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Use of histopathologic indicators on chubs (*Leuciscus cephalus*) and brown trout (*Salmo trutta fario*) in evaluating river environments

A.Paolini, M.Berti, A.D'Angelo & C.Giansante

Summary

This study was a multidisciplinary research carried out in two rivers of Abruzzo (Italy). It underlines the importance of histopathologic investigations for evaluating environmental impact on fish. Brown trouts *(Salmo trutta fario)* from the river Aterno and chubs *(Leuciscus cephalus)* from the river Vomano were sampled in winter and then again in spring. Histopathologic investigations involved gills, kidneys and livers and revealed inflammatory and degenerative lesions, early warning signals of environmental stress. Lesions were evaluated semiquantitatively and a ranking of findings was performed. The histopathologic features were compared with the results obtained from the analysis of water samples and macroinvertebrates, collected in the aforesaid rivers (Extended Biotic Index). All the results achieved confirmed alterations of the observed environment.

Keywords

EBI - Fish - Histology - Pollution.

Introduction

Fish are often exposed to water heavily polluted by industrial, agricultural and urban discharges, expecially in areas where the flow is poor or in any case unable to dilute and to allow the purification of the toxicants discharged. Harmful effects are particularly evident when toxicants are little or no biodegradable at all, when they have a high biological activity, a high accumulation trend or synergistic effects.

Fish deaths are registered in the Abruzzi and Molise rivers; therefore, in this study, histological, macroinvertebrates and water analyses were carried out aimed at identifying early warnings, i.e. pollution early signals to prevent mortality events (22,23,24,25).

Histological analysis observes cell structures and assesses lesions that are not always visible to the naked-eye. Therefore, it provides a useful tool to check pollution sub-lethal and chronic effects.

Histological investigation so may be considered as a biomarker, i.e. an indicator of the impact of a xeno-biotc compound on different levels of biological organisations (cell, organ, individual and population) (17).

EBI (Extended Biotic Index) aims at formulating a diagnosis of the biological quality of surface running waters. It is based on the fact that effluents as well as other stress elements in the aquatic environment can alterate the composition of macroinvertebrate communities.

This method relies on the different sensitivity of

[1]Istituto Zooprofilattico Sperimentale dell'Abruzzo & del Molise «G. Caporale» (IZSA&M), Teramo - Italia
a.paolini@izs.it

some faunistic groups to pollutants and on the Taxa abundance of the benthic community. The indicators are represented by macrozoobenthos populations colonizing every stream substratum. These Species usually occupy all trophic levels, being themselves fish food. The macroinvertebrate life cycle can last over a year during which they mainly live tied to the water bottom, thus being unable to escape changes in the water quality.
For these reasons macroinvertebrates are largely used as water quality indicators (4).

Materials and methods

Sampling sites

Sampling was carried out in the Aterno (Popoli - Pescara) river and in the Vomano (Villa Vomano –Teramo) river (Figure1).

Fish

Sampling was carried out in Winter and hen again in Spring, from 13th November 2000 to 4th June 2001; each sampling consisted in 10 fish, brown trouts (*Salmo trutta fario*) from the Aterno river and chubs (*Leuciscus cephalus*) from the Vomano river. Fish were immediately denervated; immediately, in order to prevent post-mortal alterations (16, 19),

Figura 1
Abruzzo (Italy): Sampling sites (Δ) from Vomano river in Villa Vomano (Teramo) and the Aterno river in Popoli (Pescara), Abruzzo region of Italy

liver, kidney and gills were removed from each fish. Then, 3 small samples for each organ were taken, fixed by buffered 10% v/v formalin and processed according to usual histological methods (3). In this way, 360 organ sections were examined.
In order to assess the presence of any parasitic and infective disease and to discriminate between histological lesions caused by such diseases and histological lesions caused by pollution (22), each sample underwent a diagnostic screening, consisting in necroscopy, parasitic analysis (18), microbiological analysis (1). Histopathological lesions (11, 19, 26) were evaluated by ranking the severity of the alterations, from grade 1 (no pathological alterations) to 3 (extended/severe pathological alterations) (2, 23).

