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Innovative eco-toxicological monitoring strategies for the aquatic ecosystems protection and the implementation of the Water Framework Directive (WFD)



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OVERVIEW

The **Water Framework Directive** (WFD – 2000/60/EC) commits the EU member states to ensure good qualitative and quantitative status of all water bodies. Despite the efforts to reduce the release of chemicals into aquatic environments, new emerging substances still make pollution one of the main challenges across Europe. The overall goal of this project is to identify innovative methods to assess how the exposure to different pollutants affects the aquatic ecosystems and human health. The study will also aim to **provide recommendations to enhance the monitoring strategies** of the WFD built around the **Effect-Based Methods**.

This project is realised thanks to the European Social Funding 2014 – 2020, within the framework of *Torno Subito*, the Operational Programme sponsored by Lazio Region.



METHODS

Several bio-assays, both *in vitro* and *in vivo* (e.g. FET, behavioural analysis, Ames test, Micronucleus test, etc.) have been studied **to investigate the effects of the most common chemicals and pharmaceuticals observed in aquatic ecosystems**, and to adopt the suitable monitoring strategies for their protection. At the same time, a literature review of such substances on the development of zebrafish (*Danio rerio*) has been conducted to test the efficacy of this *in vivo* assay. **The aim is promoting the use of Fish Embryo Acute Toxicity Test (FET) with zebrafish** at early stages as it represents an excellent tool for the eco-toxicological studies.



Ames test in 384-wells plate

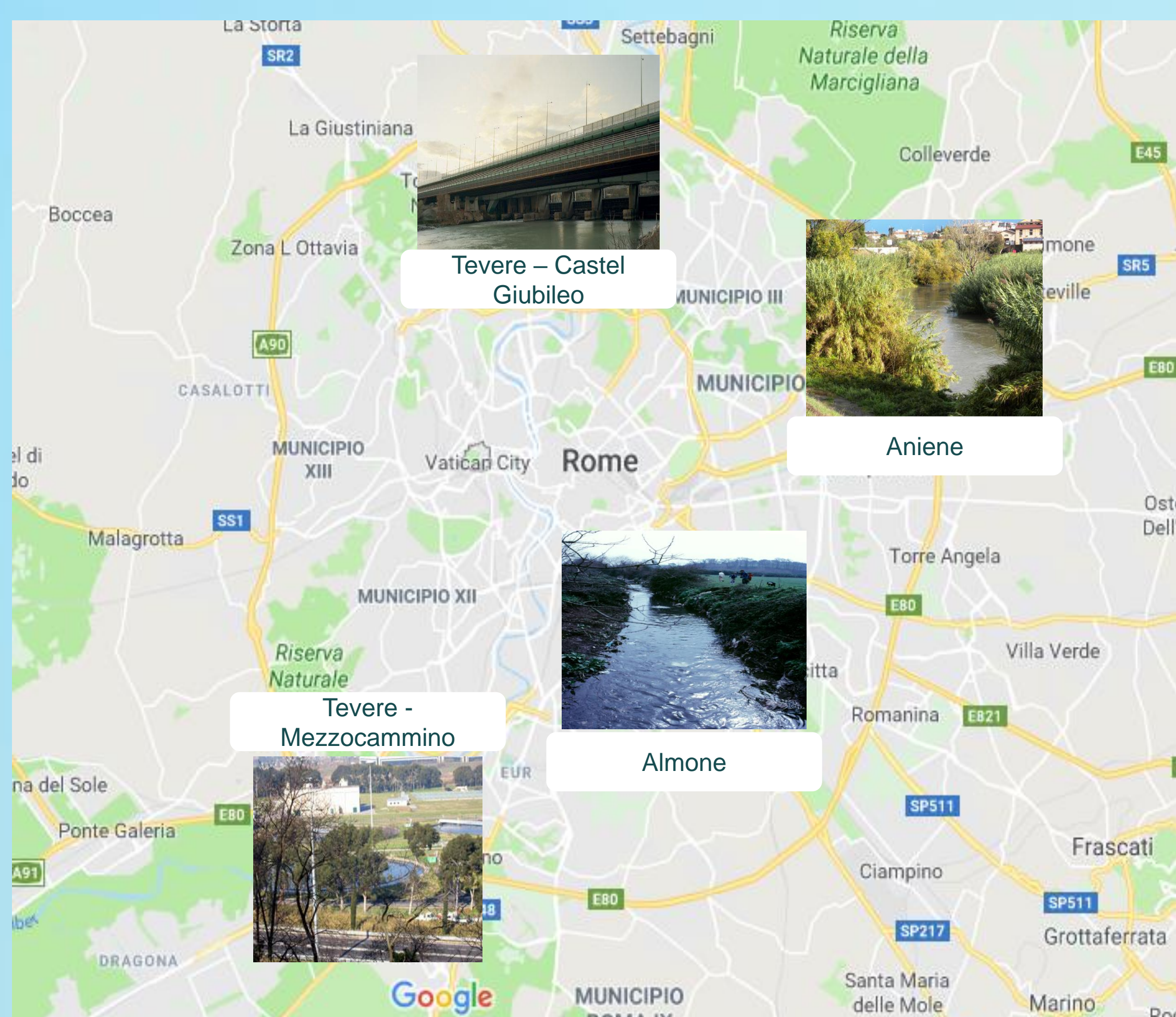


FET test – normal embryo at 48 hpf

SAMPLING

Water samples from four different sites in Rome have been collected in March 2018 to detect the effects of pollution in local river ecosystems. **Tevere river** has been sampled both in the north and in the south of the city, while samples from **Aniene** and **Almone rivers** have been collected only in one site.

Sampling sites in the metropolitan area of Rome



PRELIMINARY RESULTS

Zebrafish embryos have been exposed for 96 hours (96 hpf) to the water samples right after the fertilisation. At the end of the exposure period, all the endpoints identified by the OECD N. 236 (Fish Embryo Acute Toxicity Test) have been investigated, but any morphological effect has been observed. Afterwards, the locomotor activity of the 96 hpf treated larvae has been recorded to assess the potential neurotoxicity of the samples. The results have not shown any abnormal **swimming behaviour**.

The preliminary results suggest a possible presence of mutagenic compounds in **the urban area of the Aniene river**. Indeed, the **Ames fluctuation assay** detected a positive result on the **mutagenicity** of bacteria (two lineages of *Salmonella*). Further investigations are currently under development.

FUTURE INVESTIGATIONS

To assess the general effects of the pollutants in the mentioned river ecosystems, other **bioassays on different trophic levels (bacteria, algae, crustaceans, fish)** will be performed. Moreover, new samplings will be also performed in the next coming months.