

First eco-toxicological survey in the Miramare Marine Reserve (Gulf of Trieste, Italy)

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Abstract - This study is a survey aiming at applying and evaluate different analysis methodologies in the Miramare Marine Reserve, rather than being the result of an analysis campaign. Marine bacteria, rotifers, marine and fresh water crustaceans and fresh water algae were used as ecotoxicological tests. Biomarkers in decapod crustaceans and the assessment of chemical analysis in mussel tissues were used, respectively, as biosensors and bioaccumulators. Acute toxicity signals and not negligible PAHs levels in mussels were detected in the small port of Grignano area, and freshwater inputs in this area provide signals of eutrophic conditions. The acute toxicity level is not high, but it certainly requires an enhancement of the monitoring activities.

1. Introduction

The Reserve of Miramare, located in the High Adriatic basin, is a safeguarded site whose basic objective is monitoring. In the present survey there are two parallel objectives:

1. to have an eco-toxicological overview of the protected area, in order to plan future monitoring activities;
2. to try to select a battery of indicators using toxicity bioassays, biomarkers and bioaccumulation analysis.

Eco-toxicological research in the Marine Reserve of Miramare has not been carried out before. Physical water quality evaluations have been performed *in continuum* for several years by the Marine reserve management (Stravisi et al., 2000; Vinzi and Bussani, 2000). Several chemical studies were conducted over the last years in the Northern Adriatic Sea and Gulf of Trieste, giving evidence of the presence of heavy metals in the sediment (mainly mercury) and their bioconcentration in mussels of some areas, mainly in the lagoons (Faganelli et al., 1991; Covelli et al., 1999; ICRAM, 2000). It is helpful to the Reserve management to start

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with bioassays, since there are tourist activities all around the protected area and suburban settlements together with a small port, where sailing and fishing boats usually dock. The hydro-dynamism of the area, with its promontory and shallow water, may favour the sedimentation of the particles in suspension. A pre-requisite for monitoring a protected area is to have detection techniques, which should be reliable, effective, repeatable and, if possible, cheap (Janssen and Persoone, 1992). Most of these requisites are guaranteed by the use of standard populations (stalled culture, spores, seeds or encysted larvae) (Persoone and Janssen, 1993) and the use of standard protocols (UNI EN ISO, ASTM, IRSA, EPA, etc.) (Gentile et al., 1984; U.S.EPA, 1993). It is also necessary to have statistical significance and resolution and to maintain a certain consistency at bioethical level. Therefore, microbial and invertebrate organisms are used, cultured, if possible, or collected for commercial purposes (Persoone et al., 2000), choosing species representing those present in the surveyed ecosystem, in terms of *trophic role* or habitat (Vanhaecke and Persone, 1984; Persoone et al., 1989).

