Biological monitoring system continues in Scarlino

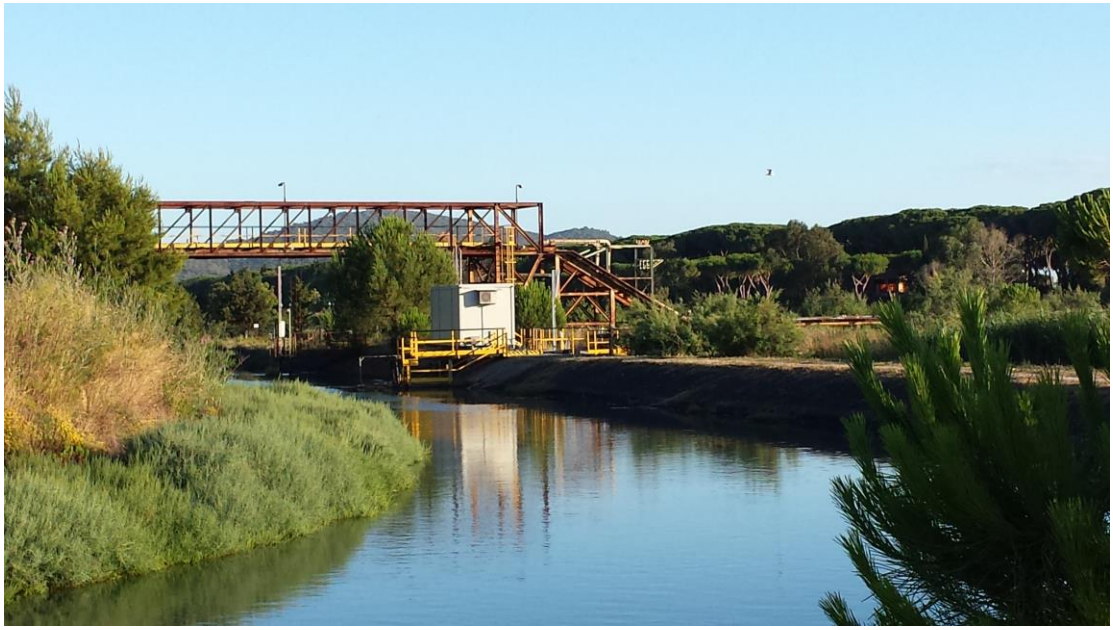

Figure 1 - The returning canal at the sea of Scarlino

The recorded death of fish in the river that flows out of the industrial zone of Scarlino has created the need of the installation of a biological alarm system. The decision was made to work with an automatic system, iTOXcontrol created by microLAN-The Netherlands represented in Italy by Ecotox Lds, which uses marine bacteria. The system compares the values of the light emission from these organisms at the entrance and exit of the canal, generating alarms in the presence of toxic substances.

An innovative monitoring system has been installed in 2014 at the end of June, inside the river that flows out of the industrial zone of Scarlino (Province of Grosseto) in the zone of Casone. This area is the home of the most important industrial zone of the province, which includes production facilities of the following companies: Nuova Solmine, Tioxide Europe and Scarlino Energia. The plant uses only seawater for cooling, and this is pumped from an artificial river above the ground named delivery canal. After the industrial use, the water flows back into the sea using above ground canals, which are known as the returning canals. Within the main stream, different drains from the numerous purification plants unite as well as the communal ones of Scarlino and Follonica.

The canal, provided by Nuova Solmine is from the '60 and is located at the border between the two municipality of Scarlino and Follonica (GR).

The study of the most adaptable monitoring system.

In 2001, ARPAT assimilated these waters as seawater due to the enormous quantity of water, which runs through these canals.
The return canal, named as a receiving water facility and not anymore as a private sewer, has been strictly controlled as required by the legislation, meanwhile each singular unloading within the main canal is authorised by the D.Lgs 152/99 legislation.

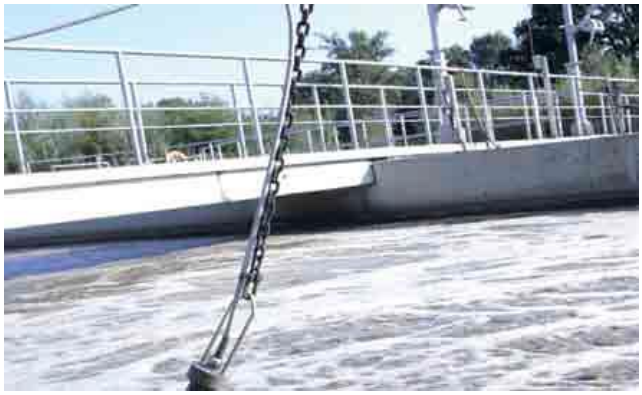

Figure 2- The monitoring of the water

In the past years episodes of dead fishes found in numerous quantities created a certain level of preoccupation within the population, which is very sensitive when talking about these events, also due to the large presence of tourists during the year.
Even though the numerous inspections made by ARPAT and another institute it has never been possible to determine the main cause of this water pollution, and furthermore highlights the ecotoxical effects from the samples taken in the location of the disaster. The investigation didn't take place contemporarily during the events and therefore the quantities of toxicological substances haven't been quantified, and therefore the main substance or group of substances related to the death of the fishes couldn't be identified.

