Pharmaceutical pollution drives changes in the composition and the functionality of the aquatic microbial biotic and abiotic communities

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INTRODUCTION

Nowadays, pollution is a worrying problem that affects all ecosystems. The impact on aquatic ecosystems is one of the most worrisome, as they are a sink for pollutants from various sources. Among the main chemical pollutants threatening the stability of aquatic ecosystems, pharmaceuticals stand out due to their exponential increase in use for both human treatment and intensive livestock farming. As environments are exposed to multiple stressors, we have used multispecies assays, including the bivalve *Scrobicularia plana (S. plana)*, in artificial controlled microcosms to assess the specific impact of different pharmaceutical compounds on the microbiome (**Fig. 1**). The effects of two pharmaceutical compounds on both the abiotic and biotic matrices of the ecosystems were analyzed using metagenomic techniques. We used 16S rDNA sequencing to determine changes in the structure, diversity, and functionality of the bacterial populations from sediments and the digestive gland of *S. plana*.

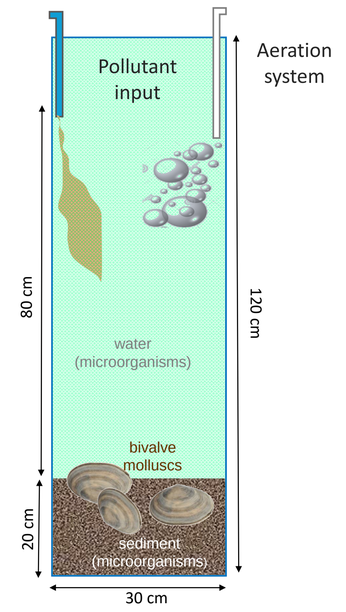

Fig 1. Experimental design of the microcosm used to study the effect of drugs on the microbiome of sediment and digestive gland samples.

OBJECTIVES

- To assess the changes in the microbiota of aquatic sediments and the digestive gland of *S. plana* after exposure to pharmaceutical compounds.
- To identify biomarkers of response to pharmaceutical pollution.

MATERIAL AND METHODS

Here we present the effects of the drugs carbamazepine (CBZ), an anticonvulsant used to treat epilepsy and bipolar disorder, and sulfamethoxazole (SMZ), a synthetic antibiotic of the sulfonamide family, when exposed individually and in combination to a concentration of 1 µg/L, which is an environmentally relevant concentration, for 10 days. An unexposed control was also included. Three biological replicates were set per condition. The ZymoBIOMICS™ DNA Miniprep Kit was used to isolate gDNAs. 16S rDNA was sequenced and bacteria taxa were identified by database search (Ion Torrent, SCAI-UCO).

RESULTS AND DISCUSSION

The phylum with the highest number of identifications in both types of samples was Proteobacteria. It is worth high-lightning the differences in the number of identifications of the Tenericutes phylum between sediment and digestive gland samples (**Fig. 2**). The Cyanobacteria phylum significantly increased in the mixture CBZ+SMZ when compared to SMZ in sediment samples. In the case of digestive gland samples, the Chloroflexi phylum significantly decreased in the SMZ exposure when compared to the control (**Table 1**). The families *Ectothiorhodospiraceae* and *Flavobacteriaceae* were highly represented in both types of samples (**Fig. 3**). Exposure to SMZ in the digestive gland samples resulted in a significant decrease in families related to nitrogen fixation, such as *Thioalkalispiraceae* and *Xanthomonadaceae,* when compared to the control. The *Clostridiaceae* and *Pseudonocardiaceae* families significantly increased in the mixture when compared to SMZ (**Table 2**). No significant changes were observed at the family level in the sediment samples.

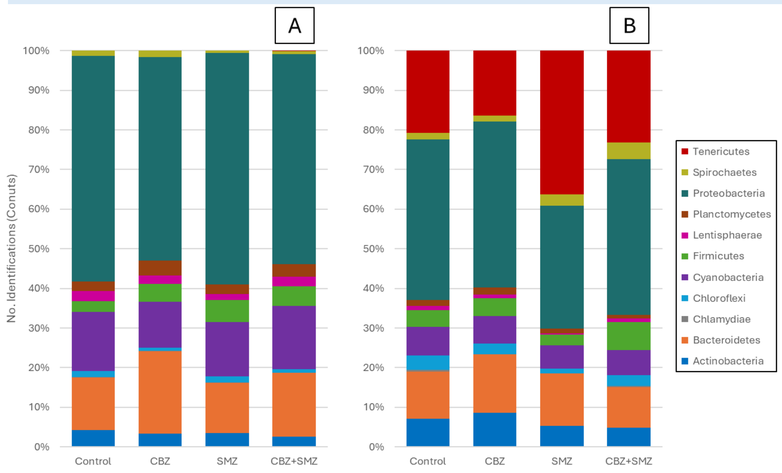

Fig 2. Taxonomic identification at the phylum level of microorganisms present in sediment (A) and digestive gland (B) samples exposed to drugs. The number of identifications is represented as a percentage of 100%.