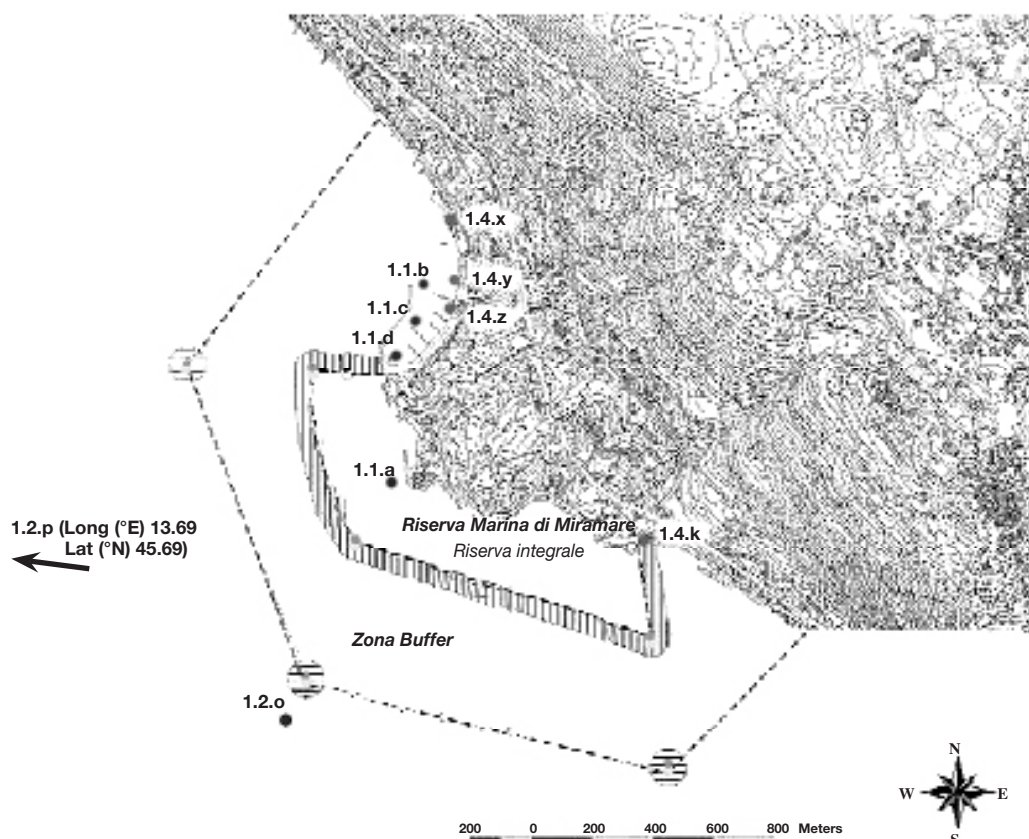
2. Materials and methods

In the present survey, marine bacteria *Vibrio fischeri*, according to the Microtox[®] procedure (Johnson, 1998), rotifers (Francesse e Traldi, 2001), marine and fresh water crustaceans (Van Steertegem and Persoone, 1993; Centeno et al., 1995) and fresh water algae (Persoone, 1998) were used as toxicity bioassays (Stroscher, 1984). They were all applied according to standard protocols and with genetically controlled populations, using SDI (Strategic Diagnostics Inc., Newark, DE USA) reagents for Microtox[®] and Microbiotest[®] kits. More experimentally, the effects of the exposure to the samples were assessed on decapod crustaceans (*Palaemon elegans*) through biomarkers tests (Migliore and de Nicola Giudici, 1990; Ahsanullah and Ying, 1995; Lorenzon et al., 2001/a). The assessment of bioaccumulation was carried out through a chemical analysis of mussel tissues (*Mytilus galloprovincialis*), in two different seasons (winter and summer) in order to detect possible different seasonal toxicant concentrations (Lobel et al., 1991; Regoli, 1994; Pellegrini et al., 2001).

These choices permitted us to carry out surveys on three matrices (U.S.EPA, 2002): fresh water, sediment, thus obtaining the elutriate, and biota, thus identifying the mussels as bioaccumulator species. Standard water parameters were controlled during the sampling moment; concentration of organic toxic compounds was investigated by ICP/MS (U.S.EPA 1996, 1998). The bioavailability of heavy metals in sediments was tested by means of a leaching test using CO₂ saturated water (IRSA-CNR, 1984). The exclusion of marine water monitoring at ecotoxicological level was decided because the bottom is the main acceptor and accumulator compartment for xenobiotic substances in any marine water basin (Volpi, Ghirardini and Pellegrini, 2001).

Sediment sampling sites are those signed in the map (Fig. 1), clustered into two groups: one to compare sites with different anthropic impact (1.1.a, two samples: August and September 2001; 1.1.b, two samples: August and September 2001) and the other, based on the previous one, focusing investigation along a transect within the small port area (1.1.c, two samples:

January 2002; 1.1.d, two samples: January and May 2002) where there is a direct anthropic impact. Previous surveys (data not reported) gave us the opportunity to identify sampling sites for negative (1.2.o, one sample: December 2001) and positive (1.2.p, one sample: December 2001) reference sediments in the Gulf of Trieste. Fresh water samples were collected from two little creeks (1.4.k, two samples: January and July 2002; 1.4.x, two samples: January and July 2002) and two small sewage pipes (1.4.y, two samples: December 2001; 1.4.z, two samples December 2001) within the protected area. Waste water samples were collected only in winter in



Elaborazione GIS della Riserva Marina di Miramare

Fig. 1 - Sampling points Map.