Macroinvertebrates

The macroinvertebrate sampling was carried out during two different hydrobiological cicles "High Flow " (Autumn/Spring) and "Low Flow" (Winter/Summer).
The Aterno and Vomano rivers are located in the Abruzzo mountains known as the "Appennine Chain", thus are largely affected by local meteorological conditions.
The collection of benthic organisms was carried out by means of a macroinvertebrate - net (21 meshes/cm), and their separation from the detritus was performed on the field.
The benthic organisms were identified at a family or genus level, called "Systematic Units" or "Taxon", according to the official method (4). Their presence or absence can reflect the stream general condition, thus being important to determine the river biological Quality Class .
A minimum number of organisms present in the sample, necessary to consider the caught organism as steadily belonging to the macroinvertebrate community, was fixed for every Taxon. Below this value, the caught organism was considered as "drift", that means it is casually present in the

Table I
Histopathological lesions observed in chubs caught in the Vomano river (winter sampling 8th March 2001)

Sample	Gill		Kidney		Liver	
	Lesions	grade	Lesions	grade	Lesions	grade
1	II V L F P M	3	R	1.5		1
2	I S L	3	V	1.5		1
3	S L P	3	V L	2.5		1
4	S	2	V L	2.5	C	1.5
5	S V	2.5	V L	2.5	C	1.5
6	V	2	V L	2.5	C	1.5
7	S	2	V L	2.5		1
8	V S	2.5	V	1.5		1
9	S L P	3	R	1.5	C	1.5
10	S V	2.5	V L	2		1
MEAN		2.5		2		1
ST. DEV.		0.43		0.49		0.25

Legend: I hypertrophia, II hyperplasia, F fusion of secondary lamella, V vacuolar degeneration, E hemorrhage, P picnosi, N necrosis, T telengiectasia, S epithelial lifting, L leukocyte infiltration, M mucous cell hyperplasia, m macrophages increase, D increase of granules deposits (melanin/hemosiderin), R regenerating cells, C congestion, p parasites, b bacteria, r rupture

sample and in this case, the Taxon was not calculated with reference to the assessment of the index.

Water

Water samples were carried out monthly for a year (April 2000 to March 2001) to analyse: pH, dissolved oxygen, suspended load, BOD5, total phosphorus, nitrites, phenol compounds, hydrocarbons, ammonia not-ionized, total ammonia, total active chlorine, anionic surface-active agent, arsenic, cadmium, chromium, mercury, nickel, lead, copper, zinc, organochlorine pesticides (Aldrin, Dieldrin, Endosulfan α, Endosulfan β, Hexachlorobenzene, HCH α, HCH β, Eptachlor, Eptachlor epoxide, Lindane, Methoxychlor, Mirex, o-pDDD,o-pDDE, o-pDDT, p-pDDD, p-p-DDE, p-pDDT), organophosphorus pesticides (Azinphos ethyl, Azinphos methyl, Diazinon, Dimethoat, Phenthion, Matathion, Mevinphos, Parathion ethyl, Parathion methyl, Phorate, Ronnel). Chemical analysis were carried out by water analytic methods I.R.S.A.-

Table II
Histopathological lesions observed in chubs caught in the Vomano river (summer sampling 18th May 2001)

Sample	Gill		Kidney		Liver	
	Lesions	grade	Lesions	grade	Lesions	grade
1	T S E	3	L V p	2	L	1.5
2	I V F L	3	L V	2.5	L	1.5
3	II V S T M	3	L V b	2.5		1
4	V S	2.5	L V p	2.5		1
5	V S	2.5	L V r	3		1
6	I II M L p	2.5	L R p	2.5	N	2
7	S I M	3	L V	2.5		1
8	T E S	3	L V	3	L	1.5
9	V S	2.5	L V	2.5	N	2
10	I II L	2.5	L R p	3	L	1.5
MEAN		2.7		2.6		1.4
ST.DEV.		0.26		0.31		0.39

Legend: I hypertrophia, II hyperplasia, F fusion of secondary lamella, V vacuolar degeneration, E hemorrhage, P picnosi, N necrosis, T telengiectasia, S epithelial lifting, L leukocyte infiltration, M mucous cell hyperplasia, m macrophages increase, D increase of granules deposits (melanin/hemosiderin), R regenerating cells, C congestion, p parasites, b bacteria, r rupture

Table III
Histopathological lesions observed in brown trouts caught in the Aterno river (winter sampling 11thJanuary 2001)

