Highlights

- Plastic litter collected by commercial fishing trawlers was analyzed.
- The microbiota on plastic litter, in seawater, and in sediment was determined.
- *Vibrio parahaemolyticus* and *Vibrio alginolyticus* were detected on plastic litter.
- Multiple resistance among *Vibrio* isolates was detected.

A preliminary study of the cultivable microbiota on the plastic litter collected by commercial fishing trawlers in the south-eastern Adriatic Sea, with emphasis on *Vibrio* isolates and their antibiotic resistance

Damir Kapetanović [a,*], Irena Vardić Smrzlić [a], Snježana Kazazić [a], Dario Omanović [a], Neven Cukrov [a], Ana-Marija Cindrić [a], Ana Rapljenović [a], Lorena Perić [a], Karla Orlić [a], Tatjana Mijošek [a], Zuzana Redžović [a], Ana Gavrilović [b], Tena Radočaj [b], Vlatka Filipović Marijić [a]

[a] Ruđer Bošković Institute, Bijenička cesta 54, 10000 Zagreb, Croatia

[b] University of Zagreb Faculty of Agriculture, Svetošimunska cesta 25, 10000 Zagreb, Croatia

*Corresponding author. E-mail: kada@irb.hr

ABSTRACT

Mediterranean Sea is the sixth largest area of marine litter accumulation in the world, and plastic pollution is a growing problem in its Adriatic sub-basin. The aim of the present study was to evaluate the cultivable microbiota associated with plastic litter collected by commercial fishing trawlers in the south-eastern Adriatic Sea in comparison with microbiota in seawater and sediment. Plastic litter in the sea contains an autochthonous microbiota that is different from that of the surrounding seawater and sediment. *Vibrio* abundance was higher on plastic litter than in surrounding seawater and sediment. All isolated *Vibrio* showing resistance to ampicillin and vancomycin, while resistance to other antibiotics depended on the isolated species. Overall, this study provides for the first time information on the cultivable microbiota associated with plastic litter collected by commercial fishing trawlers and provides a data base for further studies.

Keywords: macroplastics, microplastics, Mediterranean, pathogens, marine fishing, antibiotic resistance

1. Introduction

In recent years, plastics in the ocean have become a significant global environmental problem (Moore et al., 2020), as plastics are the largest, most harmful, and most persistent fraction of marine litter (Carlson et al., 2017; Zeri et al., 2018; Mokos et al., 2020; UNEP, 2021). The Mediterranean Sea is the sixth largest accumulation area for marine litter, and the Adriatic Sea has been described as one of the areas most affected by marine plastic litter (MPL) pollution (Palatinus et al., 2019; UNEP 2021). Recent studies of marine litter in the Adriatic Sea have revealed high concentrations of macro- and micro-litter at the sea surface, on beaches, on the sea bottom, in sediments, and in biota (Palatinus et al., 2019; Mokos et al. 2020; Mistri et al., 2022). Methods for sampling of plastic litter on the sea bottom vary widely, ranging from observation by divers or remotely operated underwater vehicles to actual collecting with trawls or grabs (Schmid et al., 2021).

A bacterial biofilm has been shown to develop rapidly on MPL (UNEP, 2021), which is taxonomically distinct from that of surrounding seawater (Bowley et al., 2021). MPL may act as vectors and indirectly promote the transmission of microorganisms through the marine environment (Meng et al., 2021), pathogens (Barboza et al., 2018), and even infections (Zhang et al., 2020), and cause antimicrobial resistance (Bowley et al., 2021). Bacterial communities on MPL have been shown to contain pathogenic organisms such as *Vibrio* (Bowley et al., 2021), *Escherichia coli*, *Morganella morganii*, *Stenotrophomonas maltophilia*, *Bacillus cereus*, *Aeromonas popoffii*, and *Aeromonas salmonicida* (Barboza et al., 2018; Sathicq et al., 2021).

The genus *Vibrio* includes more than 63 species that are autochthonous and ubiquitous aquatic microorganisms in marine environments (Kapetanović et al., 2013). *Vibrio* has been found in large numbers on MPL, especially during the summer months (Bowley et al., 2021), with pathogenic potential for human and aquatic organisms (Laverty et al., 2020; Sathicq et al., 2021). Previous studies of marine microplastics have detected high levels of *V. parahaemolyticus*, *V. vulnificus*, and *V. cholerae*, which can cause septicemia or diarrhea in humans (Meng et al., 2021). *Vibrio* pathogens can also cause losses in aquaculture (Laverty et al., 2020), because *Vibrio* strains can cause vibriosis (Kapetanović et al., 2013). MALDI-TOF MS is a highly specific and sensitive option for *Vibrio* species identification (Dieckmann et al., 2010; Zhenyu et al., 2013, Kirstein et al., 2016; Bronzato et al., 2018; Håkonsholm et al., 2020; Moussa et al., 2021).

Increasing human activities along coasts, especially in the surrounding semi-enclosed basins such as the Adriatic Sea, make them ideal hotspots for microbial pollution (Manini et al.,

2022.). Variations in bacterial abundance in the marine environment are controlled by the interactions of complex physico-chemical and biological parameters (Perkins et al., 2014). Monitoring fecal pollution is essential to identify reservoirs and hotspots that promote the spread of fecal bacteria in coastal areas (Manini et al., 2022), including microbial contamination of MPL. Microbial contamination may pose an additional risk due to the potential spread of antibiotic resistance. There is concern that MPL serves as a vector for pathogens and that antibiotic resistance could spread through bacteria on MPL (Laverty et al., 2020).

Litter, including MPL, lying on the sea bottom is often caught by bottom trawls and makes up a variable part of fishermen's daily catch (Ronchi et al., 2019). Although there are numerous studies on the composition of marine litter collected during bottom trawl surveys in the Adriatic Sea (Strafella et al., 2015; Anastasopoulou et al., 2018; Ronchi et al., 2019; Schmid et al., 2021) and beyond (Pham et al., 2014; Neves et al., 2015; Moriarty et al., 2016; García-Rivera et al., 2017; Lopez-Lopez et al., 2017; Urban-Malinga et al., 2018; Galgani et al., 2000), no previous study has examined the cultivable bacteria associated with MPL collected by bottom trawl.

The objective of this study was to determine the cultivable bacteria associated with MPL collected by commercial fishing trawlers compared to seawater and sediments from the same habitat, focusing on *Vibrio* and their antibacterial resistance.

2. Materials and methods

The studied fishing area is located between the Pelješac peninsula in the west and the mainland in the east, with an average depth of about 35 m, in the area of the town of Ploče, one of the largest ports in the south-eastern part of the Adriatic Sea, Croatia (Fig. 1). This area is characterized by several underground freshwater springs reaching the sea, and the main influence from the land is the Neretva River.