- Beacon buoy
- Sediment sampling points
- Freshwater sampling points
- Marine Reserve borderline
- - - Buffer zone borderline

1.1.a, 1.1.b) comparison different anthropic impact: sediment and mussels samples in the centre of Reserve and at the entrance of the port

1.1.c, 1.1.d) focusing along a small transect: sediment and mussels samples in central and internal part of the port

1.2.o, 1.2.p) reference sampling: sediment on the border of the Reserve and spiked sediment far from the shore sampling sites

1.4.x, 1.4.k) monitoring freshwater input: little creeks samples, Grignano Creek and Sticco creek

1.4.y, 1.4.z) monitoring freshwater input: small sewage pipes samples, near reserve basin and in port.

order to detect the direct anthropic impact of the local resident community.

Moreover, in order to have a picture of the most recent impact and not to incur in interferences belonging to the geologic history of that place, the analysis focused on the topsoil, reaching up to 5 cm in depth. Scuba diver sampling were performed for sediment, water and mussels. Two litre glass pots were used to collect both sediment and water; about 40 mussels of the same average size (i.e. 7.2 cm) were collected by hand. All samples were immediately shipped to the laboratory in ice-cooled boxes. Once in the laboratory, the samples were stored at 4° C. Elutriate was obtained within 24 hours and bioassays performed within 48 hours (ASTM 1994; U.S.EPA 2000). Elutriate was prepared using US EPA protocol (U.S.EPA- 1998/1; U.S.EPA 1998/2). Mixing was performed in 2 litre bottles for 30 minutes with an overhead mixer at 15° C. Elutriate samples were centrifuged (15 minutes at 2000 g) and the supernatant was used to perform bioassays. Before performing Microtox[®] bioassay samples were filtered with 0,45 micron pore size cellulose acetate filters. Mussels (*Mytilus galloprovincialis*) were sent immediately to an external laboratory for bioaccumulation analysis, by gas chromatography and atomic absorption spectroscopy, without lipid normalisation (APHA, 1992).

Bioassays with *Vibrio fischeri* (Microtox[®], 1992), *Brachionus plicatilis* (ASTM, 1991), *Artemia franciscana* (Artoxkit FTM), were conducted with marine elutriate samples, and bioassays with *Palaemon elegans* were performed with no-modified sediment samples. Toxicity tests using *Vibrio fischeri* (Microtox[®], 1992), *Daphnia magna* (Daphtoxkit FTM, 1996; UNI EN ISO, 1999), *Thamnocephalus platyurus* (Thamnotoxkit FTM) and *Selenastrum capricornutum* (renamed *Pseudokirchneriella subcapitata*) (ASTM, 1990) were performed on fresh water samples. All bioassays, except *Palaemon elegans*, were conducted by using commercial kits (Microtox[®] and Toxkits[®]) containing cists, ehippia or immobilized algae and bacteria. *S. capricornutum* bioassay was performed at 72 hours instead of 96, because kit's batch was tested with reference after a 72-hour exposure. The use of commercial kits, instead of home cultured organisms, was preferred because they are easy to use, they have a low cost and give good quality results. These tests could be performed even in small laboratories, at low cost, without trained technicians.

A. franciscana and *B. plicatilis*, which are not as sensitive as others (Ruck et al., 2000), were used for different reasons: these kinds of tests are very similar to the others used (*D. magna*, *T. platyurus*) and some sampling sites (inside the port) were supposed to be at least moderately polluted. Moreover, the Shoreline laboratory used *B. plicatilis* for testing other polluted matrices. No analyses on pore water were performed, because diver sampling could not avoid mixing sediment with overlaying water.

Palaemon elegans were supplied by a fisherman and maintained in large holding tanks with natural photoperiod and aerated circulating seawater. Each test was carried out by using 30 animals in three replicates, after acclimatisation (29 ‰, 16° C), in 6 litres of standard seawater with about one kilo of sample sediment on the bottom. Hemolymph samples were taken from pericardic sinus at 0, 1, 2, 3, 5, 8, 24 hours and tested for glycemia (Lorenzon et al., 2000/b).