Sample	Gill		Kidney		Liver	
	Lesions	grade	Lesions	grade	Lesions	grade
1	I T	2	m	1.5	L	1.5
2	I	1.5	m	1.5	L	2
3	I	1.5	D	2	L	1.5
4	I	1.5	m	1.5	L	2
5	I L T	1.5	D	2		1
6	I	1.5	m	1.5	L	2
7	M	1.5	D L	2	L	2
8	T	2	m	1.5		1
9	M	1.5	m	1.5	L	1.5
10	I	1.5	m	1.5		1
MEDIA		1.6		1.6		1.5
DEV. ST.		0.21		0.24		0.43

Legenda: I hypertrophia, II hyperplasia, F fusion of secondary lamella, V vacuolar degeneration, E hemorrhage, P picnosi, N necrosis, T telengiectasia, S epithelial lifting, L leukocyte infiltration, M mucous cell hyperplasia, m macrophages increase, D increase of granules deposits (melanin/hemosiderin), R regenerating cells, C congestion, p parasites, b bacteria, r rupture

C.N.R. (13) and UNICHIM methods (15). Faecal choliformes, faecal streptococci and salmonellas were detected according with Quaderni methods I.R.S.A.-C.N.R. methods (12).

Chemical results were assessed in compliance with the limits prescribed by the D.L.vo N°152/99, Attachment 2, Section B concerning the general criteria for the assessment of superficial freshwater qualitative features for trouts and minnows (5). Bacteriologic results were assessed in compliance with D.P.R. N° 470/82 concerning bathing water quality.

Results

Fish

Histologic lesions are described in Table I-IV.

Chubs from the Vomano river:

- Gills: epithelial lifting and vacuolar degeneration ranked a severity of 2.5 grade; this stress index rises in spring (grade 2.7), though it is not possible to distinguish how much that was due to the presence of Dactylogyris.
- Kidneys: vacuolar degeneration in renal tubule epithelial cells, lymphocytes and granulocytes infiltration (Figure 2) in winter (grade 2) which rises in spring (grade 2.6) also as a consequence of the presence of parasites belonging to the Genus Spherospora.
- Liver: narrow zones of necrosis, observed only in two chubs (grade 1.2 in winter and 1.4 in spring).

Trouts from the Pescara river:

- Gills: light hypertrophy and telangiectasia (grade 1.6) in winter, more severe in spring (grade 4) with secondary lamella edema and epithelial lifting (Figure 3).
- Kidneys: macrophage infiltration (grade 1.6) that rises in spring (grade 1.9) with lymphocyte

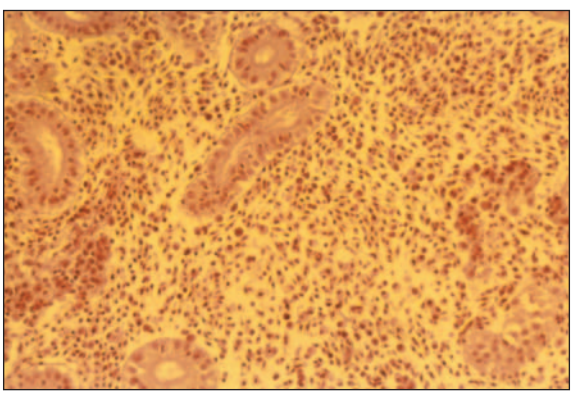

Figure 2
Chub kidney (25x) vacuolization in the epithelial cells of renal tubules, leukocytes infiltration of interstitial tissue