Marine plastic litter (MPL) was collected from the seabed in May 2021 using a commercial trawl with a cod end mesh size of 40 mm. After trawling, MPL was separated from the catch and identified. After excess water and mud were removed, MPL was counted, weighed, and classified into the different categories (Neves et al, 2015; Strafella et al., 2015; Lopez-Lopez et al, 2017; Strafella et al., 2019). Six different plastic litter items were selected for microbial analysis, three from the PET bottles and three from the nylon bags made of polyethylene. Swabs were taken from the surface of each plastic item in duplicate, for a total of 12 swabs for isolation and subsequent bacterial identification (Ismaïl et al., 2013). The swabs were then serially diluted in 10 mL of sterile 0.9% NaCl B. Braun solution (Pliva).

The vertical distribution of physico-chemical parameters (temperature, salinity, dissolved oxygen, and chlorophyll-a) was measured using a multiparameter CTD probe EXO2 (YSI, Xylem). Sediment samples (10 g of the top sediment layer) were collected using a UWITEC gravity corer, while seawater samples were collected using the single sampling method with a homemade water sampler and placed in sterile 1-litre bottles. All samples were serially diluted in 10 mL of sterile 0.9% NaCl B. Braun solution.

Total coliforms and *E. coli* were determined with Colilert-18™ and enterococci with Enterolert-E™ (IDEXX, Westbrook, ME, USA) (Ramljak et al., 2022). Heterotrophic plate counts (HPC) and *Vibrio* bacteria from MPL swabs, seawater and sediment samples were determined by the spread plate method on Difco™ Marine Agar (MA) 2216 medium (BD) and on selective Thiosulfate-Citrate-Bile-Sucrose (TCBS) medium (BD), respectively. Both plates were incubated for 24-48 hours at 35 °C and 3-5 days at 22 °C. Results are expressed as the mean number of colony-forming units (CFU) per 1 cm^2 of plastic surface and in 1 mL of sediment and seawater ± the standard deviation of two technical replicates per sample type. Bacterial colonies representing different morphologies per plate on MA and TCBS Agar were then selected and transferred to Difco™ Tryptic Soy Agar (TSA) (BD) with the addition of 1% NaCl (Kemika) purification plates. The purified colonies were then identified using matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) and analyzed for antimicrobial resistance.

Sample preparation for MALDI-TOF MS analysis was performed according to the Bruker protocol for the extended direct transfer method (Kapetanović et al., 2022). The Kirby–Bauer disk diffusion method on BBL™ Mueller Hinton II agar (BD) was used to determine the antimicrobial susceptibility of the isolated bacterial strains (Ramljak et al., 2022). The following antimicrobial disks were used: ampicillin, streptomycin, gentamicin, chloramphenicol, ciprofloxacin, erythromycin, imipenem, oxytetracycline, sulfamethoxazole/trimethoprim, vancomycin, enrofloxacin, florfenicol, and flumequine. The multiple antibiotic resistance index (MAR) was calculated for each isolate studied by summing the number of antibiotics to which the isolate was resistant and dividing by the number of 13 antibiotics (Letchumanan et al., 2015).

The nonparametric Mann-Whitney test using SigmaPlot version 14.0 (Systat Software Inc., San Jose, CA, USA) was used for statistical analyses of bacterial content between MPL swabs, seawater, and sediment samples. Comparison between sampling sites was performed using Kruskal-Wallis one-way analysis of variance based on ranks, followed by post hoc Dunn test. The observed differences were statistically significant at $p < 0.05$. To visualize the cultivable

bacteria, Venn diagrams were generated using a freely available web tool (http: //bioinformatics.psb.ugent.be/webtools/Venn/, accessed August 11, 2022).

3. Results

The vertical profiles of the main physico-chemical parameters are shown in Fig. S1, while the table of trawl transect numerical data can be found in Table S1. The temperature profiles reflect the expected behaviour considering the season, i.e., solar radiation and air temperature, as well as the flow of the water masses. Higher temperatures were determined at the surface than at deeper layers, indicating a weak thermocline below 14 m. The salinity profiles show a clear distinction of sites close to the Neretva River mouth, exhibiting lower salinity in the surface layer due to the mixing of freshwater with seawater. The concentrations of dissolved oxygen in the water were always above 100% at all stations in spring. With the exception of station PL1, where an increase in chlorophyll-a was recorded in deeper layers and an increase in dissolved oxygen in the halocline, the concentration of dissolved oxygen at the other stations ranged between 105% and 110%, while the vertical profiles of chlorophyll-a were uniform.

The total length of the fishing trawl transect was 796.36 km, and transect began 2150 m from the nearest coast and ended 1670 m from the nearest coast. During the fishing trawl, 130 kg of material was collected from the bottom. Of that, there were 100 kg of fish. The wet mass of litter of anthropogenic origin was 22 kg, or 17% of the total catch. Litter consisted of 2 big plastic bins (20%) made of Polypropylene (PP), and 79 whole and partially destroyed PET bottles (40%), nylon bags made of Polyethylene (PE) (10 %) and one low-density polyethylene (LDPE) bottle, the rest were tin and aluminum cans and aluminum remains of tetrapaks (Fig. S2). Most of the bags did not have an intelligible inscription and their origin could not be determined, although those with an inscription were found to be of local origin.

Results of microbial analysis of seawater and sediment at all sampling sites showed low levels of fecal indicator bacteria (Fig. S3). Total coliform results indicated statistically higher abundance in seawater than in sediment ($p < 0.05$). The abundance of total coliforms in seawater ranged from < 10.0 to 9606.0 MPN/100ml, while the maximum abundance in sediments was 1467 MPN/100ml, with no statistically significant differences among sampling sites ($p > 0.05$). On the other hand, the abundance of *E. coli* and enterococci was higher in sediments than in seawater, but statistically significantly different only for enterococci ($p < 0.05$).

All analyzed bacteria were most abundant on MPL, except HPC at 35 °C (Table 1). Nevertheless, only a statistically significant difference was found in the number of *Vibrio* on

MPL compared to their number in seawater at both incubation temperatures ($p<0.05$), while there was no statistically significant difference with respect to sediment ($p>0.05$). Lower incubation temperatures (22 °C) consistently resulted in higher levels of HPC in seawater and sediment and *Vibrio* on MPL, seawater, and sediment. The differences were statistically significant for higher HPC values obtained at 22 °C for seawater and *Vibrio* counts in seawater and sediment ($p < 0.05$).