Judgements, relevant to the results, are given on a generic scale for EC₅₀ (Oddo, 1998; Persoone, 1999; Lapa et al., 2002). If EC₅₀ cannot be calculated, the effect (% of mortality or inhibition) and the IC index (% of growth inhibition) was used to express the results of the

tests. 20% is the threshold, above which it is possible to consider the effect of that matrix toxic. We did not consider other values of EC_{xx}, because moving away from the central value of the dose-effect curve, the statistical reliability deteriorates (Oddo, 1998). Phenomena like *hormesis*, i.e. a high increase in metabolic level in the examined animals, which is particularly true for the *V. fischeri*, could be considered an attempt of the animals to free themselves from the stress (Oddo, 2001); as regards the inhibition, 20% would be the threshold, after which we consider the hormesis effect probable.

3. Results and discussion

Tables 1 to 3 report results obtained from toxicity bioassays applied respectively to marine sediment samples (elutriates) and freshwater samples.

As a comment to Table 1, first of all, we have to consider results of reference and spiked sediments; both of them are collected and sampled in the same gulf, but show us two good examples: a non-contaminated site and a polluted one. In the non-contaminated site, the whole set of tests shows a signal comparable with dilution standard water response; in the second case (polluted sediment, probably due to an oil spill or tank cleaning), we have a high toxicity signal with biomarker tests (bio-luminescence and glycemia) and medium toxicity considering

Table 1 - Results of toxicity bioassay testing marine sediment samples.

EC₅₀ = concentration expected to effect 50% of the test population; LC₅₀ = concentration expected to kill 50% of the test population; ΔG = Increase Glycemia; n.c. = not calculable; TQ = sample not diluted; Endpoints: *V. fischeri* EC₅₀ after 30 min; *B. plicatilis* LC₅₀ after 48 h; *A. franciscana*, LC₅₀ after 24 h. *P. elegans* Increase Glycemia during 24 h. Effect is significant if ≥ 20%; For *V. fischeri* negative value indicates biostimulation.

Ecotoxicological test → Sampling site ↓	<i>V. fischeri</i> EC ₅₀ 30 min Average of 5 replicates	<i>B. plicatilis</i> LC ₅₀ 48 h Average of 2 replicates	<i>A. franciscana</i> LC ₅₀ 24 h Average of 2 replicates	<i>P. elegans</i> ΔG 24 h Average of 3 replicates
1.2.o - Reference sediment out of the Marine Reserve 1 sample	Effect in TQ = 5% LC ₅₀ = n.c. Not toxic	Effect in TQ < 10% LC ₅₀ = n.c. Not toxic	Effect in TQ < 10% LC ₅₀ = n.c. Not toxic	
1.1.a In the centre of the Marine Reserve 2 samples	Effect in TQ = 16% EC ₅₀ = n.c. Not toxic	Effect in TQ < 20% LC ₅₀ = n.c. Not toxic	Effect in TQ < 20% LC ₅₀ = n.c. Not toxic	
1.1.b - Entrance part of the port 2 samples	Effect in TQ = 18% EC ₅₀ = n.c. Not toxic	Effect in TQ < 20% LC ₅₀ = n.c. Not toxic	Effect in TQ < 20% LC ₅₀ = n.c. Not toxic	
1.1.c - Central part of the port 2 samples	Effect in TQ = -12% EC ₅₀ = n.c. Bio-stimulation	Effect in TQ = 20% LC ₅₀ = n.c. Toxic signal		ΔG = 0,5 Not toxic
1.1.d - Internal part of the port 2 samples	Effect in TQ = -12% EC ₅₀ = n.c. Bio-stimulation	Effect in TQ = 23% LC ₅₀ = >> 100% Toxic signal		ΔG = 0,5 Not toxic
1.2.p - Spiked	Effect in TQ = 100%	Effect in TQ = 23%		ΔG = 1

