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An Ecotoxicological Approach to Evaluate the Environmental Quality of Inland Waters

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1. Introduction

Water is traditionally considered a renewable resource as the quantity theoretically available depends essentially on meteoric water contributions. Modern theories consider river ecosystem as a central element of the environment but, actually, water ecosystems are more and more polluted because of agricultural and industrial activities. In Italy, where there is a widespread urbanization, most part of river ecosystems are exposed to a severe risk of damage, with consequent loss of biodiversity. The institution of national and regional parks makes possible the preservation of significant natural value areas. In 1974, the UNESCO established a "World Network of Biosphere Reserves" (WNBR) aimed to preserve areas, where there is a close relation between man and nature, through environmental preservation and sustainable development (UNESCO,2010). Nevertheless, a constant monitoring along the whole watercourse (from spring to mouth) is needed as, even in a protected area, human activities could cause, directly or indirectly, damages to valuable ecosystems.

1.1 The ecotoxicology

In recent years, ecotoxicology started a new approach to the environmental analysis as it covers:

- chemistry, i.e. the fate of chemicals in the environment
- environmental toxicology, dealing with the evaluation of toxic effects of a pollutant at different levels of biological integration
- ecology, which provides indications on regulation of both structure and function of ecosystems and, at the same time, on interactions between biotic and abiotic components (De Castro et al., 2007).

Ecotoxicology is based on the use of bio-indicators belonging to different levels of an ecosystem trophic chain. Mathematical models are also used to foresee the environmental

fate of chemicals and their effects on exposed organisms (man included) and ecosystems. An organism can be considered a "bio-indicator" when, in presence of pollutants, shows detectable variations from its natural state. Well defined responses to different concentrations of pollutants are also needed. Moreover, a good bio-indicator should be sensitive to pollutants and have a wide distribution in the investigated environment, low mobility, long life cycle and genetic uniformity. Actually, *Daphnia magna*, *Lepidium sativum*, *Cucumis sativus*, *Sorghum saccharatum*, *Pseudokirchneriella subcapitata*, *Vibrio fischeri* are widely used to evaluate water and soil quality as well as the toxicity of chemicals, wastes, pharmaceutical products which have to be processed in a wastewater plant or directly dumped in the environment. The main symptoms or endpoints of an ecotoxicological test could be:

- change in community structure;
- morphological changes;
- change in vitality;
- damage to genes.

Actually, a contemporary utilization of different bio-indicators in order to evaluate the ecotoxicity of a matrix (wastewater, contaminated soil, pharmaceutical by-products, etc.) let the researchers able to gain useful data about the possible toxic effects on an ecosystem (Guida et al., 2006). In the next lines, informations about biology of some test-organisms adopted in ecotoxicological analyses and about the main indices and parameters used in the evaluation of a river ecosystem quality are resumed.

1.1.1 *Daphnia magna*

Daphnia magna is a freshwater crustacean belonging to the class Brachiopoda, order Cladocera, phylum Arthropoda. It has a small size (not more than 5 mm in length) with an oval body compressed laterally. It is dorsally characterized by a welded bivalve structure (called "carapace"), which encloses the entire animal except for the head. Its body has a single compound eye, sessile, strongly pigmented; a small ocellus and two pairs of antennae. One pair of antennae is greedy and very developed, having essentially a swimming function. The transparency of carapace allows an observer to notice some internal organs: heart, located dorsally in the post-cephalic region, middle intestine (clearly visible when it contains food) and ovaries, that are located laterally. At a temperature of 20°C, the life cycle of *D. magna* is 60-100 days. Under favourable environmental conditions, the population is exclusively composed of female animals, with a parthenogenetic reproduction. This species is very sensitive to many pollutants able to cause variations to aquatic ecosystem, since it is considered the "perfect" bioindicator and test-organisms in ecotoxicological essays (Guida et al., 2004).

1.1.2 *Vibrio fischeri*

Vibrio fischeri is a rod-shaped Proteobacterium, gram negative, characterized by polar flagella. It is widespread in marine environments, living as symbiotic of various marine animals, such as the bobtail squid. *V. fischeri* is most often found as a symbiont of *Euprymna scolopes*, a small shallow water squid found on the shores of Hawaii.

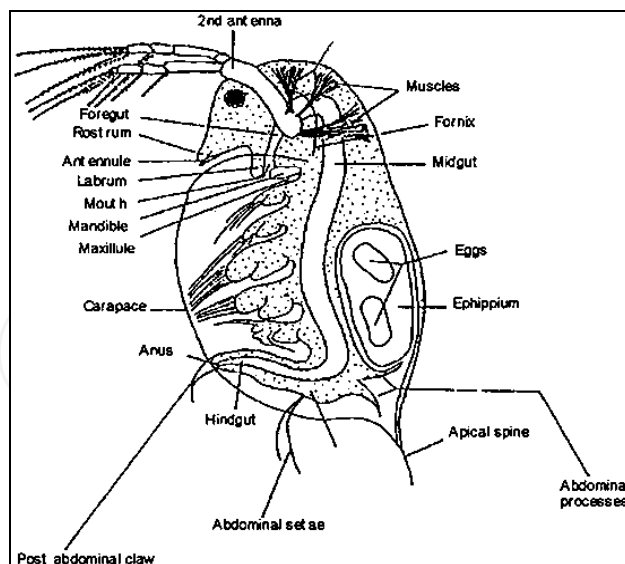


Fig. 1. A female individual of *Daphnia magna*: internal and external anatomy. (FAO, 1996)

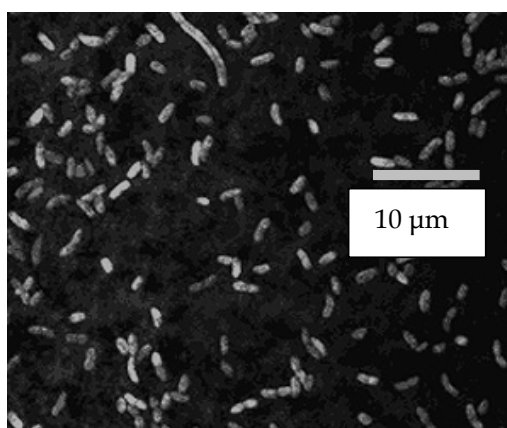


Fig. 2. The bioluminescence of *Vibrio fischeri* (1000X). Image taken by E. Nelson and L. Sycuro (<http://microbewiki.kenyon.edu>; last accessed: September 2011).

The bioluminescent bacterium *Vibrio fischeri* and juveniles of *Euprymna scolopes* specifically recognize and respond to each other during the formation of a persistent colonization within the host's nascent light-emitting organ. The bioluminescence depends on metabolic activity of bacteria so, damaged microorganisms less in bioluminescence. This feature has been used to study toxicity in marine samples.

1.1.3 *Pseudokirchneriella subcapitata*

Pseudokirchneriella subcapitata (previously called *Selenastrum capricornutum*) is a single-celled freshwater algae, belonging to the *Chlorococcales* family. It is a representative species of oligotrophic and eutrophic aquatic system. The cells, sickle-shaped, have a volume of 40-60 μm^3 , size of 6-7 μm , and its life cycle is very short. This alga shows a good level of sensitivity to toxicants and it is commonly used to perform multispecies tests (Chen C.Y. et al., 2007).

