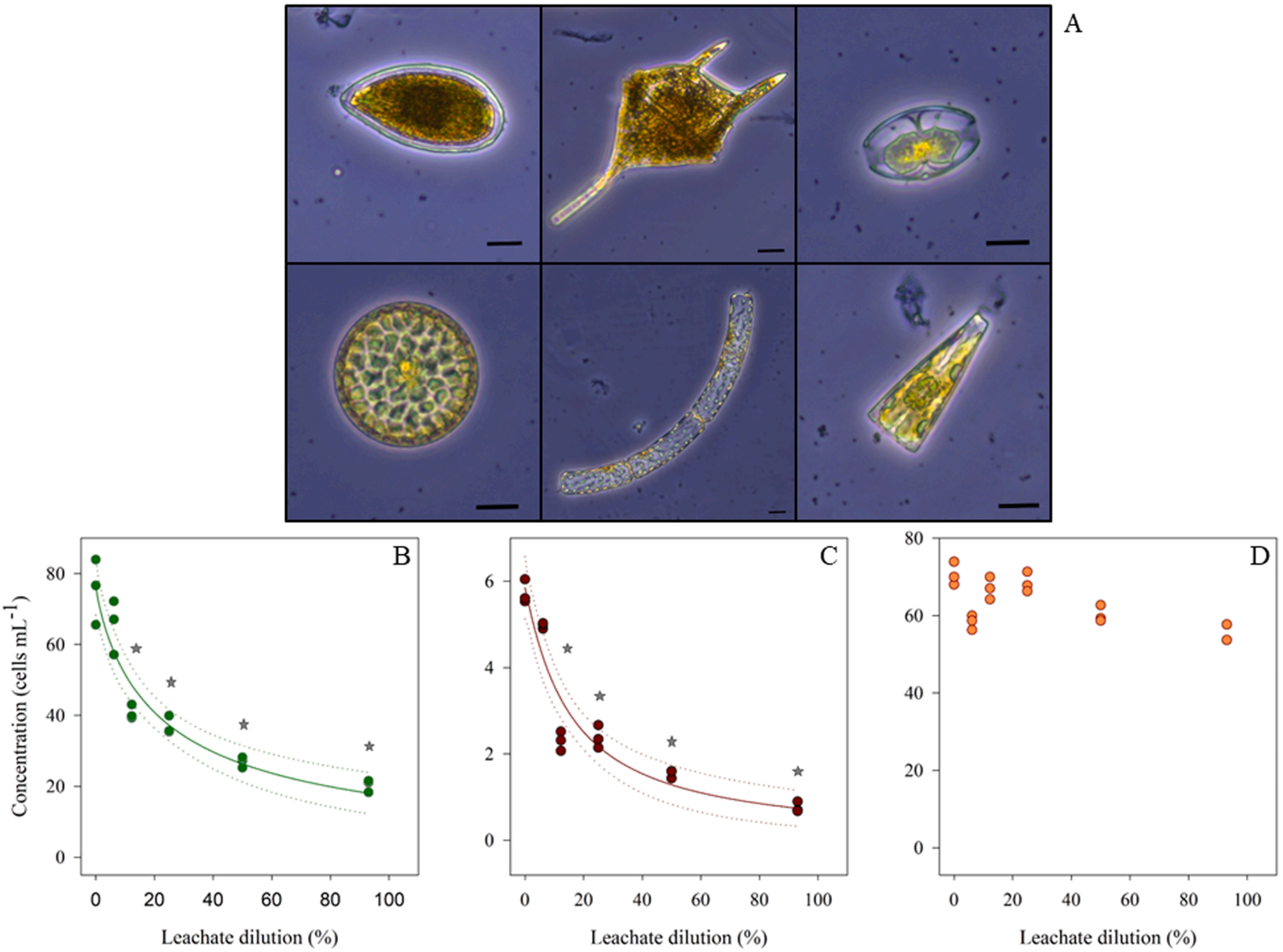

Fig. 2. A: Microscope images of representative species from the microplankton assemblage in E2 (Table 1), displayed from left to right and top to bottom: *O. cf. ovata., C. pentagonum, Ampora sp., Actinocyclus octonarius, Guinardia striata, and Licmophora* sp. (A). Abundances of total diatoms (B), other dinoflagellates (C), and *O. cf. ovata* (D) after 72 h of exposure to different leachate dilutions. The solid lines represent the fitted curves based on Eq. 1., with the dotted lines showing the 95 % confidence bands. The estimated model parameters are presented in Table 2. The stars denote statistically significant differences in cell concentration compared to the control ($p < 0.05$).

PS (12.9 g L^{-1}) and PE (16.5 g L^{-1}). Studies on the cyanobacteria Prochlorococcus exposed to PVC and high-density polyethylene (HDPE) leachates found EC$_{50}$ values ranging from 6.25–25 g L^{-1} for HDPE and 0.125–5 g L^{-1} for PVC (Sarker et al., 2020; Tetu et al., 2019). Comparing these EC$_{50}$ values with those from our study (0.03–0.70 g L^{-1}; Table 2), tire particle leachates are significantly more toxic than conventional microplastic leachates to marine microplankton. This suggests that tire wear leachates represent one of the most harmful types of microplastics to marine plankton food web.

4.3. Toxic compounds in tire particle leachates

The toxicity of conventional plastic leachates to plankton is primarily driven by leached additives, as demonstrated by the microalgae *Tisochrysis lutea* (Beiras et al., 2021). Plastic leachates, such as those from HDPE and PVC, disrupt key physiological processes in marine phytoplankton like *Prochlorococcus*, affecting photosynthetic rates, oxygen