lethality as the endpoint. About survey samples, in Table 1, it is evident that acute toxicity signals (more than 20%) detected by *B. plicatilis* regard only the sampling sites inside the small port of Grignano (1.1.c and 1.1.d). *V. fischeri* shows inhibition signals (not yet affected) with sediment collected out of the port entrance and biostimulation with sediment inside the area. Increasing luminescence would be a possible hormesis due to the same contamination level, detected with rotifers. *P. elegans*, on the contrary, does not show signals of toxicity. Since sensitivity of the biomarker used towards heavy metals has been tested (Lorenzon, 2000), an explanation for the results obtained could be the scarce heavy metal bioavailability in undiluted sediment, supported also by results obtained by means of leaching test with CO₂ saturated water (all heavy metal concentrations under 0.280 mg/l – data not reported). However, chemical analyses of bulk sediment (US-EPA, 1998) reveal increasing concentrations of heavy metals in the samples coming from the most internal part of the port, particularly as far as copper, lead, zinc, chromium and mercury (data not reported) are concerned. As regards bioaccumulation, size, weight (average of 28 g) and esteemed age of animals were comparable. Table 2 shows a tendency for concentration (dry weight) of PAHs (Polycyclic Aromatic Hydrocarbons) in mussel tissues. It was possible to detect increasing concentration (from the outside to the inside of the port) of Naphthalene, Phenanthrene, Phluoranthene, Benzo(b) phluoranthene and Pyrene. Mussels, which stay within the tide zone, can collect hydrocarbon film or pyrolysis products lying on the water surface. It is clear that toxicant availability outside the small port is similar, but its concentration increases vertically within this area. No significant concentration of PCBs (Polychlorinated biphenyls), TBT (Tributyltin) and Heavy Metals was found in mussel tissues (data not reported).

Table 3 considers the fresh water bodies (waste waters and surface waters) flowing into the Reserve. The Microtox[®] test shows that waste waters, in periods of low density of residents (winter), might not be acceptable, having an evident hormetic effect. Although *D. magna* and

Table 2 - Concentrations of PAH (Polycyclic Aromatic Hydrocarbons) in mussel tissues. There was no lipid normalisation. Concentration expressed as µg/kg dry weight.

Chemical compound → Sampling site ↓	Naphthalene	Phluorene	Phenanthrene	Phluoranthene	Benzo(b) phluoranthene	Pyrene
1.1.a - In the centre of the Reserve 1 sample	0,12	1,75	2,42	2,71	0,16	0,81
1.1.b Entrance part of the port 1 sample	0,19	1,83	2,41	6,01	< 0,01	2,48
1.1.c - Central part of the port 1 sample	11,05	5,21	13,56	14,63	7,99	14,76
Chemical compound → Sampling site ↓	Benzo (a) pyrene	Anthracene	Benzo anthracene	Dibenzo anthracene	Chrysene	
1.1.a - In the centre of the Reserve 1 sample	0,06	0,36	0,59	< 0,01	1,82	
1.1.b - Entrance part of the port 1 sample	0,33	0,14	1,33	0,03	3,18	
1.1.c - Central part of the port 1 sample	4,04	1,88	2,14	0,72	6,84	

Table 3 - Results of toxicity bioassay testing freshwater samples.

EC₅₀ = concentration expected to effect 50% of the test population; LC₅₀ = concentration expected to kill 50% of the test population; IC = Index of growth inhibition; n.c. = not calculable; TQ = sample not diluted; Endpoints: *V. fischeri* EC₅₀ after 30 min; *D. magna* EC₅₀ after 48h; *T. platyurus*, LC₅₀ after 24h. Effect is significant if ≥ 20%; For *V. fischeri* negative value indicates biostimulation. For *S. capricornutum* negative value indicates eutrophic effect.

Ecotoxicological test ↓	<i>V. fischeri</i> EC ₅₀ 30 min	<i>D. magna</i> EC ₅₀ 24 h	<i>T. platyurus</i> LC ₅₀ 24 h	<i>S. capricornutum</i> (<i>P. subcapitata</i>) IC ₅₀ 72 h
Sampling site	Average of 2 replicates	Average of 2 replicates	Average of 2 replicates	Average of 2 replicates
1.4.y - Waste water near Reserve basin 2 samples	Effect in TQ = -38.44% EC ₅₀ = n.c. Light toxic, Hormesis			
1.4.z - Waste water in port 2 samples	Effect in TQ = -26.27% EC ₅₀ = n.c. Light toxic, Hormesis			
1.4.x - Grignano Creek	Effect in TQ = -12.5% EC ₅₀ = n.c.	Effect in TQ < 20% EC ₅₀ = n.c.	Effect in TQ < 20% EC ₅₀ = n.c.	IC _{72h} in TQ = - 49.44 % EC ₅₀ = n.c.