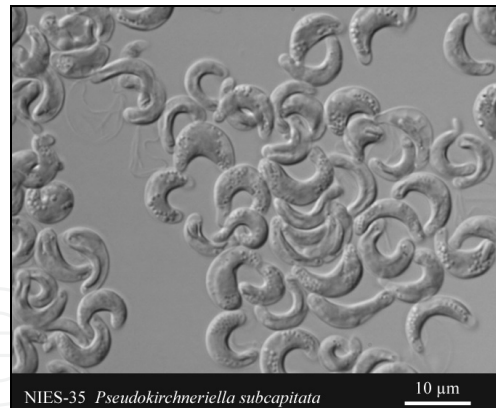


Fig. 3. Image at light microscope of *P.subcapitata* (1000x). (<http://www.shigen.nig.ac.jp>; last accessed: September 2011)

1.1.4 Phytotoxicity essays

The toxicity of a pollutant on a soil can be assessed by specific plants which, in natural ecosystems, can be considered good bioindicators. Ecotoxicological bioassays on plants consists generally in simple methods. It is possible to assess different endpoints for each essay such as seeds survival, germination rate, growth speed in the light and in the dark. Different plants could be used in ecotoxicological tests depending on the investigated matrix. Nevertheless, plants very commonly used in such essays in Italy and in some other Countries are *Lepidium sativum* and *Cucumis sativus* (Youn-Joo An; 2004).

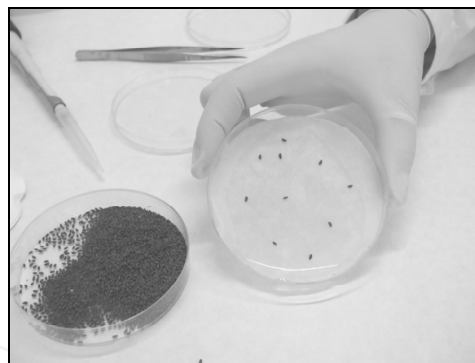


Fig. 4. Phytotoxicity test on *L. sativum*.

1.2 The evaluation of watercourse ecological quality: Extended Biotic Index (EBI) and Fluvial Functional Index (FFI)

If ecotoxicology allows scientists to gain informations about the potential toxicity of a single pollutant or a mixture of chemicals (like industrial wastewater) through the implementations of laboratorial tests, a complete study of a water ecosystems requires the collection of data directly on site. So, analyses of microfauna (small invertebrates colonizing water ecosystems) and ichthyofauna, the measure of parameters like water Dissolved Oxygen (DO), Turbidity, pH, Conductivity, Temperature, besides of quality of riverside flora and evaluation of watercourse erosion are needed to evaluate the environmental quality of a water ecosystem like a river. The collection and characterization of microfauna is at the basis

of the Extended Biotic index (EBI), whose value is used to classify the quality of freshwater courses in Italy (Ghetti P., 1997). Further biotic indices based on the quali-quantitative analysis of invertebrates community are also used with the same aim (Kalyoncu H. and Zeybek M., 2011). Once calculated the EBI value (ranging from 0 to 12), a class of environmental quality is attributed to each stream or river or part of it. There are 5 classes available, corresponding to a well defined colour (Table 1). Colours are used to report the classification of environmental quality on a map of the watercourse. Like EBI, Fluvial Functioning Index (FFI) values correspond to well defined quality classes (Negri P.et al., 2011). Recently, National Environmental Protection Agency of Italy suggested FFI for a correct evaluation of the health of river ecosystems (APAT, 2007). It must be underlined that FFI categories are as recommended by Water Framework Directive 2000/60 (Negri P. et al., 2011).

Classes	E.B.I. (Values)	Outcome	Colour
Class I	10-11-12...	Watercourse not significantly polluted or not polluted at all	Blue
Class II	8-9	Watercourse moderately polluted or altered	Green
Class III	6-7	Watercourse polluted or altered	Yellow
Class IV	4-5	Watercourse highly polluted or altered	Brown
Class V	1-2-3	Watercourse extraordinarily polluted of altered	Red

Table 1. Classes of environmental quality corresponding to EBI values.

The above mentioned indices are based on a simply concept: a watercourse is a dynamic system, formed by different habitats which continuously follow each other from the source down to the mouth and which interconnect with the surrounding terrestrial ecosystems. It is important to notice how, along the course of a river, the environmental conditions (like morphological, hydrodynamic, physical and chemical parameters) change and, with respect to these, the biological populations vary too. That’s why the state of a river ecosystem must be evaluated along the whole course in pre-determinate stations. The study of the whole territory crossed by the river and the identification of possible critical situation is of a great importance as it could affect heavily the results of a monitoring activity. The hydro-geologic conformation of the river is also important as it could affect the accessibility to sampling stations. So, an adequate preliminary study, a good experience in the choice of sampling sites, besides of a suitable equipment, are necessary.

1.3 Other indices

According to EU Regulation 2000/60/CE published on 10/23/2000 (the Water Framework Directive), the quality of a watercourse has to be evaluated through both Ecological and Chemical States indices. From the elaboration of these indices, the Ecological State of Watercourse (ESW Index) could be calculated. Another index widely used is the Evaluation of Macro-descriptors Pollution Level (EMPL) which is calculated from the following parameters: Chemical Oxygen Demand (COD), Biochemical Oxygen Demand at 5 days (BOD₅), NO₃⁻, NH₄⁺, Total Phosphorous, *Escherichia coli*, Oxygen saturation rate.

1.4 Evaluation of freshwaters microbial quality

Water quality evaluation can't exempt from a microbiological characterization of water itself. The presence of some bacterial species like *Escherichia coli* gives important informations about the grade of a watercourse pollution (Edberg S.C. et al., 2000). Different microbial parameters were considered in the present survey as more parameters led more informations about the organic pollution of a river ecosystem. *E. coli* and fecal coliforms are indicators of recent fecal pollution. Streptococci and Clostridia, instead, are abundant in case of a past fecal pollution. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Aeromonas hydrophila* are potential pathogens of both fishes and men (Pathak S.P. et al., 2008; Health Canada, 2011; Fazli M., 2009).

1.5 Phytoplankton

Phytoplankton includes different algal species belonging to different taxa (*Bacillariophyceae*, *Dinophyceae*, *Chrysophyceae*, *Cryptophyceae*, *Dictyochophyceae*, *Prymnesiophyceae*, *Raphidophyceae* and *Euglenophyceae*, *Prasinophyceae* and *Chlorophyceae*). The analysis of the phytoplanktonic component of a water ecosystem, gives important informations about the amount of nutrients (mainly nitrates and phosphates), the presence of toxics and some chemical-physical factors as water temperature and turbidity. Moreover, phytoplankton populations and corporations oscillate widely even in short time or in small space according to the environmental conditions (as nutrients, dissolved oxygen, turbidity, temperature, etc.). That's why phytoplankton is considered, since long time, an important indicator of the trophic level of an aquatic ecosystem (freshwater and marine waters) (Greene J.C et al., 1975; Mahoney J.B., 1983).

2. Aim of the study

Ecotoxicological tests are usually carried out to assess the potential toxicity of wastes or chemicals before their introduction in the environment. In the course of two years, we tried to apply an ecotoxicological approach to evaluate the environmental quality of Tanagro and Bussento rivers flowing through a WNBR area (UNESCO, 2010), in Southern Italy. We used ecotoxicological tests findings to identify possible critic situations (due to a possible chemical pollution) along both river courses. But, since a river is a complex system, a polyphasic approach in a correct environmental quality evaluation was needed. In fact, the real challenge researchers have to face consists in consider all possible elements of river ecosystem (water quality, fauna and flora composition, erosion phenomena, etc.) in order to have a realistic picture of the ecological state of a watercourse. So, besides of ecotoxicological essays, we collected data about chemical, microbiological and physical characteristics of water while ecological quality of watercourses was evaluated by the use of EBI. Moreover, further important data were gained through the interpolation of different parameters, calculating, this way, ecological indices like Evaluation of Macro-descriptors Pollution Level (EMPL) and Ecological State of Watercourses (ESW). At last, we tried to obtain a complete picture of the river environmental quality. The dependability of ecotoxicological tests for river quater quality evaluation was assessed.