The cultivable microbiota on MA at 22 °C from swabs of MPL consisted of the following genera: *Vibrio* (54%), *Pseudomonas* (23%), *Exiguobacterium* (15%), and *Psychrobacter* (8%) (Fig. 2). *Vibrio* (34%), *Pseudomonas* (22%), and *Psychrobacter* (22%) accounted for 78% of the total microbiota isolated from MPL at 35 °C, while the rest of the microbiota were: *Microbacterium* (11%) and *Shewanella* (11%). The microbiota obtained from seawater on MA at 22 °C consisted of the genera: *Vibrio* (89%) and *Streptococcus* (11%), while *V. ostreicida* and *S. hominis* were isolated and identified at 35 °C. *Vibrio* (50%), *Bacillus* (25%), *Shewanella* (15%) and *Fictibacillus* (10%) were isolated from sediment samples on MA at 22 °C (Fig. 2). Two species of the genera *Bacillus* (*B. cereus*, *B. indicus*) and *Vibrio* (*V. pomeroyi*, *V. gigantis*) were identified among the isolates, as was *F. arsenicus*. In contrast, *Bacillus* (65%), *Fictibacillus* (22%), *Vibrio* (9%) and *Solibacillus* (4%) were identified from the sediment at 35 °C. *B. cereus*, *B. idriensis*, *B. licheninformis*, *B. pumilus* and *B. megaterium* are the dominant microbiota. In addition, *F. arsenicus* and *V. harveyi* were also identified.

V. alginolyticus was the predominant species on MPL at both incubation temperatures with a relatively higher abundance at 35 °C and the other was *V. parahameolyticus* (Fig. 3). *V. gigantis* was the only cultivable *Vibrio* species on TCBS from seawater at 22 °C, while no bacteria of the genus *Vibrio* were identified at 35 °C. Two bacteria of the genus *Vibrio* were isolated from sediment at 22 °C on TCBS, and *V. gigantis* was also abundant in the seawater samples, while *V. pomeroyi* was dominant in the sediment. At the same time, two *Vibrio* species were identified from the sediment at 35 °C: *V. alginolyticus* and *V. parahaemolyticus*, which were common with those on MPL.

The potential pathogens *V. parahaemolyticus* and *V. alginolyticus* were detected on the MPL and in sediment, while *V. gigantis* was identified in seawater and sediment (Fig. 5). The list of bacteria that enter fish from the environment and cause fish spoilage is well known (Comi et al. 2021). Our study identified spoilage bacteria on MPL (*Pseudomonas*, *Shewanella*, *Vibrio*), in seawater (*Vibrio*), and in sediment (*Bacillus*, *Vibrio*, *Shewanella*).

All bacterial isolates of the genus *Vibrio* obtained from MPL and sediments showed resistance to ampicillin and vancomycin (Table 2). *V. alginolyticus* isolated from MPL showed multiple

resistance from two to five antibiotics used and consequently an MAR index between 0.15 and 0.38. *V. parahaemolyticus* from MPL showed that all isolates were resistant only to ampicillin and vancomycin (MAR = 0.15). Interestingly, the greatest resistance of *Vibrio* strains was found in sediment samples. One isolate of *V. alginolyticus* showed resistance only to ampicillin and vancomycin (MAR = 0.15), while the other isolate was resistant to five other antibiotics (MAR = 0.54). *V. pomeroyi* and *V. gigantis* showed multiple antibiotic resistance; they were resistant to five (MAR = 0.38) and eight (MAR = 0.61) antibiotics, respectively.

4. Discussion

Fecal pollution was not evident in our sampling in May 2021 compared to the October 2010 sampling by Kušpilić et al. (2010) in the same area. The assessment of the impact of MPL on the survival of fecal indicator organisms in Scotland, in May showed an abundance of *E. coli* in seawater of 140 CFU/100ml (Quilliam et al., 2014), while in our study the abundance of *E. coli* and enterococci was 10.0 MPN/100ml each.

MPL has higher bacterial counts than surrounding waters previously reported by Silva et al. (2019). Our observations partially agree with those of Quilliam et al. (2014), where *Vibrio* was predominant on MPL compared to water, but differ with respect to HPC, which was higher in seawater (at 25 °C). Results are consistent with previous reports on HPC (Kapetanović et al., 2013) and *Vibrio* isolation, which indicated higher bacterial counts in vitro at a temperature around 25 °C (Larsen et al., 1984; Takemura et al., 2014; Ina-Salwany et al., 2019).

It is well known that plastic waste can contribute to microbial pollution and water quality (Quilliam et al., 2014). In the aquatic environment, the surfaces of MPL are rapidly covered by organic material, which degrades their hydrophobic surface and facilitates microbial colonization (Wright et al., 2020). Under these stable environmental conditions in the spring season, different numbers of bacterial genera were found on MPL, seawater, and sediment. Water temperature as an indicator of seasonality was positively correlated with the number of cultivable bacteria (Kopprio et al., 2020), and the effect of temperature on *Vibrio* is known to be greatest in the spring (Takemura et al., 2014). High water temperature and low salinity best explained the *Vibrio* counts in spring (Oberbeckmann et al., 2012). In the spring, as temperatures warm, growth conditions favor chlorophyll a, which also affects *Vibrio* abundance (Oberbeckmann et al. 2012; Takemura et al., 2014). The occurrence of bacterial species is affected by the quality of seawater (Onianwah et al., 2018), which is often affected by sewage pollution from neighboring cities like Ploče (Manini et al., 2022). At the same time, sediments represent more stable conditions that are less subject to change than surrounding

seawater (Perkins et al. 2014). This may have influenced the nearly equal representation of species in the sediments, but in varying numbers, likely due to the influence of incubation temperature (Perkins et al. 2014; Manini et al., 2022), as microbial indicators suggested an environmental condition without fecal pollution and limited municipal influence at the mouth of the Neretva River compared to previous studies (Kušpilić et al., 2010).

The microbiota on MPL is taxonomically distinct from that of the surrounding seawater (Bowley et al., 2021). The microbiota of each sample type was composed of different genera, and the different genera were dominant in the different sample types (Fig. 2). *Vibrio* was the dominant genus in each sample type at 22 °C (Bowley et al., 2021; Meng et al., 2021), while *Shewanella* spp. were found on MPL and sediment (Fig. 4). At both incubation temperatures, the collected MPL contained significant numbers of bacteria from the genera *Vibrio*, *Pseudomonas*, and *Psychrobacter*. However, the surrounding seawater and sediments did not contain this microbiota as on MPL, with the exception of *Vibrio*. *Vibrio* is thought to attach to and degrade biological surfaces and polymeric substrates, suggesting that specific attachment to surfaces is an important growth strategy of *Vibrio* (Takemura et al., 2014), just as sediments are a potential reservoir for *V. parahaemolyticus* (Vezzulli et al., 2009).

