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# Heavy Metal Monitoring in Fish, Bivalve Molluscs, Water, and Sediments from Varano Lagoon, Italy

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Lagoons and coastal ponds, environments of transition between fresh water and sea, represent very important ecosystems because of the complexity, high productivity and markedly variety of habitats that is possible to meet in the same basin. The Varano lagoon, located at the south of Italy (Fig.1), is about 10 km long and about 7 km wide. Its area is 60.5 km$^2$ ant it is separated from the Adriatic sea by a slim isle. The maximum depth is about 6 m. The lagoon mixes continually with the Adriatic sea through two inlets: foce Varano and foce Capoiale. The Ramsar Convention of 2 February 1971 and the Environmental Program of the Health World Organization, promoted in the frame of the Barcellona Convention between EEC and some Mediterranean Countries, suggested the introduction of this Lagoon in the list of the humid zones of international interest as well as of the protected natural areas. The Italian Government, with the law n.° 394 of 6/12/1991, declared this area a Protected Natural Area within the "Parco Nazionale del Gargano". Surveys on Varano Lagoon concerned mainly the physical and chemical conditions of the water (De Angelis, 1963, De Angelis, 1964; Marolla, 1988; Tolomio *et al.*, 1990), while lacking are the data on the presence of pollutants such as metals, except for a paper of 1991 (Morini *et al.*, 1992). The present study presents data on the concentrations of heavy metals (Hg, Pb, Cd and Cr) in water, sediments, molluscs bivalve (*Mytilus galloprovincialis, Tapes decussata*) and fish (*Gobius ophiocephalus, Atherina boyeri, Blennius pavo)* from the Varano lagoon. The metal concentrations detected in this study are compared with values from a previous survey in order to asses changes in pollution levels.

## MATERIALS AND METHODS

Samples of different fish (*Gobius ophiocephalus, Atherina boyeri, Blennius pavo*) and bivalve molluscs (*Mytilus galloprovincialis* and *Tapes decussata*) were collected in June 1997 from three sites in the Lagoon of Varano. *Gobius ophiocephalus* and *Blennius pavo* samples, after remark of morphometric data (length and weight) were gathered in pools, each formed of samples of similar weight and length, and from each one were taken muscle and liver. As for *Atherina boyeri* species, a distribution in pools was impossible because of the homogeneity in weight of the samples; more, the little size of samples did not allow the

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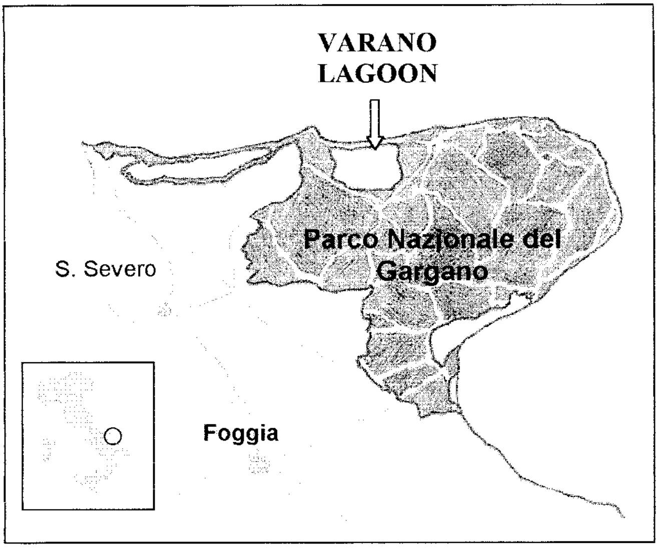


**Figure 1.** Varano Lagoon

separation of liver and the analytical determination of pollutants was done on the whole body. Samples of *Mytilus galloprovincialis* and *Tapes decussata* were analysed in pools of 80 and 40 specimens for each sites, respectively. From each site, molluscs bivalve of the same size were selected to minimise variation in metal concentrations due to individual difference. The soft parts were carefully removed with a plastic knife and homogenised in a mixer to make up the sample from each sampling site. To avoid contamination, the mixer was covered with Teflon for all parts that came into contact with the sample. The blades were of a disposable stainless-steel type. Samples of fish and bivalve molluscs (about 2 gr) were digested into the reaction flask with 11 ml of the mixture $HNO_3$-$HClO_4$ (8:3) for Pb, Cr, Cd (Ciusa and Giaccio, 1984), and with 10 ml of the mixture $H_2SO_4$ - $HNO_3$ (1:1) for Hg (G.U., 1994). Sediment samples to a depth of 2 cm, were collected with a Van Veen type apparatus. After collection sediment samples were air dried for three to four days until to constant weight. Dried aliquots were ground using a mortar and pestle and sieved through a 0.5 mm screen. The samples (about 5 g), in 250 ml boiling flasks, were added with 90 ml of conc. $HNO_3$ and 10 ml conc. HCl. The contents were refluxed on a hot plate for 4 h with vigorous boiling. The acid extracts, centrifuged, filtered and brought to a fixed volume. The seawater samples were collected in 5 l. polypropylene bottles held approximately 10 cm below the surface. Filtered seawater samples were preconcentrated with CHELEX-100 (100-200 mesh) for Pb, Cd, Cr using the method of Sturgeon et al. (1980) (10) and with BAKER-10 SPE$^{TM}$ -Phenyl (3 ml) for Hg. Quantitative analysis of heavy metals were performed by atomic absorption spectrophotometry (Perkin Elmer 5000). For Pb, Cr and Cd determination, a graphite furnace (HGA- 500 Perkin Elmer) was used. Hg was determined by the cold vapour technique after reduction $SnCl_2$ (A.V.A. Thermo Jarrel Ash Corp.). Acid washed glassware, analytical grade reagents and double distilled deionised water were used in the tissue analysis. In order to check on the purity of the chemical used, a number of chemicals blanks were run; there was no evidence of any contamination in these blanks. Accuracy of the analytical procedures was assessed by extractions performed on TORT-1 lobster Hepatopancreas for marine organisms, on CASS-4 for water (National Research Council of Canada), and SRM 1645 (National Bureau of Standards) for sediments. All results for data given here were within 10% of certified values.