3. Material and methods

Standardized procedures were applied in sampling procedures and water analyses. A complete list of all parameters measured and indices calculated is reported in Table 2. The choice of sampling sites was carried out according to EU Regulation 2000/60/CE recommendations, taking in account the introduction of waste waters through spillways . On the whole, 14 sampling points (stations) were chosen for each river. Collected data were stored in a database and georeferentiated by an ArcGis software (data not shown). Frequency of sampling and analytic procedures are reported in Table 3.

<i>Chemical parameters</i>	<i>Microbiological parameters</i>	<i>Ecological and Ecotoxicological parameters</i>
Temperature (°C)	Aerobic Colony Count at 22°C	Daphnia magna acute toxicity essay
Dissolved Oxygen (DO)	Aerobic Colony Count at 37°C	Pseudokirchneriella subcapitata acute toxicity test
Ph	Total Coliforms	Phytotoxicity test
Specific Conductivity (SC)	Fecal Coliforms	Inihibition test on algae
NH ₄ ⁺	Escherichia coli	Fitoplankton
NO ₂ ⁻	Streptococci	Extended Biotic Index (EBI)
NO ₃ ⁻	Clostridia	Fluvial Functional Index (FFI)
PO ₄ ⁻	Staphylococci	
BOD ₅	Stafilococcus aureus	
COD	Pseudomonas aeruginosa	
	Aeromonas hydrophila	

Table 2. Complete list of analyses carried out on each water sample analyzed.

<i>Biological Parameters</i>	<i>Sampling frequency</i>	<i>Chemical-physical parameters</i>	<i>Sampling frequency</i>
Ecotoxicological essays	5 times/year	Water temperature	5 times/year
Microbiological analyses	5 times/year	Dissolved Oxygen	5 times/year
Fitoplancton	3 times/year	Salinity	5 times/year
EBI	2 times/year	pH	5 times/year
FFI	1 time/year	Nutrients (NO ₃ ⁻ ,NO ₂ ⁻)	5Times/year

Table 3. Frequency of sampling for each parameter in each station.

3.1 Chemical analyses

The chemical analyses were carried out according to Standard Methods (2006).

3.2 Microbiological analyses

All microbiological analyses were carried out according to ISO methods.

3.3 Phytoplankton

The EN 15204:2006 and the EN ISO 5667-1 and EN ISO 5667-3 were applied during this survey.

3.4 *Daphnia magna* – Acute bioassay

Daphnia magna acute toxicity test were carried out according to ISO 6341:1999. According to this method, ten newborns no older than 24 hours must be exposed to a sample. The newborns of *D. magna* are transferred in each container filled with 50 ml of sample: this operation must be carried out paying attention to don't damage the daphnides. Moreover, they must not be fed during all test procedure. After 24 hours, the number of immobile crustaceans (or of the ones showing at least a change in their usual way of swimming) is calculated. If the essay is carried out considering different sample concentrations, it is possible to calculate the EC₅₀ , which gives, for a well defined toxic, the concentration value inhibiting the 50% of test organisms.

3.5 *Vibrio fischeri* – Acute bioassay

APAT IRSA-CNR 2003 n. 8030 is the method chosen to carry out acute toxic bioassays with *V. fischeri*. The method gives an evaluation of acute toxicity of freshwater and marine samples through the evaluation of *V. fischeri*, strain NRRL-B-11177 bioluminescence inhibition. Luminescence can be measured after 5, 15 and 30 minutes of exposition to a sample by the use of a luminometer. Sample toxicity is measured as EC₅₀, which represents the sample concentration in correspondence of which there is a decrease of 50% of the light emitted by bacteria. *V. fischeri* toxicity test demonstrated a good correlation with tests carried out on other aquatic organisms like *D. magna*, *Artemia salina*, *Chlorella* sp., *Tetrahymena pyriformis* (Kaiser K.L., 1998). Its reliability for the evaluation of soil and colourful samples had been also demonstrated (Lappalainen J. et al., 2001).

3.6 Chronic bioassay

ISO 8692:2004 is the method applied for *Pseudokirchneriella subcapitata* bioassay. The bioassay analyzes the toxic effect of a sample by measuring the inhibition of algal growth. Selected cultures are exposed during their exponential phase of growth, at well defined concentrations of a sample for 72 hours. After the exposition to the sample, algae density is measured reading absorbance of the culture at 663 nm.

3.7 Phytotoxicity

Phytotoxicity test assesses the potential toxicity of a sample measuring the inhibition of germination and /or root elongation of seeds under controlled conditions. Negative controls

are prepared. Seeds of two dicotyledons (*L. sativum* and *C. sativus*) and a monocotyledon (*S. saccharatum*) are exposed to a water sample and incubated in the dark at $25 \pm 2^\circ\text{C}$ for 72 hours. Then, germinated seeds are counted and root length measured using a calibre. The effect on both germination and radical elongation is expressed as percentage germination index (GI%) (UNICHIM 1651:2003). Such tests are widely used to assess the ecotoxicological effects of soils and waters contaminated with organic molecules and/or heavy metals (An Y. et al., 2004).

4. Results

4.1 Ecotoxicological results

Ecotoxicological tests didn't show any important inhibitory effect resulting in a good quality of both river waters. So, *D. magna* didn't suffer any significant toxic effect: the percentage of immobility didn't ever overcome, on the average, the 20% of individuals. Sampling sites number 7 and 13 of Tanagro river showed a modest effect on *P.subcapitata* (an increase in cell reproduction rate of about 10% on average). As to *L. sativum*, *C. sativus* and *S. saccharatum* no toxic effects were detected in both river samples. In some cases, especially for *L. sativum* and *S. saccharatum* some kind of bio-stimulation was observed (Fig.5 and 6). The results of phytotoxicity tests didn't show any important inhibition of seeds germination or

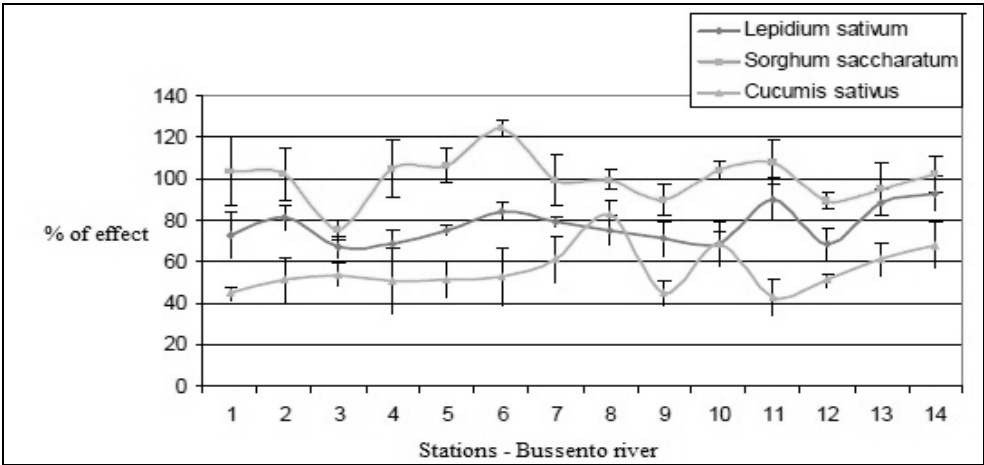


Fig. 5. Bussento river phytotoxicity tests on *L. sativum*, *S. saccharatum*, *C. Sativus*.