production, and gene expression linked to photosystem II, carbon fixation, metal transport, and cell division (Tetu et al., 2019; Amaneesh et al., 2023). Similar mechanisms are likely at play with tire rubber particles, which release a broad range of chemicals, contributing to their high toxicity to microplankton (Halsband et al., 2020; Capolupo et al., 2020; Page et al., 2022; Yang et al., 2022; Müller et al., 2022; Roubeau Dumont et al., 2023). Several toxicants were the detected in the tire particle leachates used in these studies, including polycyclic aromatic hydrocarbons, flame retardants, and metals like zinc (S.I. Table 1), in line with previous findings (Halsband et al., 2020; Capolupo et al., 2020; Page et al., 2022). These chemicals create a toxic cocktail likely impacting organisms through multiple pathways and mechanisms.

Zinc oxide is used as a vulcanization agent in tire rubber manufacturing (Councell et al., 2004). Tire particle leachates have been shown to contain high levels of zinc, reaching concentrations up to 5240 μg L^{-1} . Studies on marine nanophytoplankton and microphytoplankton species have demonstrated significant toxicity from both zinc (Jiao et al., 2023; Miao et al., 2005; Miller et al., 2010; Sarker et al., 2021) and zinc oxide (Hazeem, 2022), leading to growth reductions and impaired photosystem activity. Thus, this heavy metal has been suggested as one of the primary contributors to the toxicity of tire particle leachates on plankton (Capolupo et al., 2020; Halsband et al., 2020; Page et al., 2022). However, a recent study by Le Du-Carrée (2024) found that organic compounds, rather than zinc, were the primary toxic agents in tire leachates negatively affecting the marine microalgae *R. salina*.