In agreement with previous reports for the Adriatic Sea (Kapetanović et al. 2013; Ramljak et al. 2022) and worldwide (Zavala-Norzagaray et al. 2015), *V. alginolyticus* isolates from Adriatic seawater showed a similar pattern in the resistance to vancomycin and ampicillin. In the study of Letchumanan et al. (2015), the multiple resistance patterns of *V algynoliticus* were found as in our study. They also pointed out that MAR index more than 0.2 could be a marker of contamination from high-risk sources, indicating a potential health risk to humans. There are no other similarities in the resistance of *V. parahaemolyticus* to other antibiotics in our study compared to the available literature (Zavala-Norzagaray et al. 2015).

This study is the first report of cultivable bacteria on MPL collected by commercial fishing trawlers compared to those in seawater and sediments. The results showed that the microbiota on MPL is more diverse compared to that in seawater and sediments. The results indicate the presence of pathogenic and spoilage bacteria on MPL, as well as the presence of bacteria resistant to antibiotics, which is a potential health risk in this fishing zone.

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Table 1. Microbiological analysis of heterotrophic bacteria (HPC) and *Vibrio* counts from swabs of plastic litter, seawater and sediment obtained at two incubation temperatures (22 °C and 35 °C).

	Plastic litter	**Seawater**	**Sediment**
HPC 35 °C (CFU/mL)	1370.8 ± 1233.8	675.7 ± 717.8	1405.9 ± 1556.8
HPC 22 °C (CFU/mL)	2055.8 ± 1783.9	1182.2 ± 318.5	1330.5 ± 1061.7
Vibrio 35 °C (CFU/mL)	374.6 ± 1047.7*	0.2 ± 0.4*	10.9 ± 20.2
Vibrio 22 °C (CFU/mL)	520 ± 1544.8*	3.7 ± 9.1*	92.5 ± 63.3

*- Statistically significant differences Kruskal – Wallis test Dunns method ($p<0.05$)

Table 2. Antibiotic resistance among *V. alginolyticus* and *V. paraheaemolyticus* isolated from swabs of marine plastic litter and sediments in selected fishing zone of the southeastern Adriatic Sea.

No. of isolates	**Species**	**Source**	**Antibiotic resistance profile**	**MAR**
25	*V. alginolyticus*	Plastic	ampicillin, vancomycin	0.15
6	*V. alginolyticus*	Plastic	ampicillin, vancomycin, streptomycin	0.23
3	*V. alginolyticus*	Plastic	ampicillin, vancomycin, erythromycin	0.23
2	*V. alginolyticus*	Plastic	ampicillin, vancomycin, erythromycin, gentamicin	0.31
1	*V. alginolyticus*	Plastic	ampicillin, vancomycin, sulfamethoxazole/trimethoprim	0.23
1	*V. alginolyticus*	Plastic	ampicillin, vancomycin, streptomycin, ciprofloxacin	0.31
1	*V. alginolyticus*	Plastic	ampicillin, vancomycin, streptomycin, erythromycin	0.31
1	*V. alginolyticus*	Plastic	ampicillin, vancomycin, streptomycin, gentamicin, ciprofloxacin,	0.38
4	*V. parahaemolyticus*	Plastic	ampicillin, vancomycin	0.15
1	*V. alginolyticus*	Sediment	ampicillin, vancomycin	0.15
1	*V. alginolyticus*	Sediment	ampicillin, vancomycin, erythromycin, enrofloxacin, oxytetracycline, chloramphenicol, streptomycin	0.54
1	*V. gigantis*	Sediment	ampicillin, vancomycin, erythromycin, enrofloxacin, oxytetracycline, chloramphenicol, ciprofloxacin, flumequine	0.61
1	*V. pomeroyi*	Sediment	ampicillin, vancomycin, erythromycin, oxytetracycline, chloramphenicol	0.38

Table S1. Physico-chemical parameters (temperature, pH, salinity, dissolved oxygen, chlorophyll) of seawater on fishing trawl transect

Sites	Date	Depth (m)	Temperature (°C)	pH	Salinity (ppt)	Oxygen (%)	Oxygen (mg/L)	Chl-a (ug/L)
PL 4	25.5.2021.	0.5	18.02	8.40	38.06	107.44	8.10	0.00
PL 4	25.5.2021.	6.0	17.90	8.40	38.20	107.30	8.10	0.11
PL 4	25.5.2021.	12.0	17.87	8.40	38.31	107.39	8.10	0.77
PL 4	25.5.2021.	21.0	15.46	8.39	38.55	104.40	8.20	1.85
PL 5	25.5.2021.	0.5	18.37	8.49	37.84	107.98	8.10	0.05
PL 5	25.5.2021.	6.0	17.89	8.47	38.23	107.72	8.10	0.28
PL 5	25.5.2021.	12.0	17.57	8.47	38.34	107.47	8.20	0.30
PL 5	25.5.2021.	19.5	16.09	8.47	38.65	108.87	8.50	0.65
PL 7	25.5.2021.	0.5	18.81	8.44	37.69	108.37	8.10	0.10
PL 7	25.5.2021.	6.0	18.10	8.44	38.19	107.14	8.00	0.31
PL 7	25.5.2021.	12.0	17.71	8.44	38.32	107.39	8.10	0.41
PL 7	25.5.2021.	26.5	16.18	8.44	38.59	108.26	8.40	1.08

Figure(s)