T. platyurus tests do not show any toxicity effect with surface water samples, *S. capricornutum* bioassay shows a high eutrophic effect (the results reported in Table 3 are calculated as growth inhibition; a negative value indicates that algae population grows more in the sample than in the controls vials). We have the same results both in summer and winter. Even Microtox[®] tests have similar results in both seasons: Grignano Creek water stimulates bio-luminescence and the Sticco Creek water effect is higher, but not enough to stimulate an hormetic effect.

4. Conclusion

A gradient of anthropic impact is well evidenced by some toxicity bioassays and by bioaccumulation investigation using mussels (PAHs): it increases in a directly proportional way from the outside to the most internal point of the port (external = more natural framework; internal = more contaminated framework).

Surface freshwaters (Sticco and Grignano Creeks) are highly eutrophic for algae and this is probably due to the presence of nutrients, perhaps related to the presence upstream of many little agricultural lands. Besides the Sticco Creek collects wastewaters of dwelling houses and guesthouses (more than the Grignano Creek does), explaining the high bacteria biostimulation observed. On the other hand, waste water samples stimulate the possible hormetic effect, because they mainly come from a guesthouse (waste water near the Reserve basin) or restaurants (waste water in port).

Significant acute toxicity signals were evidenced in samples coming from the internal part

of the port, suggesting possible chronic effects in need of more accurate future investigation. An analysis for the presence of metal-thioneine and for enzyme complexes will be carried out in the future, in order to move from acute toxicity research to chronic environmental sample assessment.

References

- Ahsanullah M, Ying W.; 1995: *Toxic effects of dissolved copper on Penaeus merguensis and Penaeus monodon*. Bull. Environ Contam Toxicol **55**, 81-88.
- American Public Health Association; 1992: In: A.E. Greenberg, L.S. Clesceri and A.D. Eaton (eds). Standard methods for the examination of water and wastewater, 18th edition, Washington, D.C.
- Artoxkit FTM; 1990: *Artemia toxicity screening Test for Estuarine and Marine Waters*. Standard Operational Procedure. Creasel, Deinze, Belgium 22 pp.
- ASTM; 1990: *Standard guide for conducting static 96 h toxicity tests with microalgae*. In: American Society for Testing and Materials, Annual Book of ASTM Standards, E 1218-90, Philadelphia, PA, vol. 11.05, pp. 874-885.
- ASTM; 1991: *Standard guide for acute toxicity test with the Rotifer Brachionus*. In: American Society for Testing and Materials, Annual Book of ASTM Standards, E 1440-91, Philadelphia, PA, vol. 11.04, pp. 1210-1216.
- ASTM; 1994: *Standard guide for collection, storage, characterization and manipulation of sediments for toxicological testing*. In: American Society for Testing and Materials, Annual Book of ASTM Standards, E 1391-94, Philadelphia, PA, vol. 11.05, pp. 805-825.
- Centeno M.D., Persoone G. and Snell T.; 1995: *Cyst-based toxicity tests: IX – The potential of Thamnocephalus platyurus as test species in comparison with Streptocephalus proboscideus (Crustacea, Branchiopoda, Anostraca)* Environ. Toxicol. Water Qual. **10**, 275-282.
- Covelli S., Faganel J., Hovat M. and Brambati A.; 1999: *Mercury contamination of costal sediment as a result of a long cinnabar mining activity (Gulf of Trieste, northern Adriatic sea)*. Applied geochemistry, **16**, 541-558.
- Daphtoxkit F; 1996: *Crustacean toxicity screening test for freshwater. Standard operational procedure*. Craesel, Deinze, Belgium. 16 pp.
- Faganel J., Planic R., Pezdic J., Smodis B., Stegnar P. and Ogorelec B.; 1991: *Marine geology of the gulf of Trieste (northern Adriatic): geochemical aspects*. Marine Geology **99**, 93-108.
- Francese M. and Traldi D.; 2001: *Impiego di cisti di Brachionus plicatilis per saggi ecotossicologici in ambiente marino*. Bio. Mar. Medit., **8**, 103-109.
- Gentile J.H., Johns D.M., Cardin J.A. and Heltshe J.F.; 1984: *Marine ecotoxicology testing with crustaceans*. In: G. Persoone, E. Jaspers and C. Claus (eds), Ecotoxicology testing for marine environment. State Univ. of Ghent and Inst. Mar. Sci. Res., Bredene, Belgium, vol. **1**, pp. 479-502.
- ICRAM; 2000: *Qualità degli ambienti marini costieri italiani 1996-1999*. Ministero dell'ambiente - Servizio difesa Mare. pp. 111-124.
- IRSA-CNR; 1984: *Metodi analitici per fanghi*. Quaderno 64, Appendice 2/B test di cessione con acqua satura di CO₂.
- Janssen C.R. and Persoone G.; 1992: *Routine aquatic toxicity testing: some problems and new approaches*. In: S. Bonotto, R. Nobili and R.P. Revoltella (eds), Biological indicators for environmental monitoring. Serozo Symposia Review Rome, pp. 195-205.
- Johnson B.T.; 1998: *Microtox[®] Toxicity test System: new developments and applications*. In: P.G. Wells, K. Lee and C. Blaise (eds), Microscale testing in aquatic toxicology, CRC Press, Washington, D.C., pp. 201-218.
- Lapa N., Santos Oliveira J.F., Camacho S.L. and Circeo L.J.; 2002: *An ecotoxic risk assessment of residue materials*