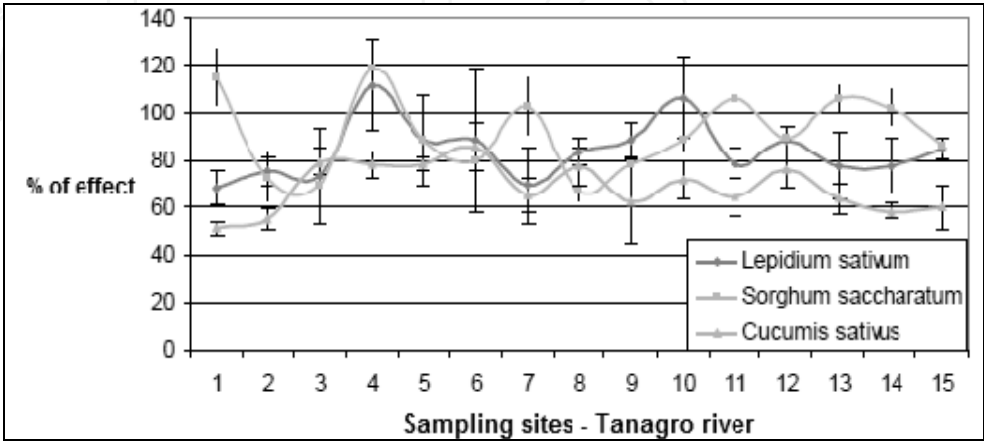


Fig. 6. Tanagro river phytotoxicity tests findings. *C.sativus* showed some inhibition effects at station 1 and 14 while *S.saccharatum* was stimulated mainly in stations 1 and 4.

root elongation on the most part of the samples, even if *C. sativus* showed a less growth rate than the other two plants. This could be explained on the base of different physiology of the species used in the test. All samples were tested also on *V. fisheri* and they never showed any inhibitory effect on bacteria. It is important to notice that, in no case and for no test organism, it was possible to calculate EC50 value because of the really low samples toxicity.

4.2 Chemical parameters and EMPL

In all stations, COD values were, on the average, always less than 20 mgO₂/mL, while BOD₅ never overcome 10 mgO₂/mL in Bussento river (Fig.7). As to Tanagro river, an increase of COD and BOD₅ was detected at station number 3, whose values overcame 20 mgO₂/L for COD and 10 mgO₂/L for BOD₅ (Fig.8). Moreover, chemical analyses showed some significant variations of nitrogenous compounds (as NO₃⁻), not only among the stations but

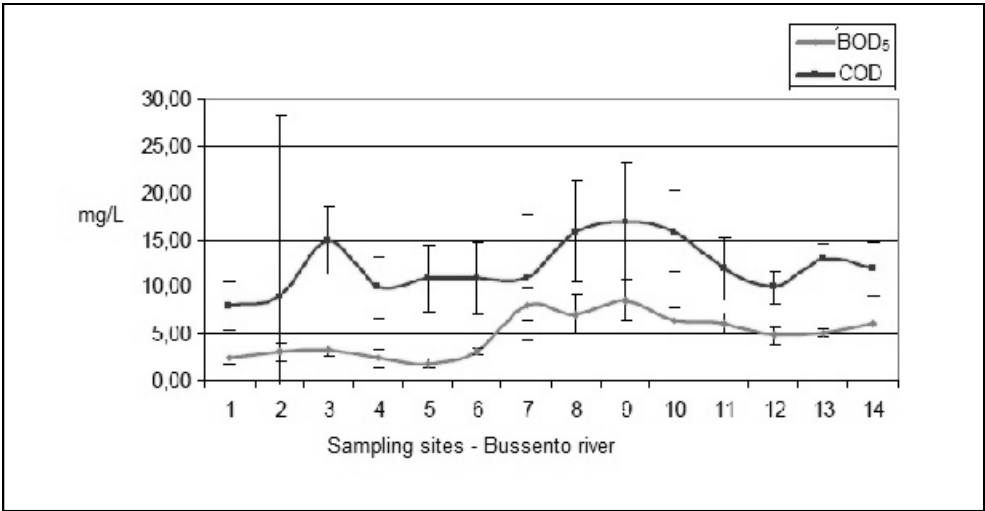


Fig. 7. BOD₅ and COD values in Bussento river. Average values and standard error are reported.

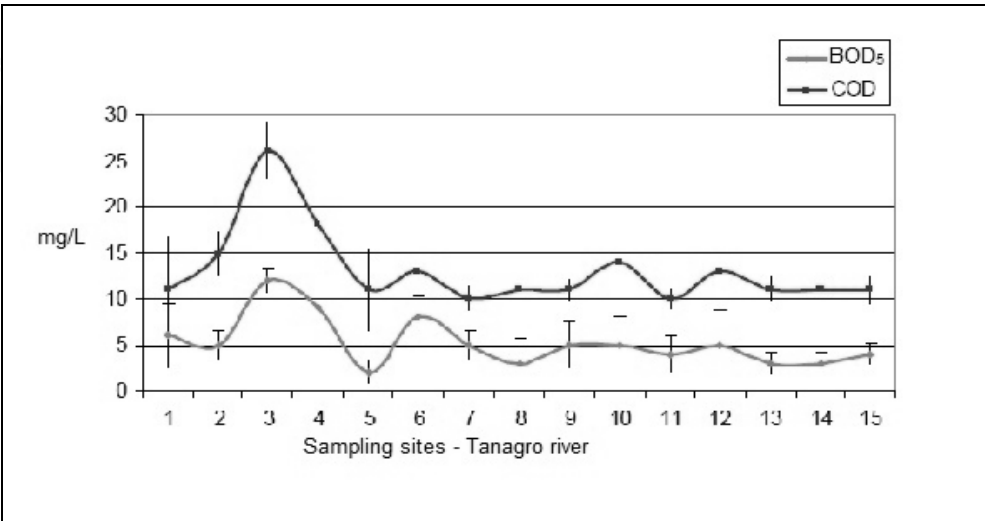


Fig. 8. Tanagro river: COD and BOD₅ average values and standard errors.

even for a single sampling site in the course of the time, causing an increase in variability (Fig. 9 and 10). So, the greatest variations were detected in correspondence of the stations number 6 and 7 of Bussento river and 3 and 4 for Tanagro ones. Nevertheless, as *E. coli* concentration never overcame the 5000 CFU/100mL limit, the increase of nitrates could be due to agricultural rather than to wastewater intakes. Both rivers, in fact, flow through a not highly urbanized land characterized by an agriculture-based economy.

If NO_2^- values were low in all samples, NH_4^+ concentration showed some different values between the two watercourses, as shown in figures 9 and 10. On the average, NH_4^+ was higher in Tanagro rather than in Bussento water. In any case, NH_4^+ concentration decreased from station number 10 till the river mouth. A similar tendency was detected for PO_4^{2-} , suggesting an agricultural origin of both nutrients.