To better understand the specific compounds or combinations responsible for the observed toxicity of tire particle leachates to plankton, and their associated mechanisms and pathways, further

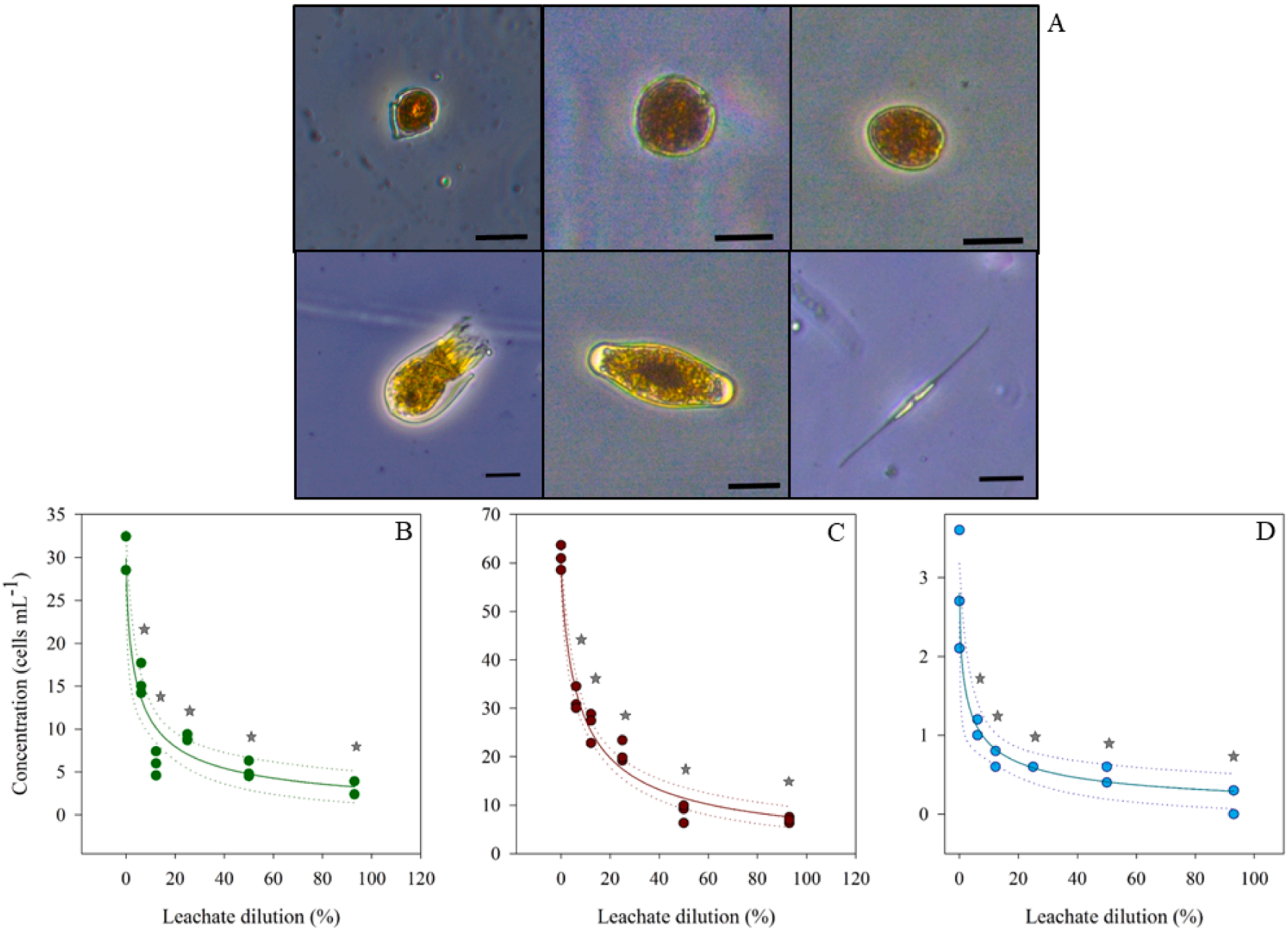

Fig. 3. A: Microscope images of the representative species from the microplankton assemblage in E3 (Table 1), displayed from left to right and top to bottom: the dinoflagellates *Scrippsiella sp., Gymnodinium sp., Prorocentrum sp., the tintinnid Ascampbelliella sp, and the diatoms Grammatophora sp., and Cylindrotheca sp*. (A). Abundances of total diatoms (B), total dinoflagellates (B), and total ciliates. (C) after 72 h of exposure to different leachate dilutions. The solid lines represent the fitted curves based on Eq. 1., with the dotted lines showing the 95 % confidence bands. The estimated model parameters are presented in Table 2. The stars denote statistically significant differences in cell concentration compared to the control ($p < 0.05$).