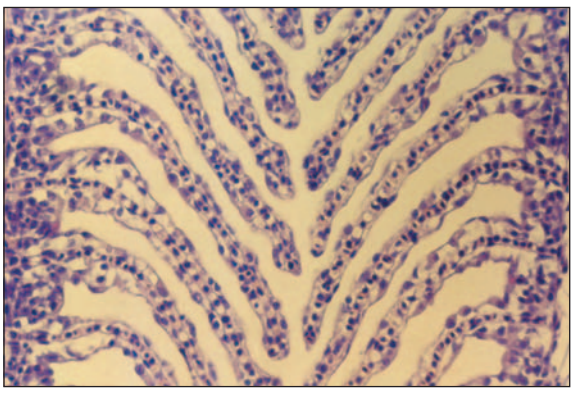

Figure 3
Trout gills (25x): epithelial lifting and vacuolization

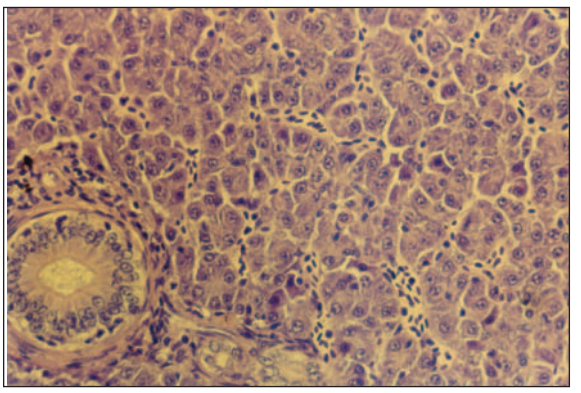

Figure 4
Trout liver(25x): light lymphocytes infiltration

infiltration and regeneration, also as a consequence of the presence of parasites belonging to the Genus Spherospora.

- Liver: light lymphocyte infiltration (Figure 4) both in winter and in spring sampling (grade 1.5).

Macroinvertebrates

The sampled macroinvertebrates and the results coming from their classification are shown in Table V

The resulting Quality Class of the Aterno and Vomano river stretches has been assessed as follows:

- Aterno (low flow), "Environment with moderate symptoms of pollution or alteration/polluted or alterated environment (Quality class: II/III).
- Aterno (high flow), "Environment with moderate symptoms of pollution or alteration (Quality class: II).
- Vomano (low flow), "Highly polluted or highly altered environment (Quality class: IV).
- Vomano (high flow), "Polluted or alterated environment (Quality class: III).

Water

Results coming from water analysis are described in tables VI-IX.

Some samples did not comply with the D.Lvo 152/99 All. 2, Sez. B: general criteria to survey the

Table IV
Histopathological lesions observed in brown trouts caught in the Vomano river (spring sampling 16th April 2001)

Sample	Gill		Kidney		Liver	
	Lesions	grade	Lesions	grade	Lesions	grade
1	S I V P	2.5	L m	1.5	L	1.5
2	S I II L	2	L m p	1.5	L	2
3	I V S P	2.5	L m p	1.5		1
4	M I	2	R P m p	2		1
5	I II V	2.5	m	1.5	L	2
6	S V	2	L R V	2		1
7	V	2.5	L R V	2.5	L P	2
8	I M V	2.5	R P	2.5	L	1.5
9	V I S P	3	L m	1.5	L	2
10	S I II L	2.5	L R m	2.5	L	1.5
MEDIA		2.4		1.9		1.5
DEV. ST.		0.31		0.45		0.43

Legend: I hypertrophia, II hyperplasia, F fusion of secondary lamella, V vacuolar degeneration, E hemorrhage, P picnosi, N necrosis, T telengiectasia, S epithelial lifting, L leukocyte infiltration, M mucous cell hyperplasia, m macrophages increase, D increase of granules deposits (melanin/hemosiderin), R regenerating cells, C congestion, p parasites, b bacteria, r rupture

Table V
Extend Biotic Index Results

Systematic Unit	Aterno River		Vomano River	
	Low flow	High flow	Low flow	High flow
PLECOPTERA				
(Genus)				
EPHEMEROPTERA				
(Genus)				
Baetis	+	+	d	+
Caenis	d		d	d
Ecdyonurus		+		d
Rithrogena		d		
Epeorus		+		
TRICHOPTERA				
(Family)				
Limnephilidae	+	+		+
Hydropsychidae	+	+		+
Rhyacophilidae		+		+
Sericostomatidae				+
COLEOPTERA				
(Family)				
Haliplidae	d			d
Elmidae	d	+		+
Gyrinidae				+
ODONATA				
(Family)				
Calopteryx	+	+		+
Onychogomphus				+
DIPTERA				
(Family)				
Simuliidae	+		+	d
Chironomidae	+	d	+	+
Limoniidae				+
HETEROPTERA				
(Family)				
CRUSTACEA				
(Family)				
Asellidae	+		+	
Gammaridae	+	+	d	d
GASTEROPODA				
(Family)				
Neritidae	+			
Bythiniidae	+	+		+
Planorbidae	+			
Lymnaeidae			+	
BIVALVIA				
(Family)				
TRICLADAE				
(Genus)				
Dugesia	+	+		
Polycelis	+			
HIRUDINEA				
(Genus)				
Dina	+	+		+
Glossiphonia		+	+	
Helobdella				
OLIGOCHAETA				
(Family)				
Lumbricidae	+	+	+	+
Naididae	+			
Tubificidae			+	
OTHERS GROUPS				
TOTAL	16	14	7	14
EXTENDED BIOTICA INDEX	8 - 7	8	5	7
QUALITY CLASS	**II - III**	**II**	**IV**	**III**