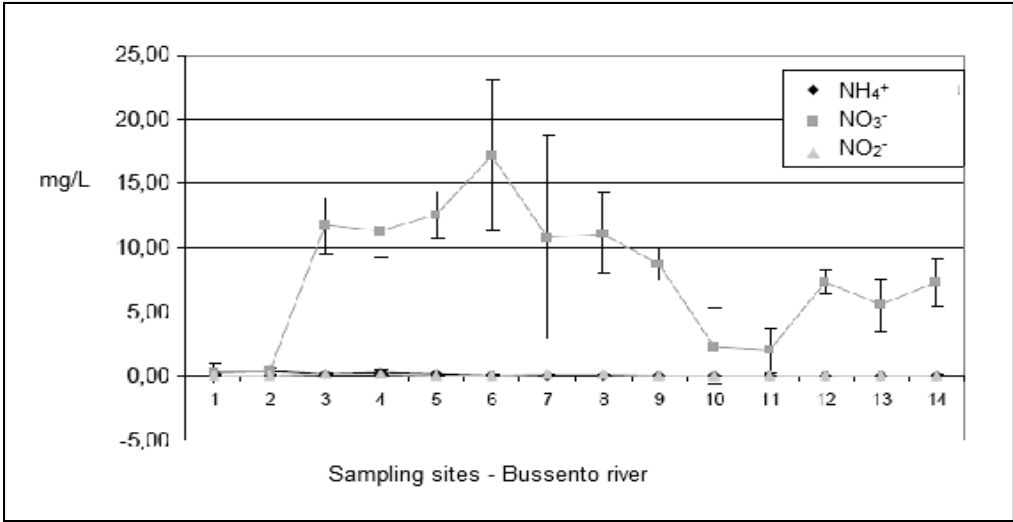


Fig. 9. Nitrogenous compounds concentrations in Bussento water samples. In the graphic, average values and standard errors are reported.

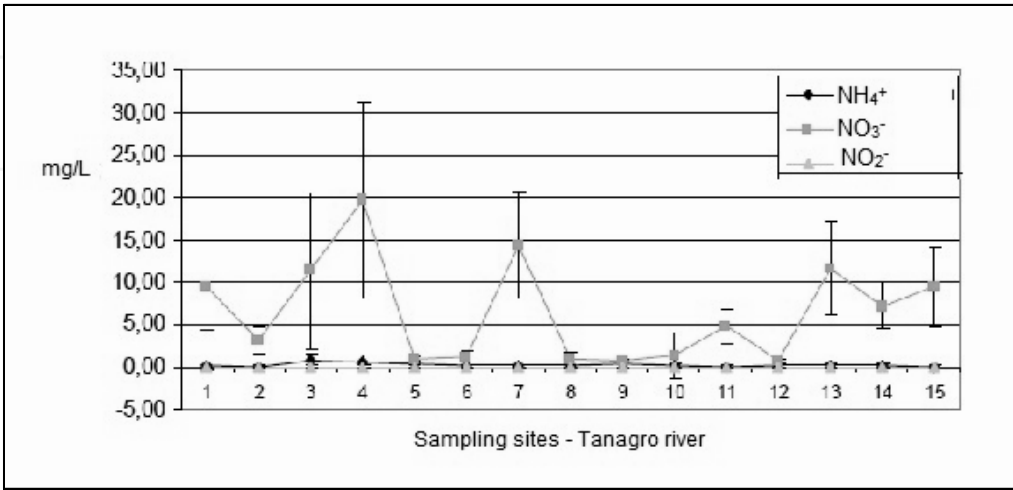


Fig. 10. Nitrogenous compounds concentrations (average ± standard error) along Tanagro river course.

No water acidification was detected in both rivers as pH values showed little variation and, in any case, they ranged between 7.5-8.5 values. The results of chemical analyses, together with *E. coli* concentrations, carried out on both rivers were compared to values shown in table 4, in order to calculate the EMPL index for both watercourses. From collected data, all stations were classified as belonging to the 2nd and 3rd levels, showing a moderate pollution. Furthermore, Tanagro river showed a better water quality than Bussento one as 73% of collected samples belonged to level 2 versus a 53% of Bussento ones. On the whole, 66% of all samples belonged to level 2.

Parameters	Level 1	Level 2	Level 3	Level 4	Level 5
100-DO (% sat.)	≤ 10	≤ 20	≤ 30	≤ 50	≥ 50
BOD ₅ (O ₂ /mg/L)	<2.5	≤4	≤8	≤15	>15
COD (O ₂ /mg/L)	<5	≤10	≤15	≤25	>25
NH ₄ ⁺ (N mg/L)	<0.03	≤0.1	≤0.5	≤1.5	>1.5
NO ₃ ⁻ (N mg/L)	<0.30	≤1.5	≤5	≤10	>10
Total-P (P mg/L)	<0.07	≤0.15	≤0.30	≤0.6	>0.6
<i>Escherichia coli</i> (CFU/mL)	<100	≤1000	≤5000	≤20000	>20000
Score referable to each parameter (75° percentile of the sampling period)	80	40	20	10	5
Evaluation of Macro-descriptors Pollution Level	480-560	240-475	120-235	60-115	<60

Table 4. Reference values of EMPL index.

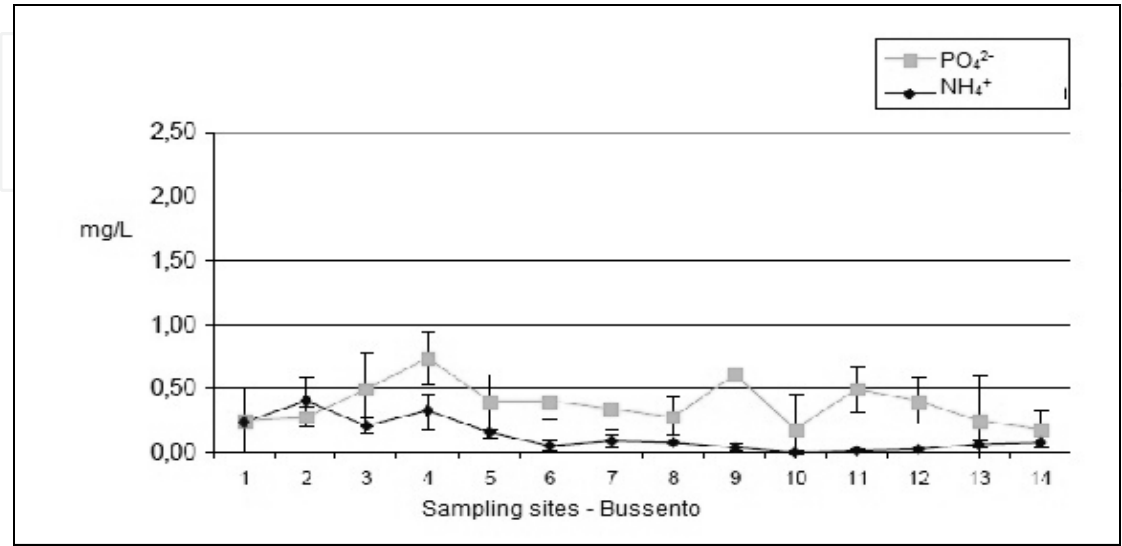


Fig. 11. PO₄²⁻ and NH₄⁺ concentrations in Bussento waters.