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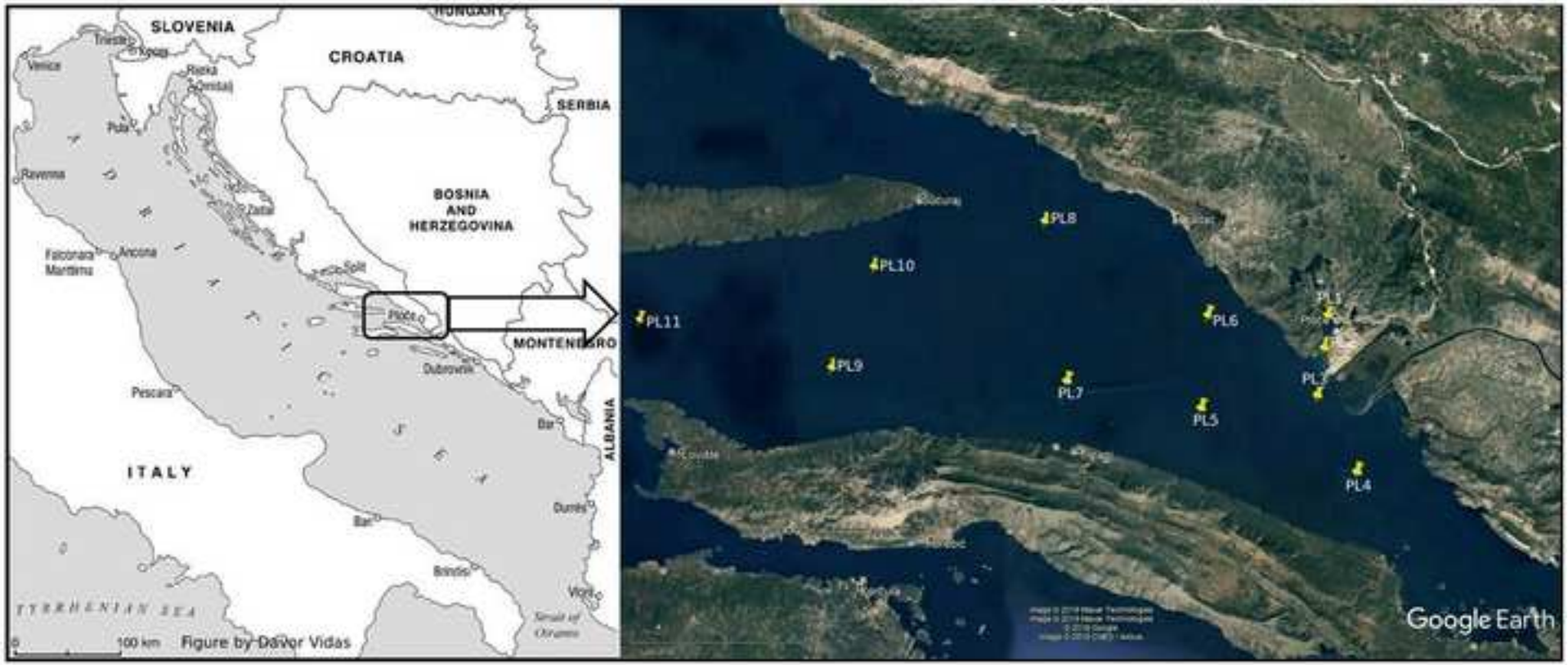

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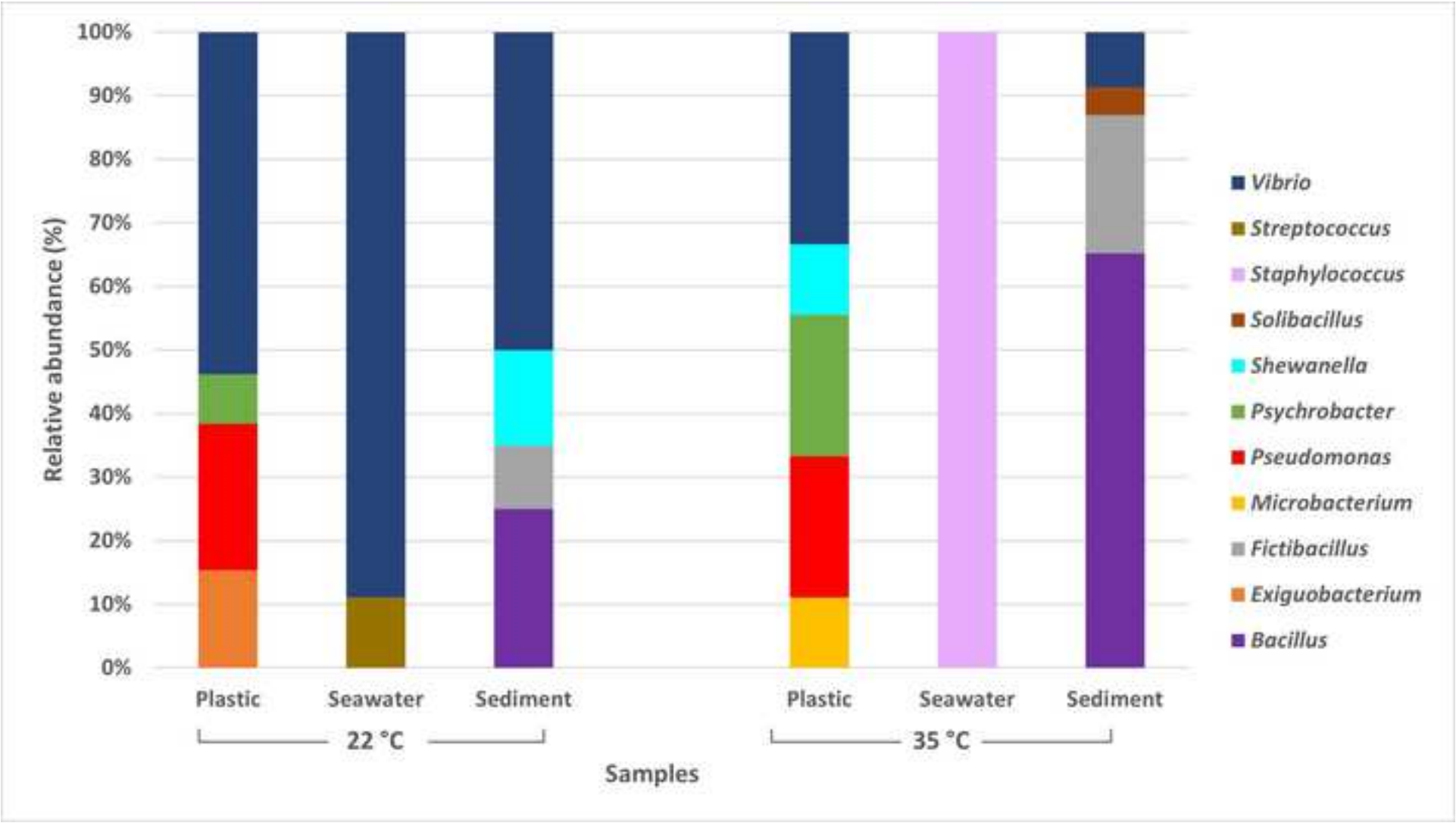