Legend: + = presence; drift: low number of organism. They don't count for Extended Biotic Index.

Table VI
Results of physical and chemical tests carried on water samples –Vomano river

Date	21/04/00	17/05	28/06	19/07	30/08	27/09	18/10	29/11	20/12	17/01/01	21/02	21/03
Dissolved oxygen (mg/l)	9,2	10,5	8,9	7,6	6,6	8,5	7,3	9,4	11,0	11,2	11,7	11,0
PH	7,80	8,00	8,68	8,94	8,84	8,77	8,50	7,97	8,48	7,73	8,10	8,36
Ammonia (mg/l)	<0,025	<0,025	<0,025	<0,025	<0,025	<0,025	<0,025	<0,025	**0,025**	<0,025	<0,025	**0,025**
Total Ammonia (mg/l)	0	0	0	0	0	0	0,37	**1,01**	**1,11**	0	0	0,54
Nitrites (mg/l)	0	0	0,04	0	0	0,92	0	0,08	0	0	0	0
Phosphorus (mg/l)	0,03	0,30	015	2,63	0	0,02	0,02	0	0,05	0,03	0,04	0,01
BOD5 (mg/l)	0,23	2,08	1,73	1,71	0,1	1,17	0,85	0,71	1,27	2,64	1,38	1,01
Suspended solids (mg/l)	1,33	2	0,88	0,04	14,59	3,33	3,05	**94,85**	1,86	8,77	10,11	1,87
Phenols (mg/l)	0	0,06	0	0	0	0	0	0	0	0	0	0
Surface-active agents (mg/l)	0	0	0	0	0	0	0	0	0	0	0	0
Total active chlorine (mg/l)	0	0	0	0	0	0	0	0	0	0	0	0
Hydrocarbons	0	0	0	0	0	0	0	0	0	0	0	0
Arsenic (ug/l)	0	0	0	0	0	0	0	0	0	0	0	0
Cadmium (ug/l)	0	0	0	0	0	0	0	0	0	0,48	0	0
Chromium (ug/l)	0	0	0	2,43	0	0	0	0	0	0	0	0
Mercury (ug/l)	0	0	0	0	0	0	0	0	0	0	0	0
Nichel (ug/l)	0	0	0	0	0	0	0	0	0	0	0	0
Lead (ug/l)	0	0	0	0	0	0	0	0	0	0	0	0
Copper (ug/l)	0	0	0	0	0	0	0	13,6	0	0	1,7	0
Zinc (ug/l)	0	0	0	0	0	0	0	0	0	0	0	0

Legend: values that exceeded limits (Directive 91/271/CE and Directive 76/160/CE) are bold-faced

superficial freshwater qualitative characteristics for trouts and minnows (8), as mentioned below:

- Aterno river: ammonia not-ionized concentration on May and suspended particles on April.
- Vomano river: total ammonia concentration on November and December, ammonia not ionized concentration on December and March. Nitrite concentration on September was good for minnows but not for trouts.

Microbiological analysis show a high concentration of faecal bacteria with faecal choliformes concentrations exceeding, from 10 to 10.000 times, the D.P.R 470/82 (6) limits. In 6 samples from Vomano river Lindane was detected, beside of organochlorine pesticides (Dieldrin, Endosulfan) and organophosphorus pesticides (Ronnel, Diazinon).

Discussion

Considering that fish was not affected by infectivous disease and that parasites (Dactylogyridae in gills of chubs sampled in spring in the Vomano river and Spherospora in kidney of fish sampled in both the rivers) were present in low abundance, it is possible to assert that inflammatory and degenerative features were caused by environmental stress, more severe in the Vomano river. This observation is confirmed by the Extended Biotic Index results and by some results of water samples.