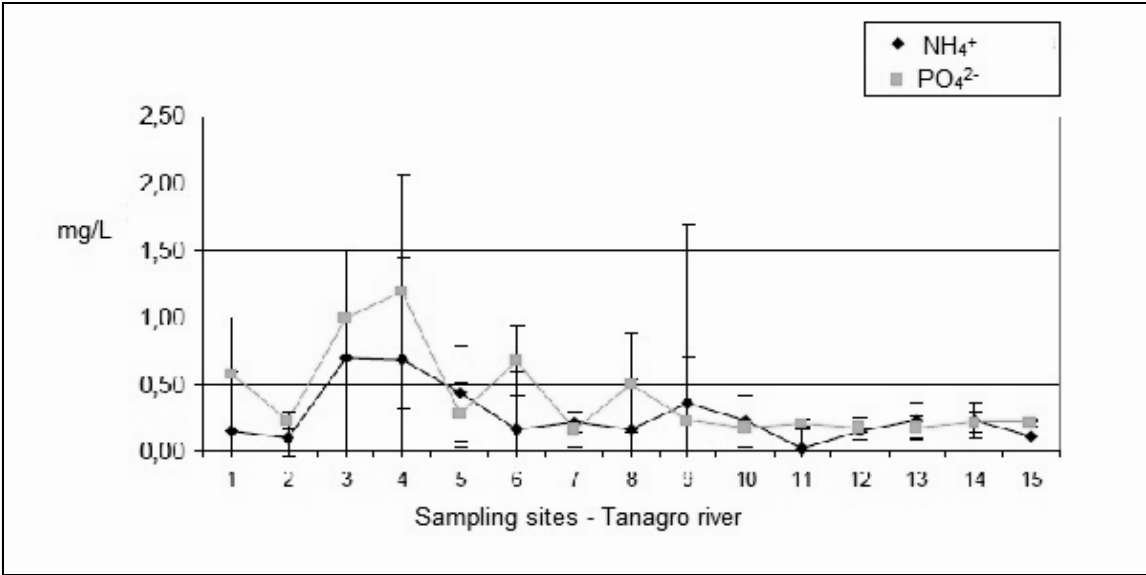


Fig. 12. PO₄²⁻ and NH₄⁺ values along Tanagro river stations.

4.3 Extended biotic index

The analysis of macro-invertebrates population were carried out two times a year (on summer and on winter) in correspondence of the same stations used to take samples for chemical-physical and microbiological analyses. Temperature, DO, pH and water conductivity were measured contemporaneously as they affect macro-invertebrates distribution heavily and a correct EBI evaluation can not exempt from a determination of the above mentioned parameters. Even if it is possible to notice a similar trend in the EBI variation along both watercourses (from spring to mouth), the second sampling campaign (on summer) showed lower values than the first one (carried out on winter). Apart from few sites showing a real deterioration of river ecosystem (especially in Tanagro river), EBI average values didn't change significantly during the sampling activity. Most part of the collected samples were classified as belonging to the second class (corresponding to moderate pollution) or to the third one (altered ecosystem).

4.4 The Ecological State of Watercourses (ESW)

The EBI values together with EMBL ones were used to gain another index, the Ecologic State of Watercourses (ESW) (Table 5). Our results showed, on the whole, a good or sufficient ecological quality. Only 36% of Bussento river sampling sites reached the 2nd ESW class of quality, suggesting a certain vulnerability of the freshwater ecosystem itself

As the remaining sites resulted just in a 3rd class. Tanagro river was characterized by a better environmental state as 46% of stations were classified as belonging to a 2nd class. Nevertheless, our results shed light on a critical environmental state regarding the station 3 of Tanagro river (4th class of quality). Even if most part of ESW values were determined by both EBI and EMBL values, it is interesting to notice how, in some cases, ESW values were affected in most part by the EBI values rather than EMBL ones as reported in Table 6.

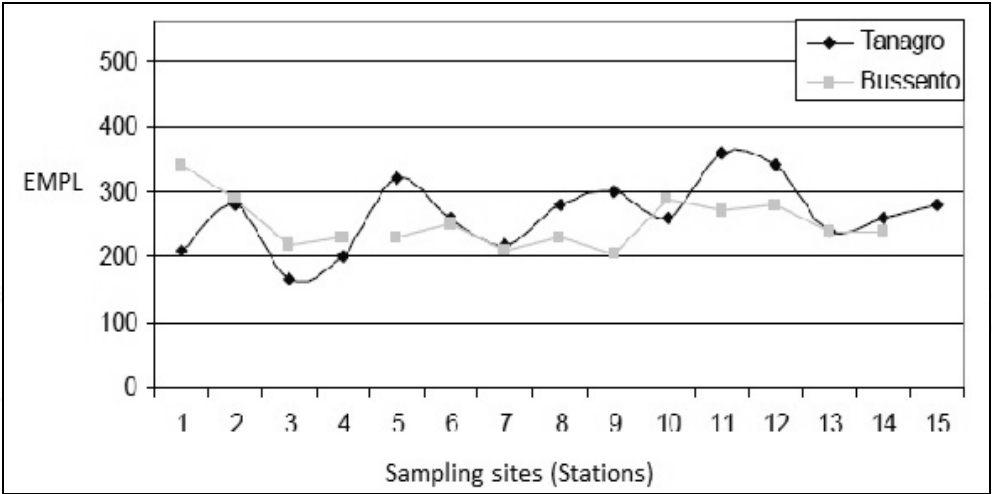


Fig. 13. EMPL values of Tanagro and Bussento river. There are not great variations in value.

The biological index not only is complementary to chemical characterization of freshwaters but it could even indicate, in advance, possible environmental criticisms.

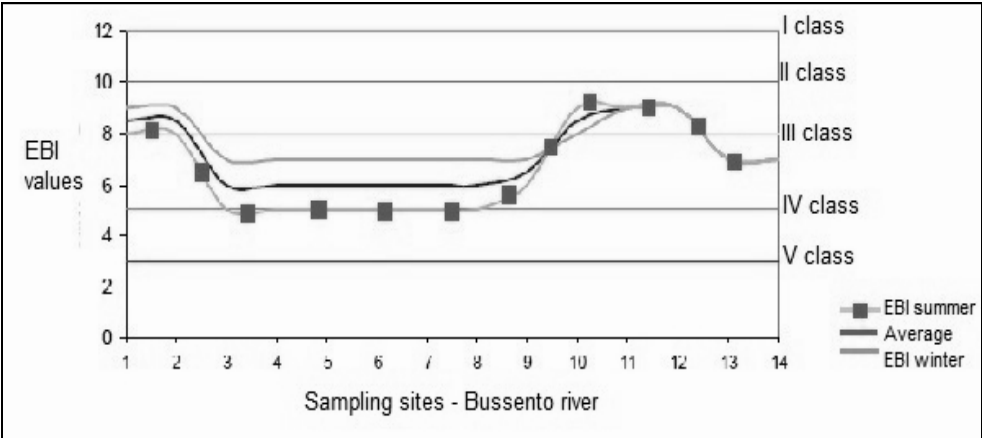


Fig. 14. EBI values of Bussento river stations. The values range from the 2nd to the 4 class of quality.

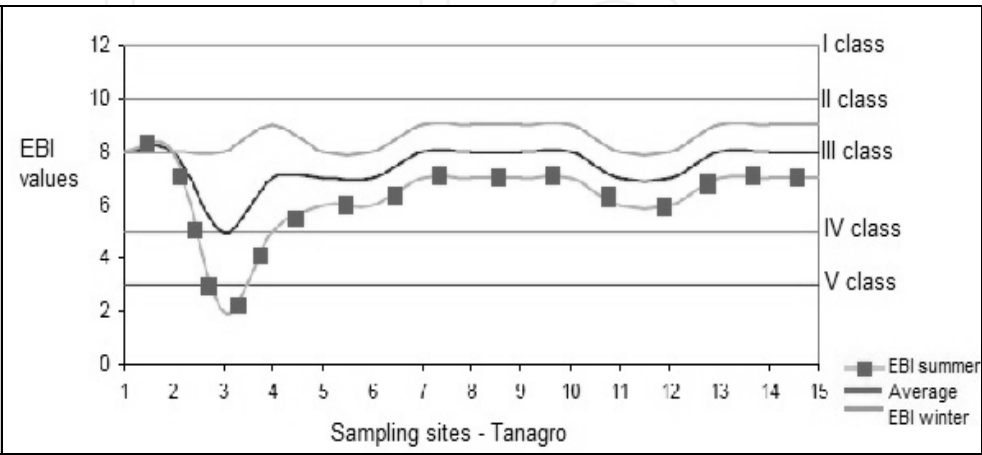


Fig. 15. Tanagro river EBI values in winter and in summer. In station n°3 the lowest value corresponding to a 5th class.