studies should focus on testing individual leachable additives alongside chemical fractionation of leachates and effect-directed analysis. More research is needed on the impact of organic toxicants from tire particles on marine microplankton, particularly alkylphenols (e.g., 4-tert-butylphenol, 4-tert-octylphenol) and the aromatic heterocyclic compound benzothiazole (Calle et al., 2025). Special attention should also be given to N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine (6PPD) and 6PPD quinone, tire rubber-derived chemicals of growing concern due to its emerging environmental toxicity, with species-specific toxicity (Calle et al., 2025). This research is critical for identifying the main drivers of toxicity and for developing strategies to mitigate the ecological impact of tire wear particle pollution.

4.4. *Are HAB-forming species more tolerant to pollution than other species?*

Previous studies have shown that certain harmful algal bloom (HAB)–forming dinoflagellates, such as *Prorocentrum* spp. and *Noctiluca scintillans*, exhibit a higher tolerance to pollution compared to other planktonic organisms (Almeda et al., 2014, 2018). Similarly, the response of *Ostreopsis* cf. *ovata* to tire particle leachates in this study highlights the relatively higher tolerance of certain HAB-forming species to pollution. One hypothesis for this resilience is the production of a mucilaginous matrix by these species. For instance, *Ostreopsis* ovata produces mucus composed of complex acidic polysaccharides, which play a crucial role in its interactions with the environment (Escalera et al., 2014; Giussani et al., 2015). While the full function of this mucus remains unclear, it has been associated with several ecological advantages, including allelopathy to reduce competition, defense against predators, metabolic regulation, and buoyancy (Reynolds, 2007). This mucus may also serve as a mechanism for retaining toxins, preventing their dilution into the surrounding environment, which could offer protection from harmful substances (Ternon et al., 2018). This ability to sequester substances could extend to toxic organic additives from tire particle leachates, limiting their diffusion into the cells and ultimately reducing their toxicity. Additionally, the rich carbohydrate content in the mucus supports a bacterial community that may contribute to detoxification processes (Pavaux et al., 2020). Given that *Ostreopsis* species are notorious for producing potent toxins like palytoxins and for generating harmful algal blooms (HABs) that have significant ecological and socio-economic impacts, their potential tolerance to pollution compared to their grazers (ciliates) and other phytoplankton requires further investigation in the context of escalating coastal pollution.

4.5. *Environmental risk and ecological implications*

There is still limited information on the concentrations of tire wear particles (TWP) and their leachates in marine environments, and current