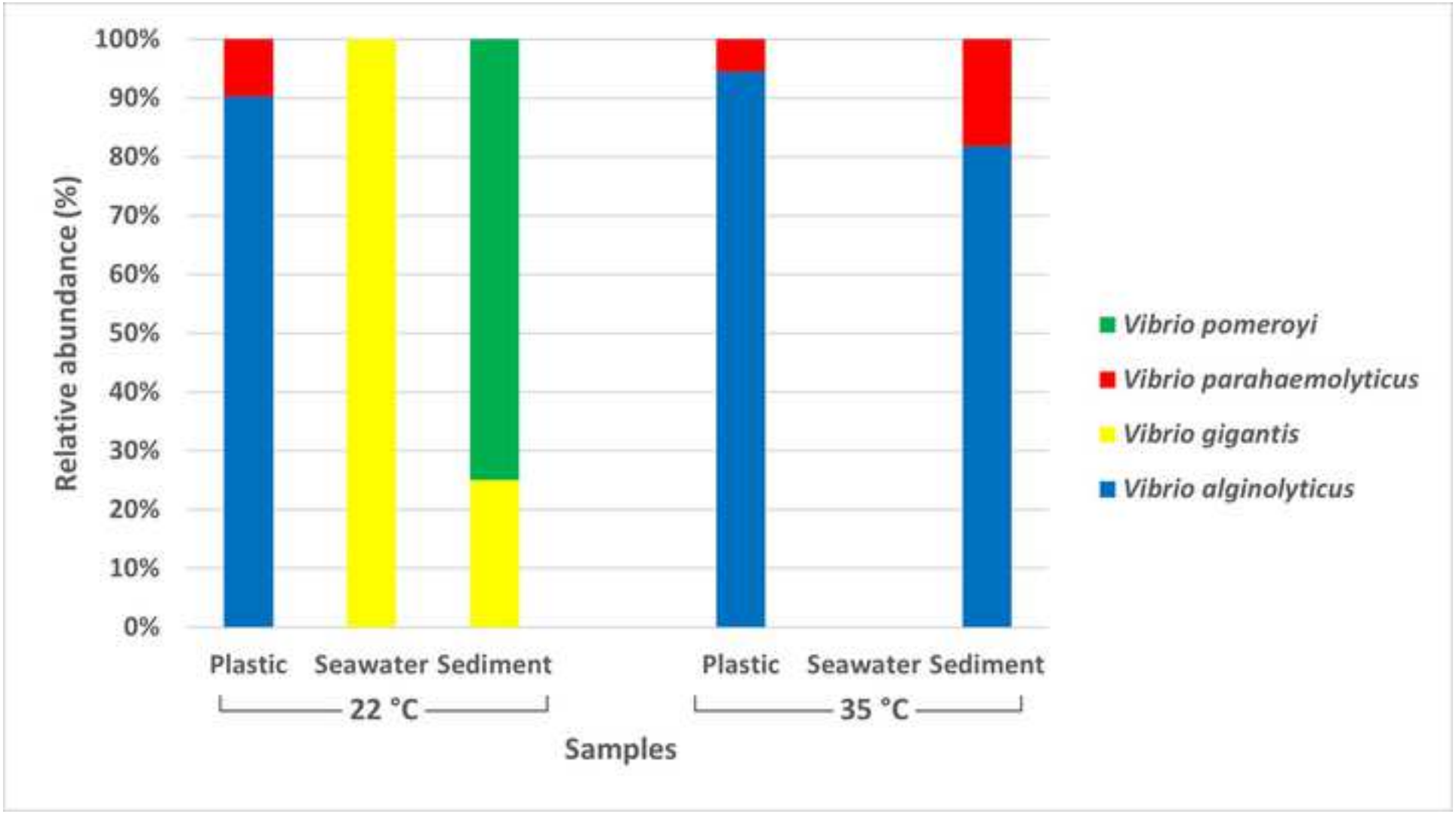

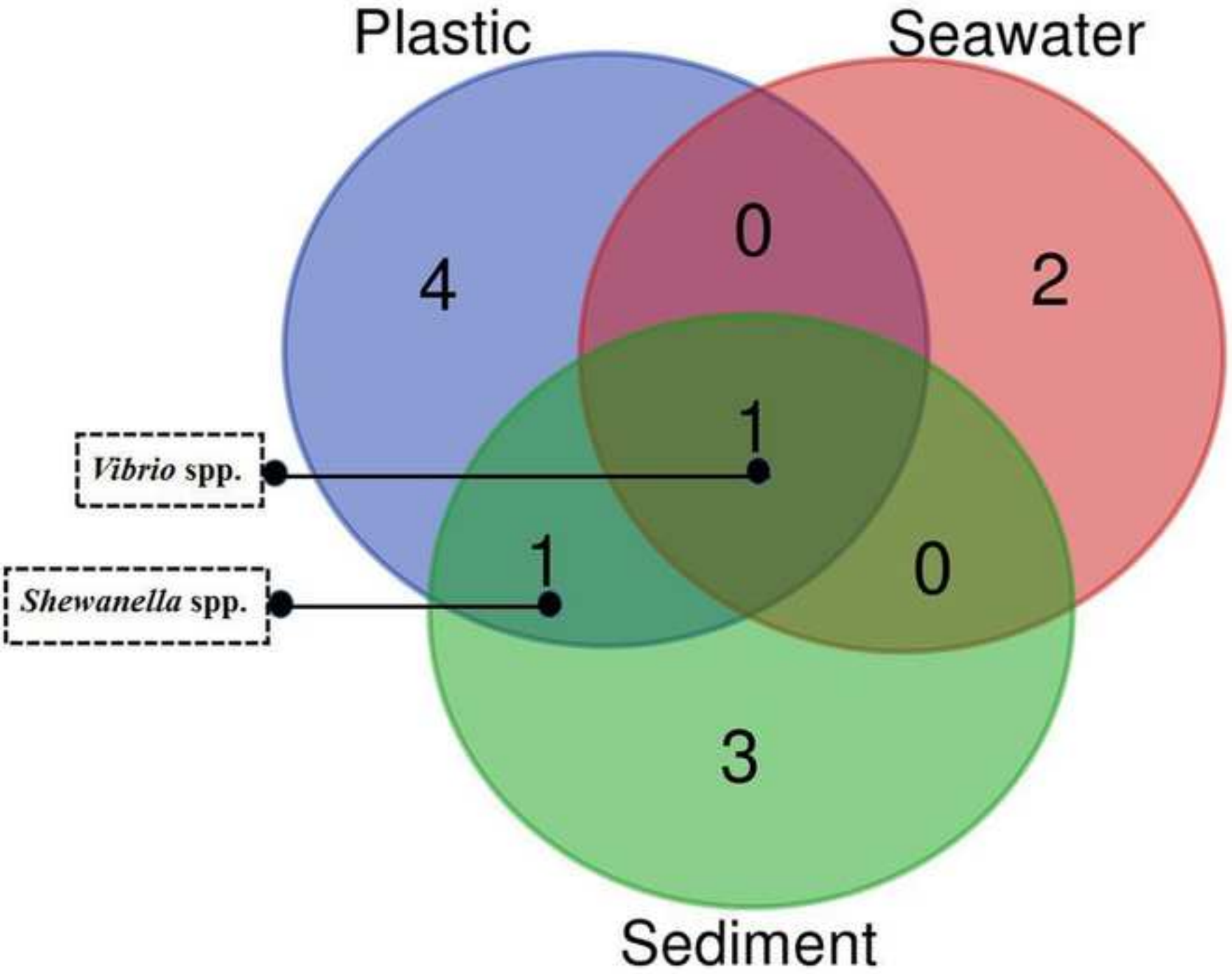

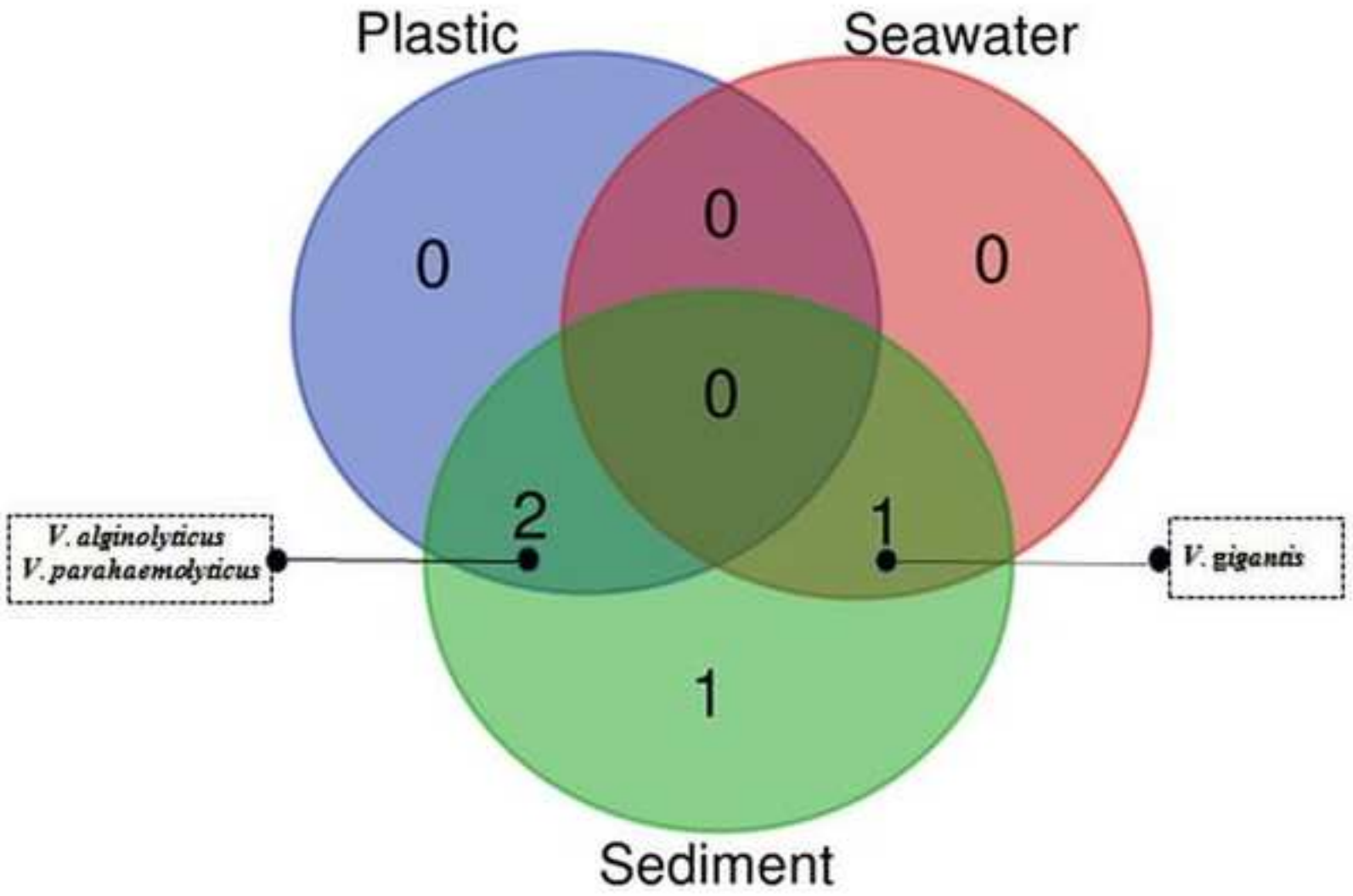