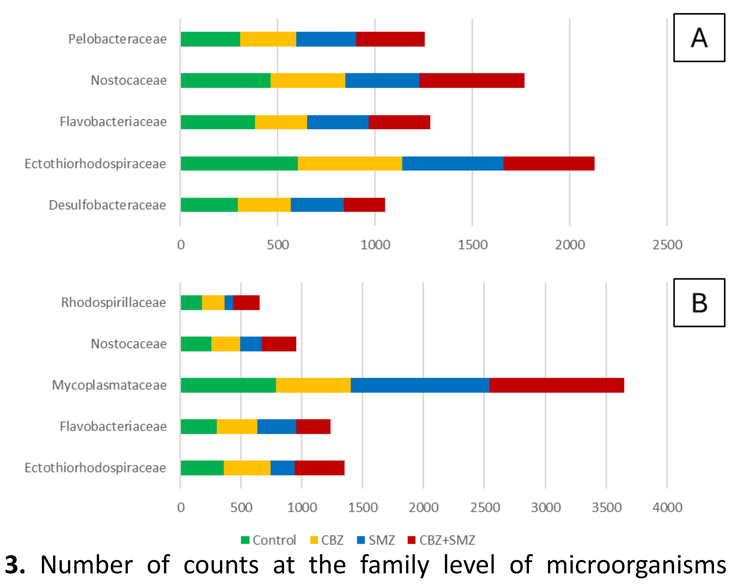

Fig 3. Number of counts at the family level of microorganisms present in sediment (A) and digestive gland (B) samples exposed to drugs. Only the 5 most represented families for each type of sample are represented.

Table 1. Statistically significant changes at the phylum level (**, $p < 0.01$; *, $p < 0.05$) in sediment and digestive gland samples.

Phyla	Sediment		Digestive gland	
	CBZ+SMZ *vs.* SMZ		SMZ *vs.* Control	
	↑	↓	↑	↓
Cyanobacteria	*			
Chloroflexi				*

Table 2. Statistically significant changes at the family level (**, $p < 0.01$; *, $p < 0.05$) in digestive gland samples.

Families	Digestive gland			
	SMZ *vs.* Control		CBZ+SMZ *vs.* SMZ	
	↑	↓	↑	↓
Clostridiaceae			*	
Pseudonocardiaceae			**	
Thioalkalispiraceae		**		
Xanthomonadaceae		*		
		*		

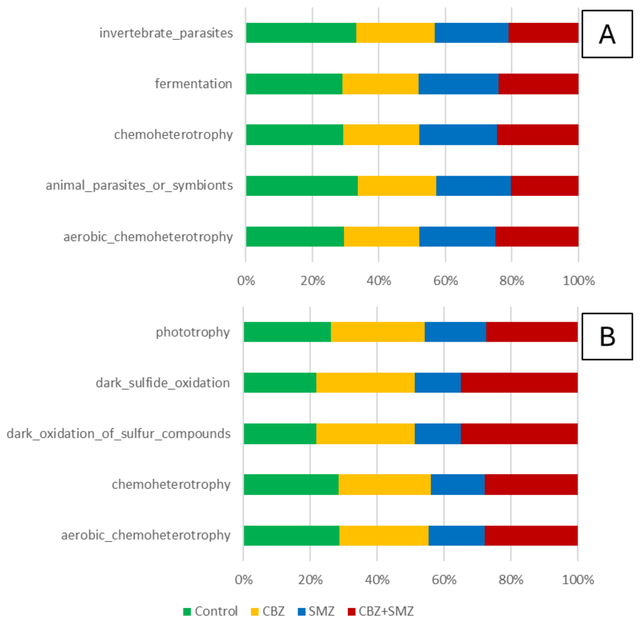

Fig 4. FAPROTAX analysis of the biological functions of the microbiome of sediment (A) and digestive gland (B) samples upon drugs exposure. The 5 most abundant functions are represented in each case. The number of identifications associated to biological functions is represented as a percentage of 100%.

The alpha diversity was calculated using the Shannon-Wiener index, but no significant changes were observed upon these pharmaceuticals exposure. The most represented functions in both types of samples were "chemoheterotrophy" and "aerobic_chemoheterotrophy". There were no significant changes in any of the exposures at the functional pattern (**Fig. 4**).

CONCLUSIONS

- The exposure to SMZ caused a significant decrease in several families, while the exposure to CBZ did not produce any significant change when compared to the control in digestive gland.
- The mixture CBZ+SMZ caused a significant increase in several families when compared to SMZ in digestive gland samples. Thus, CBZ could have an antogonistic effect on SMZ.
- Unlike the digestive gland samples, no significant changes at the family level were found in sediment samples.
- The presence of these pharmaceutical compounds did not affect either the diversity or the potential biological functions.
- Changes observed in the microbiome structure can be used as biomarkers of environmental exposure to these drugs.

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