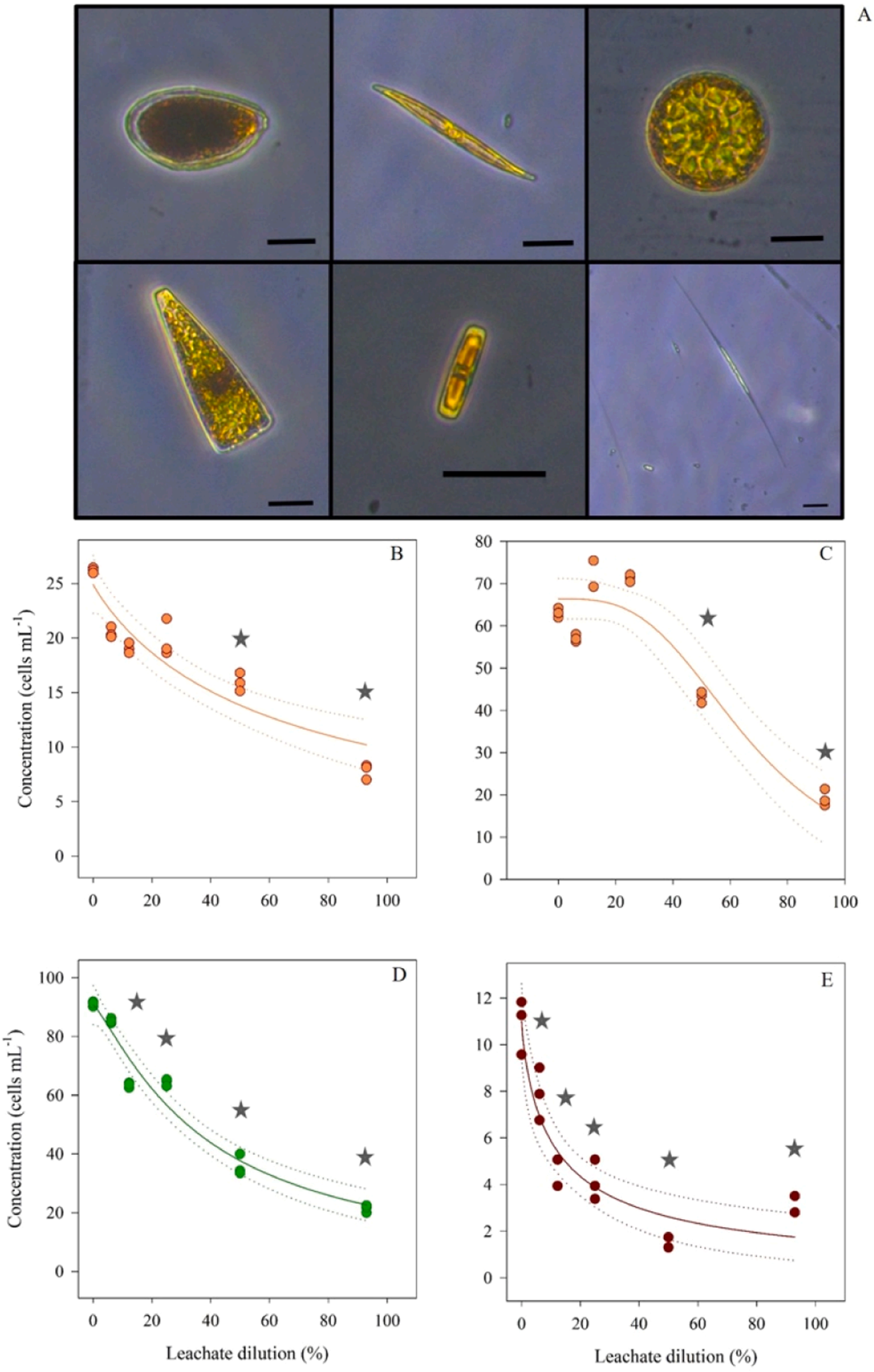

Fig. 4. A: Microscope images of representatives species from the microplankton assemblages in E4 and E5 (Table 1), displayed from left to right and top to bottom: *O. cf. ovata., Pleurosigma elongatum, Actinocyclus octonarius, Licmophora spp., Trachyneis spp., and Cylindrotheca* spp. Abundances of *O. cf. ovata* in E4 (B), *O. cf. ovata* in E5 (C), total diatoms (D) in E5, and other dinoflagellates in E5 (E) after 72 h of exposure to different leachate dilutions. The solid lines represent the fitted curves based on Eq. 1., with the dotted lines showing the 95 % confidence bands. The estimated model parameters are presented in Table 2. The stars denote statistically significant differences in cell concentration compared to the control ($p < 0.05$).

analytical methods for detecting and quantifying these pollutants remain insufficient (Mattonai et al., 2022). However, ecological impacts at current TWP pollution levels have already been observed, such as massive mortalities of coho salmon, a keystone species, following runoff events (Tian et al., 2021). Predicted concentrations of TWPs in surface waters range from 0.03 to 56 mg L^{-1} (Wik and Dave, 2009), while estimated discharges from surface water drainage vary between 12 and 179 mg L^{-1} (Baumann and Ismeier, 1998; Kumata et al., 1997, 2000, 2002; Parker-Jurd et al., 2021; Reddy and Quinn, 1997; Zeng et al., 2004). TWPs that accumulate in sediments or soil can continue to leach