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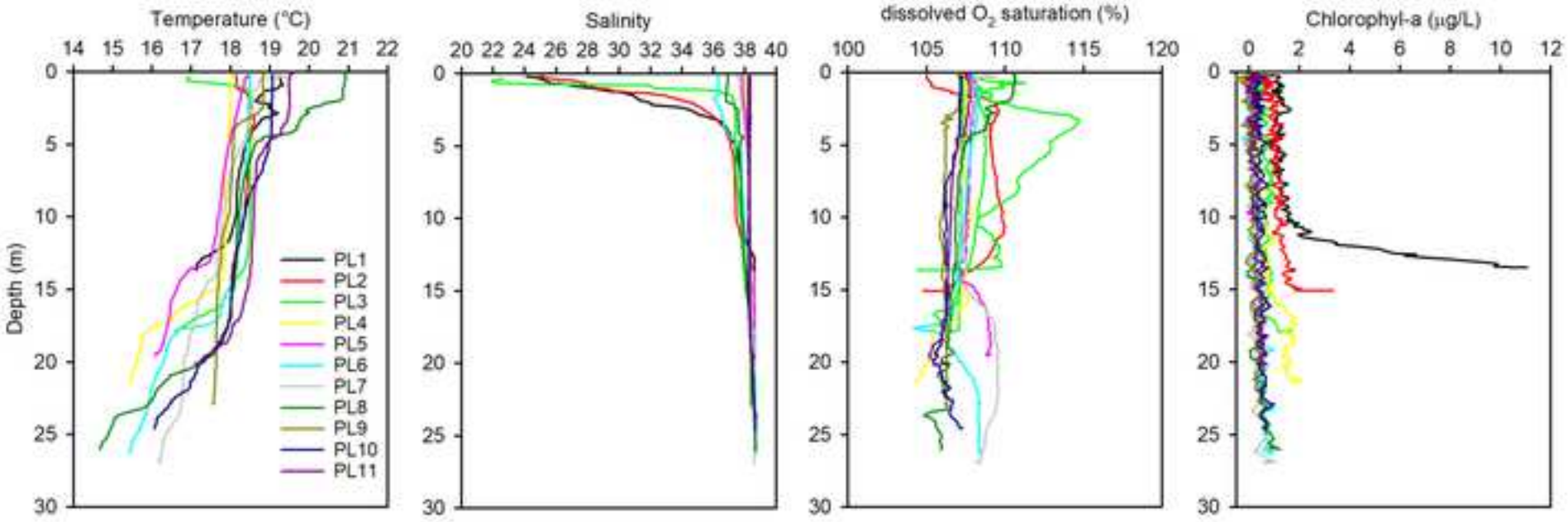