ESW	Class I	Class II	Class III	Class IV	Class V
EBI EMBL	≥10 480-560	8-9 240-475	6-7 120-235	4-5 60-115	1,2,3 <60
Overcome	High	Good	Sufficient	Poor	Very Poor
Conventional colour	Blu	Green	Yellow	Orange	Poor

Table 5. ESW class of quality and the correspondent EBI and EMBL values are reported.

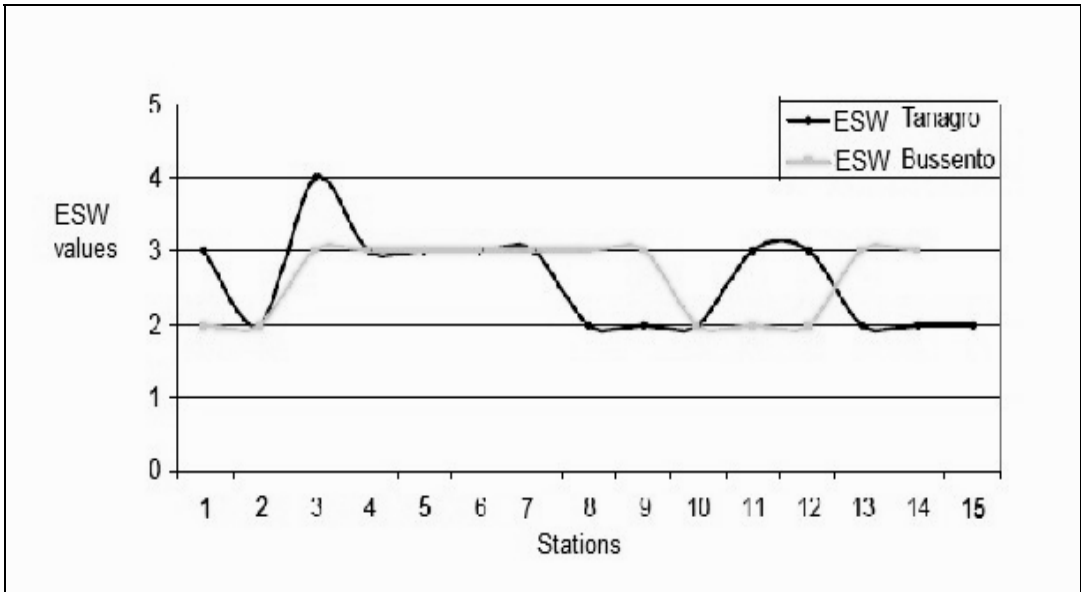


Fig. 16. ESW values: environmental quality reached the worst value just in station number 3 of Tanagro river.

	EMBL+EBI	EMBL	EBI
Tanagro	54%	13%	33%
Bussento	79%	21%	0%

Table 6. Percentage of samples whose ESW value was affected by EMBL+EBI or by just EMBL or EBI values.

FFI was applied in order to consider both hydro-morphological and biological factors in the evaluation of river courses environmental quality. Our findings, compared to some reference values, showed an environmental quality ranging from good-moderate to high (Table 7). The 57% of Tanagro river course fell into the high and good categories while Bussento river just 35%.

	High	Good	Good-moderate
Tanagro	7%	50%	43%
Bussento	21%	14%	65%

Table 7. FFI values for Tanagro and Bussento rivers.

4.5 Phytoplankton

Phytoplankton analyses didn't show any particular dystrophy, except for three stations of Bussento river, where high values of phytoplankton density were found.

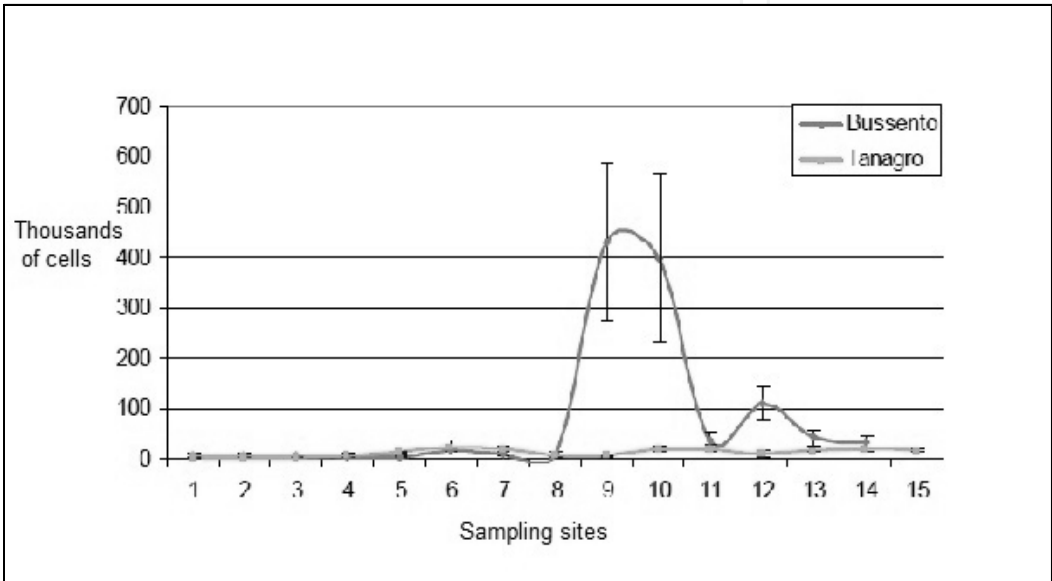


Fig. 17. Phytoplankton concentrations along the watercourses stations.

4.6 Microbiological results

Even if microbiological analyses are not strictly required to evaluate the environmental quality of a watercourse, the presence of some bacteria could be useful in order to recognize anthropogenic impacts due to wastewater intakes. As to *E. coli* concentration, Bussento water showed in all sites an amount ranging between 100 and 1000 CFU/100ml. On the other hand, most part of Tanagro samples (56%) showed a concentration ranging from 100 to 1000 CFU/100 ml, a 35% less than 100 CFU/100 ml and just a 9% of the stations was characterized by an amount overcoming 1000 CFU/100ml. These data were substantially confirmed by total and fecal coliforms, streptococci amounts. As to the other microbial parameters, no clostridia were found in both rivers water samples while *S.aureus* (typical of human and mammalian skin and mucosa) was seldom isolated. *Aeromonas hydrophyla* and *Pseudomonas aeruginosa*, instead, were widely present in water samples as they are part of environmental microflora. In Figures 18 and 19 the results concerning *A. hydrophyla* and *P. aeruginosa* are reported. From a comparison between both *A. hydrophyla*, *P.aeruginosa* and *E.coli* amounts along the rivers, there is no evidence of any correlation between such bacterial strains amounts.