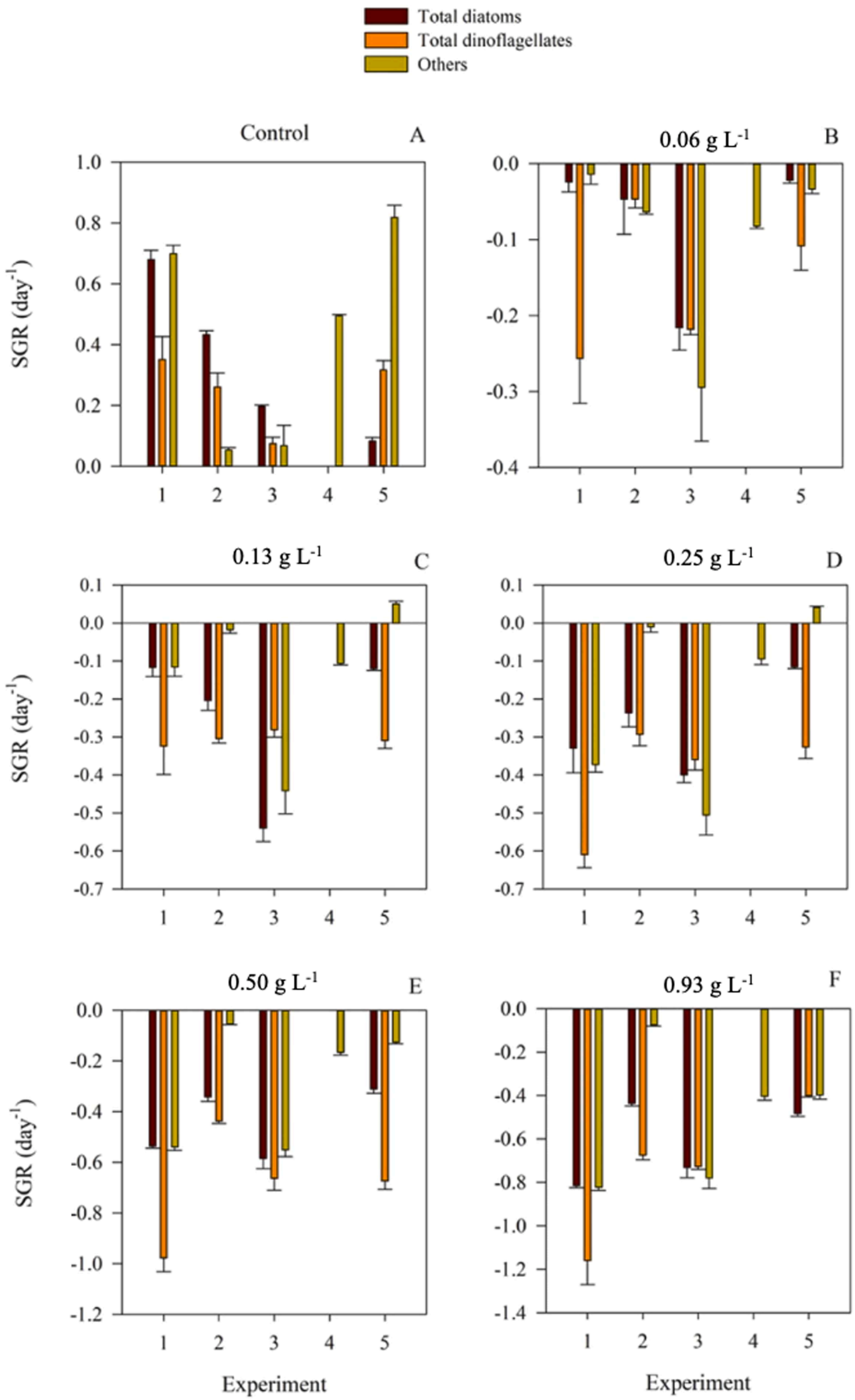

Fig. 5. Specific growth rate (SGR, d^{-1}) of each phytoplankton group and station in the control treatment (A) and the different leachate dilutions (B-F). From experiment 1 to experiment 5, the label "others" represents, respectively, the group *Chaetoceros* spp., *O. cf. ovata*, total ciliates, *O. cf. ovata,* and *O. cf. ovata*.

toxic chemicals into the water over time. Concentrations of TWPs in sediments and soils are often significantly higher than those in the water column, reaching up to 155 g kg^{-1} dry weight (DW) (Wik and Dave, 2009; Wagner et al., 2018;). Some of the EC50 and LOEC values reported in this study fall within the range of predicted TWP concentrations in surface waters and drainage discharges, suggesting a potential ecological risk to marine plankton food webs, particularly in enclosed coastal areas and bays that are exposed to wastewater effluents and runoff events. Beyond laboratory bioassays, *in situ* field studies examining the effects of TWP on plankton communities after runoff events and drainage discharges are necessary to better assess the ecological impact of TWP pollution on the coastal marine food web.

The toxic effects of TWPs on the microplankton community could significantly disrupt the structure and function of marine food webs, thereby compromising the overall health of estuarine and coastal ecosystems. Our findings indicate that tire particles are toxic to key primary producers, such as phytoplankton, at environmentally relevant concentrations, potentially leading to a reduction in primary production, a critical foundation of the marine food web. The adverse effects on ciliates, which are key grazers in marine ecosystems, could further disrupt energy transfer up the food web, particularly through the ciliate-copepod link, which plays a crucial role in the marine food chain (Calbet and Saiz, 2005). Additionally, the reduction of non-toxic phytoplankton (nutrient competitors) and major grazers due to TWP pollution could increase the proliferation of harmful algal blooms (HABs) by species with higher tolerance to TWP, such as *O. ovata*, in certain circumstances. This could contribute to more frequent and intense HABs in coastal areas, with significant ecological, health, and economic consequences.