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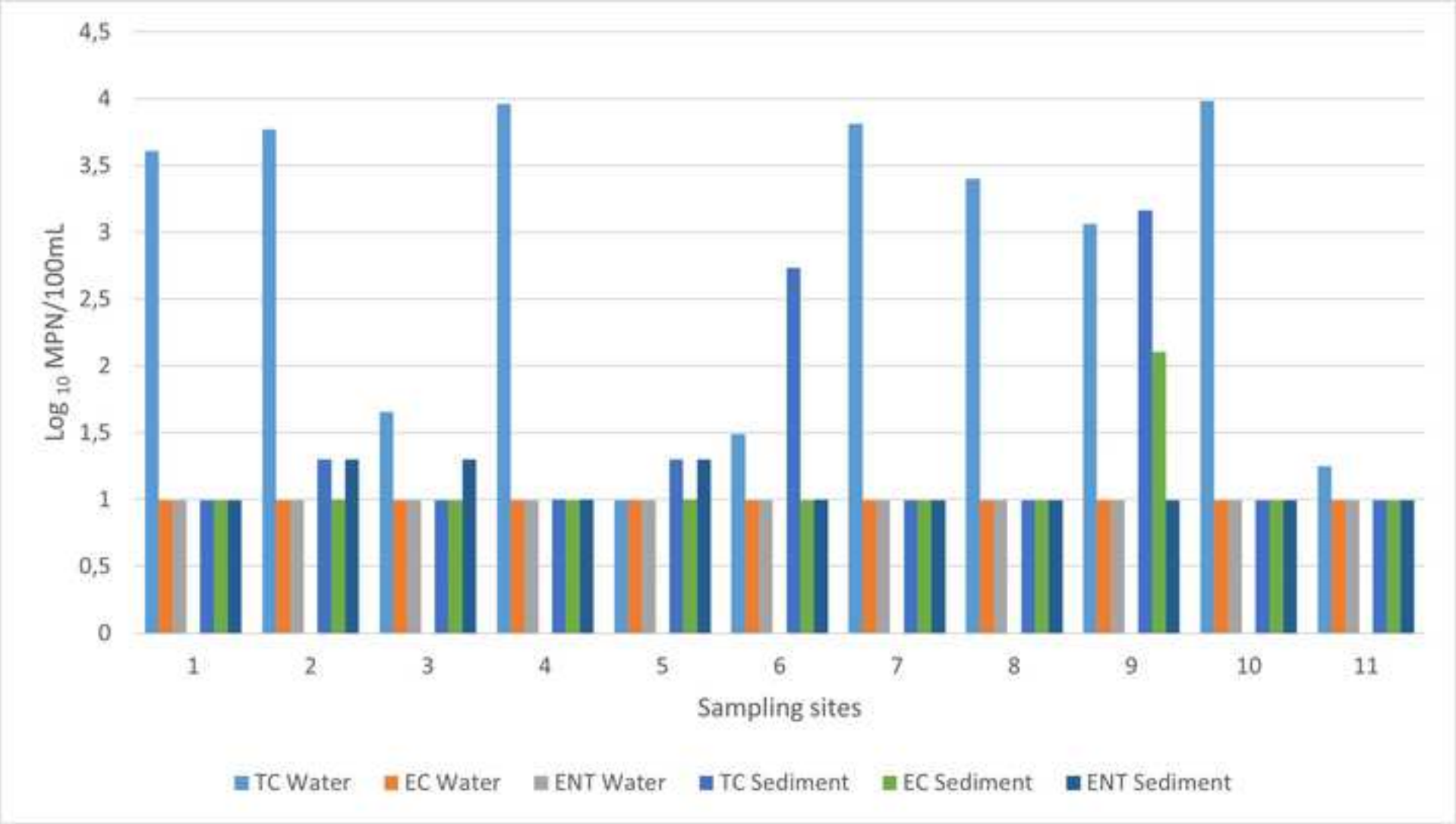

Fig. 1. Study area in south-eastern Adriatic Sea with marked 11 sampling sites.

Fig. 2. Relative abundance of bacterial genera isolated on Marine agar at incubation temperatures of 22 °C and 35 °C, and identified in each sample type: marine plastic litter, seawater and sediment.

Fig. 3. Relative abundance of *Vibrio* species isolated on TCBS agar at incubation temperatures of 22 °C and 35 °C, and identified in each sample type: marine plastic litter, seawater and sediment.

Fig. 4. Venn diagram of bacterial genera identified as cultivable microbiota from swabs of plastic litter, seawater, and sediment from the fishing zone of the southeastern Adriatic Sea.

Fig. 5. Venn diagram of *Vibrio* species identified as cultivable microbiota from swabs of plastic litters, seawater, and sediment from the fishing zone of the southeastern Adriatic Sea.

Fig. S1. Vertical profiles of temperature, salinity, dissolved oxygen and chlorophyll-a at 11 sites from the south-eastern Adriatic Sea.

Fig. S2. Composition of marine plastic litter collected by commercial fishing trawlers in the south-eastern Adriatic Sea.

Fig. S3. Microbiological analysis of seawater and sediment at 11 sites from the south-eastern Adriatic Sea. TC Water – total coliforms in seawater, EC Water – *E.coli* in seawater, ENT Water – enterococci in seawater, TC Sediment – total coliforms in sediment, EC Sediment – *E. coli* in sediment, ENT Sediment – enterococci in sediment.

Declaration of interests

☒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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Credit Author Statement

Damir Kapetanović: Conceptualization, Funding acquisition, Formal analysis, Writing - Original Draft

Irena Vardić Smrzlić : Formal analysis, Writing - Review & Editing

Snježana Kazazić: Methodology, Formal analysis

Dario Omanović: Conceptualization, Investigation, Formal analysis, Writing - Original Draft

Neven Cukrov: Conceptualization, Investigation, Formal analysis, Writing - Original Draft

Ana-Marija Cindrić: Investigation, Data Curation

Ana Rapljenović: Investigation, Formal analysis

Lorena Perić: Formal analysis, Data Curation

Karla Orlić: Formal analysis, Visualization

Tatjana Mijošek: Investigation, Formal analysis

Zuzana Redžović: Investigation, Formal analysis

Ana Gavrilović: Investigation, Writing - Review & Editing

Tena Radočaj: Investigation, Formal analysis

Vlatka Filipović Marijić: Conceptualization, Funding acquisition, Supervision, Writing - Review & Editing