- produced by the plasma pyrolysis/vitrification (PP/V) process. *Waste Management* **22**, 335-342.
- Lobel P.B., Bajdik C.D., Belkhole S.P., Jackson S.E. and Longerich H.P.; 1991: *Improved protocol for collecting Mussel watch specimens taking into account sex, size, condition, shell shape, and chronological age*. *Archives of Environmental Contamination and Toxicology*, **21**, 409-411.
- Lorenzon S., Ferrero E.A. and Francese M.; 2000/a: *Valutazione della qualità dei sedimenti delle Foci dell'Isonzo (Baia di Panzano) mediante analisi chimiche e saggi biologici*. *Hydrores annuario*, 2000.
- Lorenzon S., Francese M. and Ferrero E.A.; 2000/b: *Heavy metal toxicity and differential effects on the hyperglycemic stress response in the shrimp *Palaemon elegans**. *Arch. Environ Contam and Toxicol* **39**, 167-176.
- Microbics Corporation; 1992: *Microtox[®] manual*. Carlsbad, CA Vol. 1-5, 2008-8883.
- Migliore L., de Nicola Giudici M.; 1990: *Toxicity of heavy metal to *Asellus aquaticus* (L.) (Crustacea, Isopoda)*. *Hydrobiologia* **203**, 155-164.
- Oddo N.; 1998: *Una scala di tossicità*. *AcquaAria*, maggio 1998, pp. 85-88.
- Oddo N.; 2001: *Criteri di interpretazione di un test di tossicità*. *AcquaAria Biologi Italiani*, **2**, 45-51.
- Pellegrini D., Virno Lamberti C., Regoli F. and Focardi S.; 2001: *Indicazioni sull'utilizzo dei molluschi bivalvi nel programma di monitoraggio dell'ambiente costiero (protocollo Mussel Watch)*. In: Lo Studio Editoriale (ed), *Metodologie analitiche di riferimento. Programma di monitoraggio per il controllo dell'ambiente marino costiero (triennio 2001-2003) 2001*. Quaderno ICRAM-Ministero Ambiente.
- Persoone G.; 1998: *Development and first validation of a 'stock-culture free' algal microbiotest: the algaltokit*. In: P.G. Wells, K. Lee and C. Blaise (eds), *Microscale aquatic toxicology, advances, techniques and practice*, pp. 311-320.
- Persoone G.; 1999: *Pollution classification system*. Not published. 6.
- Persoone G. and Janssen C.R.; 1993: *Freshwater invertebrate toxicity test*. In: P. Calow (ed), *Handbook of ecotoxicology*, vol. 1, pp. 51-65.
- Persoone G., Colin R.J. and De Coen W.; 2000: *Proceedings of the International Symposium on New Microbiotests for Routine Toxicity Screening and Biomonitoring*, June 1-3 1998, Brno, Czech Republic, Ed. Kluwer Academic.
- Persoone G., Van de Vel A., Van Steertegem M. and De Nayer B.; 1989: *Predictive value of laboratory test with aquatic invertebrates: influence of experimental conditions*. *Aquatic toxicology* **14**, 149-167.
- Regoli F. and Orlando E.; 1994: *Accumulation and subcellular-distribution of metals (Cu, Fe, Mn, Pb and Zn) in the Mediterranean mussel *Mytilus galloprovincialis* during a field transplant experiment*. *Marine Pollution Bulletin* **28**, 592-600.
- Ruck J.G., Martin M. and Mabon M.; 2000: *Evaluation of toxkits as methods for monitoring water quality in New Zeland*. In: G. Persoone, C. Janssen and W. De Coen (eds), *new microbiotest for routine toxicity screening and biomonitoring*, Kluwer Academic/Plenum Publishers, New York, pp. 103-119.
- Stravisi F., Bussani A. and Odorico R.; 2000: *Andamento delle temperature nel Golfo di Trieste 1997-1999*. *Hydrores Information. Pesca Maricoltura Ambiente*. **17**, 3-63.
- Stroscher M.T.; 1984: *A comparison of biological testing methods in association with chemical analysis to evaluate toxicity of Waste Drilling Fluids in Alberta*. Canadian Petroleum Association Alberta, Calgary, vol. 1.
- Thamnotokit FTM; 1995: *Crustacean toxicity screening test for freshwaters*. Standard Operational Procedure. Creasel, Deinze, Belgium, 23 pp.
- U.S.EPA; 1996: *Method 3052 microwave assisted acid digestion of siliceous and organically based matrices*. In: *Test for evaluating solid waste*, 3rd edition, Washington, D.C.
- U.S.EPA; 1998: *Method 6020/A inductively coupled plasma-mass spectrometry*. In: *Test methods for evaluating solid waste: physical/chemical methods*, 3rd edition, Washington, D.C.