In fact the study of benthic organism communities also shows a condition of environmental imbalance which revealed to be light on the sampling site of the Aterno river and heavier on the sampling site of the Vomano river. The Extended Biotic Index results show a worsening of the aquatic ecosystem health condition during the low flow, as frequently happens, which is probably due to a lower pollutant dilution and self-depurative capacity of the stream.

Water analysis show and heavy presence of faecal bacteria, that are not dangerous for the fish but however indicate an uncontrolled sewage waste-water discharge in these areas. It is remarkable, in both the water-courses, the presence of *Salmonella thyphimurium*, that represents a sanitary hazard.

The results of pesticides analysis are alarming is

Table VII
Results of water samples physical and chemical tests – Aterno river

Data	5/04/00	10/05	21/06	12/07	23/08	20/09	25/10	22/11	18/12	31/01/01	14/02	28/03
Dissolved oxygen (mg/l)	9,6	8,8	8,8	6,8	6,3	11,8	11,5	9,3	10,0	6,5	11,3	9,7
PH	8,10	8,40	7,40	8,10	8,67	8,53	8,56	7,91	7,71	8,10	8,13	8,00
Ammonia (mg/l)	<0,025	**0,025**	<0,025	<0,025	<0,025	<0,025	<0,025	<0,025	<0,025	<0,025	<0,025	<0,025
Total Ammonia (mg/l)	0	0,53	0	0	0	0	0	0,57	0,59	0	0	0
Nitrites (mg/l)	0,47	0	0	0,85	0,53	0,83	0,19	0,15	0	0	0	0
Phosphorus (mg/l)	2,5	2,77	0,01	1,97	3,58	0,06	0,17	0,11	0,1	0,11	0	0,15
BOD5 (mg/l)	2,5	2,08	1,52	2,52	2,33	2,73	1,59	1,72	2,23	2,44	3,04	3,95
Suspended solids (mg/l)	**96,98**	2	1,43	2,13	8,73	0,29	0,31	4,25	5,6	1,38	4,77	10,25
Phenols (mg/l)	0	0	0	0	0	0	0	0	0,02	10,11	0	0
Surface-active agents (mg/l)	0	0	0	0	0	0	0	0	0	0	0	0
Total active chlorine (mg/l)	0	0	0	0	0	0	0	0	0	0	0	0
Hydrocarbons	0	0	0	0	0	0	0	0	0	0	0	0
Arsenic (ug/l)	0	0	0	0	0	0	0	0	0	0	0	0
Cadmium (ug/l)	0	0	0	0	0	0	0	0	0	0	0	0
Chromium (ug/l)	0	0	0	0	0	0	0	0	0	0	0	0
Mercury (ug/l)	0	0	0	0	0	0	0	0	0	0	0	0
Nichel (ug/l)	0	0	0	0	0	0	0	0	18,7	0	0	3,01
Lead (ug/l)	0	0	0	0	0	0	0	0	0	0	0	0
Copper (ug/l)	0	0	0	0	0	0	0	0	2,2	0	2,71	2,86
Zinc (ug/l)	0	0	0	0	0	0	0	0	0	0	0	0

Legend: values that exceeded limits (Directive 91/271/CE and Directive 76/160/CE) are bold-faced.

too, Dieldrin showed a concentration that exceeds limit fixed by D.L.vo 152/99(8) for waste-water. Jenkis *et al.* (14) assessed that Endosulfan causes acute effects and subacute effects in carp (*Cyprinus carpio*) respectively with concentration of 10 µg/l and 5 µg/l; in this study higher concentration was detect in the Vomano river. The harmless concentration of Lindane desumed in *Channa puncatus* is 5,44 µg/l (10); higher concentration was detected both in the Vomano and in the Aterno river. In *Pimephales promelas* (Cyprinids) the LC_{50}/7 day and the IC_{50}/7 day (Inhibitions of growth and reproduction) are respectively 112 µg/l and 58,5 µg/l (5). Besides, this pesticide, which use is