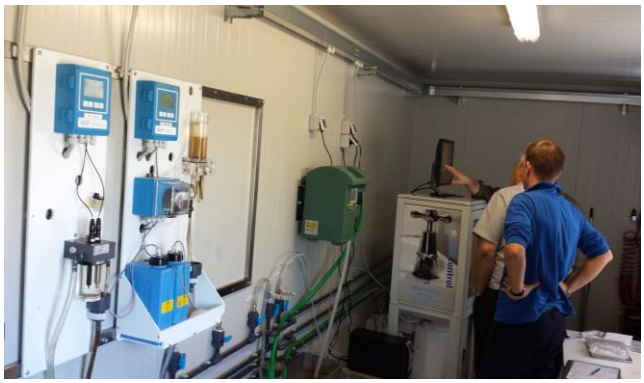

Figure 3-The container containing the Biological Early Waning System (BEWS) in Casone

Therefore, the competent authorities have decided to start searching for a system that could observe these phenomena, which, so far, have been unable to discover the causes for this. A technical committee was set up, that involved the town of Scarlino, the province of Grosseto, ARPAT and the 'Instituto Zooprofilattico', as well as representatives of the companies that unload in the canal.
The request for ARPAT to install, on the receiving body, a biological warning system was included in 2011 in the new Integrated Environmental Authorisation (AIA), issued to the companies by the competent bodies. The choice of the most suitable system had been left by the Minister of Environment and Land protection to the outcome of the debate

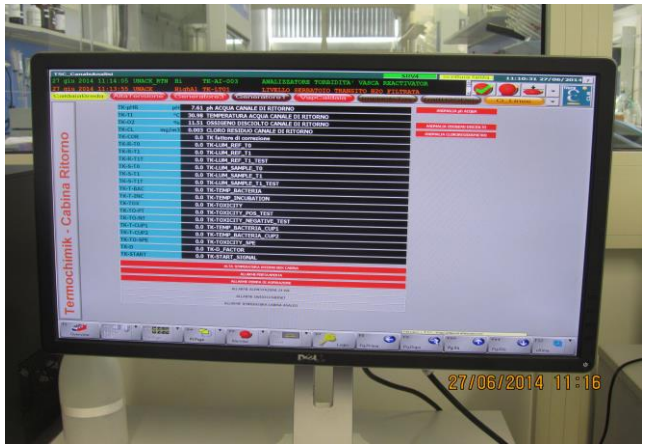

Figure 4-The monitor that monitors and integrates data remotely

between the companies and the local institutions (ARPAT, the town of Scarlino, the province of Grosseto), which are part of the technical committee.
In June 2013, the company 'Nuova Solmine' has selected Ecotox LDS From Cornaredo (MI), as part of a detailed survey on monitoring systems available on the market. Ecotox LDS has been a consulting company with regard to the methods of ecotoxicological investigation since 1992.
After the first meeting took place at the plant of Nuova Solmine with representatives of companies in the area, whose facilities unload into the returning channel to the sea and the inspection of the outflowing stream area Ecotox has provided an aggregate report by industry experts (Dr. Valeria Mezzanotte UNIMI; Dr. Rento Baudo CNR-ISE Pallanza). In addition to this, it has a working hypothesis for the detection of an automatic ecotoxicity alarm system (Biological Early Warning Systems), robust and standardized which could ensure a response in the shortest possible time.
The Biological Early Warning Systems (BEWS) assume the use of online biosensors, trough the use of whole organisms (or parts thereof such as cells and tissues) that can be monitored continuously for their biological activity that in addition can be modified due to the presence of stressors (interfering agents like physical elements, such as temperature of brightness, or chemical elements or compounds with potential toxics). Activities that can be monitored as possible answers to a stressful (toxic) event are for example: locomotion, behaviour, reactions of escape, or of physiological responses such as breathing, ventilation (fish), heart rate, the bioelectric potential, photosynthesis, growth, bioluminescence, the ion flow, the blood chemistry and haematology, or even death. Regardless of the activity monitored, the principle on which the BEWS are based, is the detection of abnormalities, with reference to the same activity measured in normal conditions (negative control). The biological monitoring systems have the essential characteristic of being able to report toxic effects in the environment, even if it is due to intermediates i.e. substances that are formed in the water sample for interactions or antagonism of molecules present, which are therefore not detectable by traditional chemical analysis.