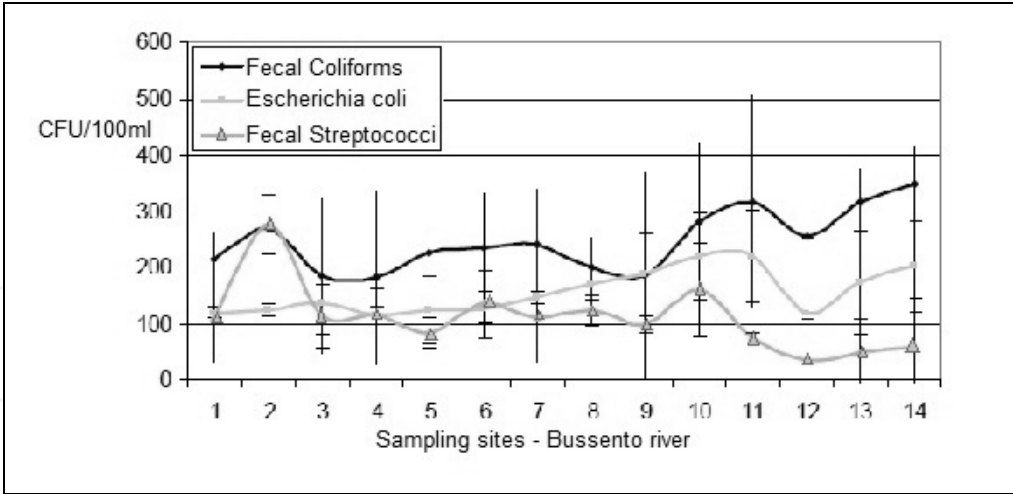


Fig. 18. Bussento river: microbiological values overcame the 100 CFU/100mL values except for streptococci in the last four stations.

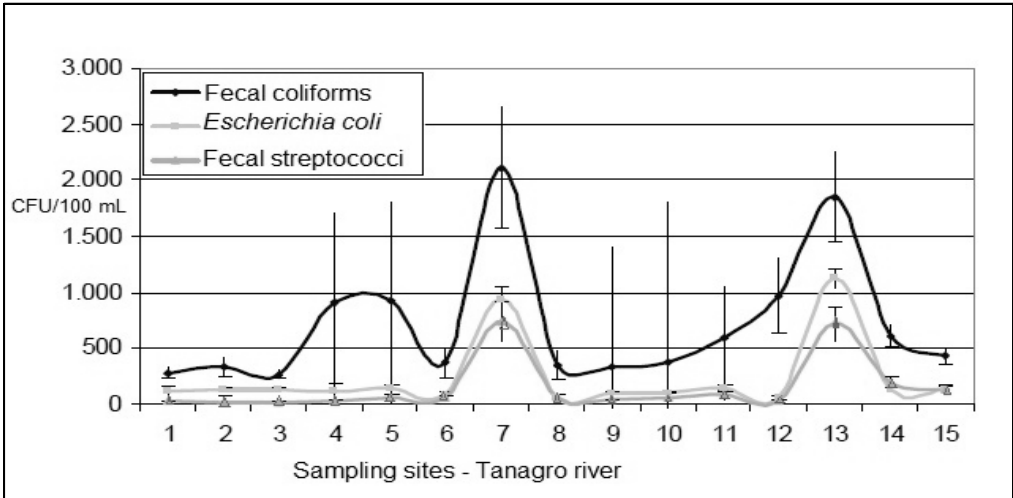


Fig. 19. *E.coli*, fecal coliforms and streptococci in Tanagro river waters. Stations 7 and 13 showed some high values.

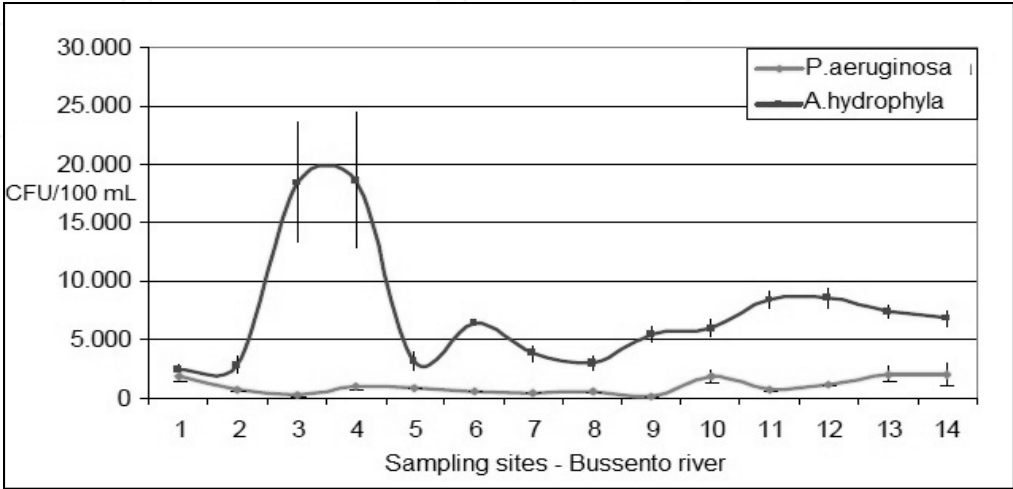


Fig. 20. *P. aeruginosa* and *A. hydrophila* concentrations in Bussento waters.

5. Conclusions

The environmental quality of both rivers resulted, on the whole, in a good or, at least, a sufficient quality, comparable, at least, to the upper course of Sele river flowing in the same area (Rizzo D. et al., 2009). These data are important as in a so highly urbanized district like the Campania one, most part of watercourses are heavily polluted and compromised by human activities. Sarno river, for example, is one of the most polluted river in Europe (Arienzo M. et al., 2000; De Pippo T. et al., 2006) and it flows in a high anthropized area. In this context of environmental degradation, the protection of high naturalistic value areas is a must and our data confirmed the efficacy of natural reserves and of a sustainable development policy. Under a strictly technical point of view, a polyphasic approach provides detailed informations about environmental quality of a river ecosystem. Nevertheless, it must be underlined that some experience in data analyses and competence in different fields are needed in order to give a right interpretation of findings yielded by so different analyses. In fact, it has to be noticed, for example, that phytotoxicity tests outcomes weren't fully overlapping with other ecotoxicological tests and of some difficult interpretation. While the *D.magna* and *P.subcapitata* tests gave no evidence of any significant toxic effect of water samples, *L.sativum*, *C.sativus*, *S.saccharatum* showed a modest inhibition of germination after 5 days (between 22% and 30% of seeds for each species didn't germinate) in correspondence to the station number 3 of Tanagro river, in accordance to the EBI, ESW, EMPL whose values were corresponding to a poor or sufficient environmental quality. It is clear that ecotoxicological tests are not enough to evaluate the environmental quality of a complex ecosystem like a river but they showed to be a useful tool in the evaluation of river environmental quality.

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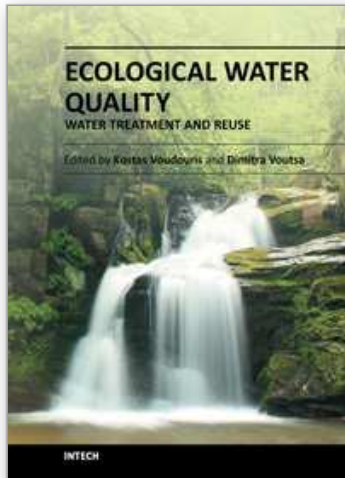
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This book attempts to cover various issues of water quality in the fields of Hydroecology and Hydrobiology and present various Water Treatment Technologies. Sustainable choices of water use that prevent water quality problems aiming at the protection of available water resources and the enhancement of the aquatic ecosystems should be our main target.

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