Table VIII
Microbiological and pesticides analysis – Vomano river

Date	Fecal Coliphorm	Fecal Streptococci	Salmonella	Organochlorine pesticides	Organophosphorus pesticides
12/04/2000	**700 u.f.c./100 ml**	100 u.f.c./100 ml	***S. newport***	N.R.	N.R
17/05/2000	**1700 u.f.c./100 ml**	**490 u.f.c./100 ml**	Absent/100 ml	N.R.	N.R.
28/06/2000	**1900 u.f.c./100 ml**	**1200 u.f.c./100 ml**	Absent/100 ml	Lindane 13 µg/l	N.R.
19/07/2000	**450 u.f.c./100 ml**	**290 u.f.c./100 ml**	Absent/100 ml	Dieldrin 13 µg/l Lindane 13 µg/l	N.R.
30/08/2000	**1300 u.f.c./100 ml**	**1100 u.f.c./100 ml**	Absent/100 ml	N.R.	N.R.
27/09/2000	**500 u.f.c./100 ml**	**400 u.f.c./100 ml**	Absent/100 ml	Lindane 9 µg/l	N.R.
18/10/2000	**2200 u.f.c./100 ml**	**900 u.f.c./100 ml**	***S. typhimurium***	Endosulfan 11 µg/l Lindane 8 µg/l	Ronnel 22 µg/l
29/11/2000	**210 u.f.c./100 ml**	**3600 u.f.c./100 ml**	Absent/100 ml	Lindane 9 µg/l	N.R.
20/12/2000	**3200 u.f.c./100 ml**	**3600 u.f.c./100 ml**	Absent/100 ml	N.R.	N.R.
17/01/2001	**2800 u.f.c./100 ml**	**2800 u.f.c./100 ml**	Absent/100 ml	N.R.	N.R.
21/02/2000	**1200 u.f.c./100 ml**	**2000 u.f.c./100 ml**	Absent/100 ml	Lindane 9 µg/l	N.R.
21/03/2000	**3100 u.f.c./100 ml**	**2700 u.f.c./100 ml**	Absent/100 ml	N.R.	Diazinon 8 µg/

Legend: Microbiological values that exceeded limits Directive 76/160/CE are bold-faced

Tabella IX
Microbiological and pesticides analysis – Aterno river

Date	Fecal Coliphorm	Fecal Streptococci	Salmonella	Organochlorine pesticides	Organophosphorus pesticides
05/04/2000	**9500 u.f.c./100 ml**	**400 u.f.c./100 ml**	***S. typhimurium***	N.R.	N.R
10/05/2000	Non effettuato	**7000 u.f.c./100 ml**	Absent/100 ml	N.R.	N.R.
26/06/2000	**5500 u.f.c./100 ml**	**30000 u.f.c./100 ml**	Absent/100 ml	N.R.	N.R.
12/07/2000	**300 u.f.c./100 ml**	**1400 u.f.c./100 ml**	Absent/100 ml	N.R.	N.R.
23/08/2000	**14200 u.f.c./100 ml**	**2900 u.f.c./100 ml**	Absent/100 ml	N.R.	N.R.
27/09/2000	**3600 u.f.c./100 ml**	**3100 u.f.c./100 ml**	Absent/100 ml	N.R.	N.R.
25/10/2000	**3800 u.f.c./100 ml**	**1200 u.f.c./100 ml**	Absent/100 ml	Esaclorobanzene 4 µg/l Dieldrin 9 µg/l	N.R.
22/11/2000	**9700 u.f.c./100 ml**	**1500 u.f.c./100 ml**	Absent/100 ml	N.R.	N.R.
19/12/2000	**2300 u.f.c./100 ml**	**1900 u.f.c./100 ml**	Absent/100 ml	Lindane 14 µg/l	N.R.
31/01/2001	**9700 u.f.c./100 ml**	**4200 u.f.c./100 ml**	Absent/100 ml	N.R.	N.R.
21/02/2000	**191000 u.f.c./100 ml**	**6700 u.f.c./100 ml**	Absent/100 ml	N.R.	N.R.
28/03/2000	**19400 u.f.c./100 ml**	**2800 u.f.c./100 ml**	Absent/100 ml	Lindane 5 µg/l	N.R.

Legend: microbiological values that exceeded limits Directive 76/160/CE are bold-faced

restricted by law (7), causes reproductive and behaviour disorders in wild fish, bird and mammalian (27,28) This multidisciplinary study confirms usefulness of bioindicators that are able to detect marks left by toxic substances (17). By means of them it is possible to monitor effectively the aquatic environment and to take corrective decision in terms of environmental management

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