The selected requirements for the system:
The fundamental requirements for the system to apply into the canal of Solmine are identified in 3 prevailing characteristics:

- The utilization of an organism suitable for the environmental condition of the site of observation (seawater)
- The speed of response
- The interface with an appropriate data processing system, capable of generating an alarm sign.

In the past, the BEWS were applied mostly to freshwater (anti-terrorism, raw surface water used for drinking, control of residential and industrial waste). The manufacturers of the instrument have been focussing on the applications that could guarantee the highest economic return until now, however, only recently they began to experiment with the use of automated systems in fresh- and brackish water, the matrixes

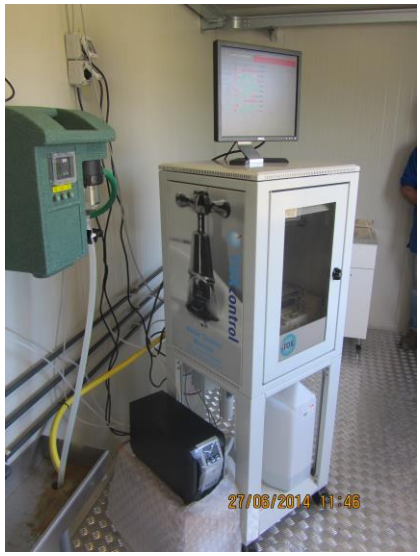

Figure 5-The iTOXcontrol system for monitoring toxicity

used on these samples only tested ecotoxicity laboratory test, and therefore not able to provide continuous data. An automatic system has been chosen (iTOXcontrol, microLAN-The Netherlands) which uses marine bacteria (*Vibrio fischeri)* which, in optimal living conditions, emits light.
If the quality of the water samples decreases due to the presence of eco-toxic products, the luminescence decreases in proportion to the concentration of the toxic substances. The light emissions are read by a photomultiplier and the data is processed according to the indication of the ISO 6341 – 3 Norm, providing an indication of the change in water quality of the sample.

The working of iTOXcontrol
The instrument uses a PC equipped with special software and an analyser that performs all commands of the software, measures it, and then sends the data back to the PC to evaluate them.
The reference for the analysis (negative control) in this application is made of the water from the entrance of the canal, where it gets pumped before being introduced into the plants.
The light emission values produced by the bacteria when getting in contact with water from the entrance of the canal are compared with those obtained from the sample (exit of the canal).
It provides an alarm signal in about 20 minutes in case of the presence of toxicity at the exit of the canal. The data analysis and possible alarm signal are sent to the control room in real time.
The bacteria (Vibrio fischeri) are stored in a lyophilized and frozen form, and activated directly at the incubation room of the iTOXcontrol about once a week.
This type of equipment is used a lot in Europe where the big rivers are used for both the shipping and handling of goods (the Netherlands, France and Germany), both for irrigation and drinking waters and large rivers and reservoirs in China.
The BEWS based on bioluminescent bacteria (iTOXcontrol), was taken in consideration for the transfer of applications in both freshwater and saltwater, since it provides the use of marine bacteria (*Vibrio fischeri)* and the project of feasibility written and performed by Ecotox LDS has received the approval of the companies in the consortium and by ARPAT.
The preliminary tests carried out, both in Cornaredo in the laboratory of quality control of Ecotox, as well as in the Netherlands at the test plants of the manufacturer, have provided encouraging and repeatable data of the applicability of the system.

Therefore it has been decided, in consultation with the technical committee, to construct and install the system in the canal returning into the sea and the industrial zone of Scarlino, whose commissioning took place at the end of June 2014, in accordance with the requirements of the integrated environmental authorization.
This monitoring system has been the first, with this application, to be used in Italy, on a body receptor of seawater and required a significant engineering effort of Nuova Soline that has taken on the management of the project. The suppliers of the equipment (Ecotox LDS) have assisted this with the used cabinet. In addition to the containment of instruments, to the distribution of the samples of the system (Opus Automazione,

Follonica, GR), and the integration of analytical data with the internal management system of Nuova Solmine, as well as sending alarm signal to the control room (Eurotherm, Guanzata, MI).
The investment by companies of industrial and civil purifiers of Scarlino and Follonica concerned both the supply of the entire system of analysis as well as the identification of an external laboratory of the company Sol.Tr.Eco, as it ensures the integrity of the daily maintenance. All laboratory staff that will deal specifically with the management of the iTOXcontrol, have attended courses in applied ecotoxicology, thus demonstrating the high sensitivity of the part of the companies to the new measurement systems and its maintenance in optimal conditions.
The innovation of the system has attracted international interest and it is forecasted that a delegation of the Korean government will visit at the end of September, as the government is interested in including the use of biological systems in their legislation.