- U.S.EPA; 2000: *Guidance for choosing a sampling design for environmental data collection*. Office of environmental information, U.S.EPA, Washington, D.C., EPA QA/G-5S.
- U.S.EPA - USACE; 1998/1: *Evaluation of dredged material proposed for ocean disposal. (Testing manual)*. US Environmental protection agency and us Army corps of Engineers, EPA 823-B-98-004, 571 pp.
- U.S.EPA - USACE; 1998/2: *Great lakes dredged material testing and evaluation manual. Appendix F*. US Environmental protection agency and us Army corps of Engineers, EPA Final draft Version 09/30/98, 564 pp.
- U.S.EPA; 1993: *Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms*. Weber C.I., Ecological monitoring research division. EPA 600-4-90-027F, 273 pp.
- UNI EN ISO; 1999: *Determinazione dell'inibizione della mobilità della Daphnia magna Straus (Cladocera, Crustacea)*. Prova di tossicità acuta UNI ISO EN 6341/99.
- Van Steertegem M. and Persoone G.; 1993: *Cyst-based tests: V. Development and critical evaluation of standardized toxicity tests with the brine shrimp Artemia (Anostraca, Crustaceae)*. In: A.M. Soares and P. Callow (eds). Progress in Standardization of aquatic toxicity tests. pp. 81-97.
- Vanhhaecke P. and Persoone G.; 1984: *The Arc-test: A standardized short-term routine toxicity test with Artemia naupli. Methodology and evaluation*. In: G. Persoone, E. Jasper and C. Claus (eds). Ecotoxicological testing for the Marine Environment. State University of Ghent and Institute Marine Scientific Research Belgium; **2**, pp. 143-151.
- Vinzi E. and Bussani A.; 2000: *Risultati del monitoraggio delle caratteristiche termoaline in una stazione presso la Riserva Marina di Miramare (Trieste) nel periodo 1997-2000*. Hydrores information. Pesca Maricoltura Ambiente. **20**, 85-102.
- Volpi Ghirardini A. and Pellegrini D.; 2001: *I saggi di tossicità nella valutazione della qualità di acque e sedimenti di ambienti marini e di transizione: Indicazioni per la scelta, la messa a punto, la valutazione e l'utilizzo dei metodi*. Biol. Mar. Medit: **8**, 1-16.