Globally, approximately six million tons of TWPs are estimated to be emitted annually, with up to 20 % entering aquatic ecosystems (Baensch-Baltruschat et al., 2021). As global population and vehicle usage continue to rise, TWP pollution and its associated ecological impacts are likely to increase. Therefore, more field studies and continuous monitoring are urgently needed to better understand the distribution, transformation, and toxicity of TWPs and their leachates in coastal waters. Overall, our findings highlight the critical need for the development of ecologically safer tire rubber additives and effective strategies to reduce traffic-related TWP emissions. Addressing these issues is essential to mitigate the effects of TWPs into aquatic ecosystems and prevent further damage to marine biodiversity and ecosystem services.

5. Conclusions

Our study reveals that tire particle leachates have a detrimental impact on unicellular microplankton communities, leading to reduced growth across various groups at environmentally relevant concentrations. The main microplankton groups (diatoms, dinoflagellates, and ciliates) exhibited different sensitivities to tire particle leachates. Ciliates were the most sensitive, while the HAB-forming dinoflagellate *Ostreopsis cf. ovata* showed the highest tolerance. These findings suggest that tire particle leachates have the potential to disrupt marine food web dynamics by altering both the structure and function of microplankton communities. This could have broader ecological implications, particularly in coastal environments where tire particle pollution is more concentrated.

CRediT authorship contribution statement

ALMEDA RODRIGO: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Sampalo Marta:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **Gómez May:** Writing – review & editing, Supervision, Resources.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the revision of this work, the author(s) used ChatGPT, a language model developed by OpenAI, in order to revise the grammar and language fluency. After using this tool/ service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2025.117787.

Data availability

Data will be made available on request.

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