## RESULTS AND DISCUSSIONS

Table 1 reports metal residue concentrations in fish, bivalve molluscs, water and sediments. In *G. ophiocephalus* muscle, Pb was detected in 23% of the sample analysed with mean highest levels, followed by Cr found in 66% of the samples, by Hg and Cd. The same trend was observed in muscle of *B. pavo* and in whole body of *A. boyeri*. Comparative analysis of the data related to the presence of metal residues in the muscle of the three species analysed did not show significant differences for Hg, Cd and Cr. On the contrary, Pb is present at higher levels in *G. ophiocephalus* and *A. boyeri* ($p< 0.02$) than in *B. pavo*.

Metal residue determination, was not limited to muscle tissue, but it was extended to liver (only for the species *G. ophiocephalus* and *B. pavo*) because this organ plays an important role in the accumulation, often concentrating some contaminants to higher levels than muscle, in the redistribution and transformation of these pollutants and it is also a site of pathologic effects induced by the presence of toxic products.

In *G. ophiocephalus* liver Pb, encountered in 23.8% of the examined samples, was the element present to highest concentrations, followed by Cr, Cd and Hg, this latter present in 28.6% of the examined samples. A similar pattern was found in liver of *B. pavo* in the following order: Pb>Cr>Hg≅Cd. In the liver of *G. ophiocephalus* e *B. pavo* specimens, Hg and Cr levels were similar, while Cd concentrations were higher in *G. ophiocephalus* than in *B. pavo* ($p < 0.002$). In *G. ophiocephalus* highly significant concentration differences between muscle and liver were detected for all metals (Hg: $p< 0.01$; Cd: $p< 0.0001$; Pb: $p< 0.04$; Cr: $p< 0.005$); in *B. pavo* significant concentration differences between the two considered tissues ($p< 0.001$) were observed only for Pb.

The analysis of the data related to *M. galloprovincialis* specimens showed higher Cd concentrations, followed by Cr, Pb present in 85% of the analysed samples and by Hg. The similar trend was observed in *T. decussata* with highest values for Cd and Cr, followed by Pb present in 54.6% of the examined samples and Hg. Between the two examined species were observed significant differences only for Cd ($p< 0.007$) which presented higher levels in *M. galloprovincialis* than in *T. decussata*. A comparative assay of the data obtained with those reported in literature showed that Hg and Pb concentrations found in *M. galloprovincialis* were sensibly inferior to those reported for the same species sampled in the waters of the lagoons Varano in 1991 (Morini *et al.*, 1992).

Higher Pb concentrations were detected also in *M. galloprovincialis* from northern Adriatic sea lagoon zones (Sacca di Scardovari) (Pb: 0.43-0.98 mg/kg wet wt.; Hg: 0.17-0.42 mg/kg wet wt) (Crisetig *et al.*, 1984). As for Cd and Cr the values found in the present survey in *M. galloprovincialis* were markedly higher than those found in the same species from the same lagoon zone in 1991 (Morini *et al.*,

**Table 1**. Range, mean±standard deviation in samples of fish, molluscs bivalve (mg/kg wet wt.), water (µg/l) and sediments (mg/kg dry wt) and comparison of the present data with the previous results (1991) in the same area.

| Samples | | Hg | Pb | Cd | Cr |
|---|---|---|---|---|---|
| *G. ophiocephalus* | Muscle | 0.03-0.14<br>0.07±0.03 | N.D.-0.39<br>0.26±0.10 | 0.01-0.05<br>0.03±0.01 | N.D.-0.32<br>0.10±0.07 |
| | Liver | N.D.-0.14<br>0.11±0.03 | N.D.-1.12<br>0.61±0.30 | 0.06-0.20<br>0.13±0.04 | 0.05-1.05<br>0.32±0.28 |
| *A. boyeri* | Whole body | N.D.-0.08<br>0.06±0.01 | 0.10-0.39<br>0.22±0.14 | 0.02-0.03<br>0.02±0.00 | 0.08-0.32<br>0.16±0.09 |
| *B. pavo* | Muscle | 0.04-0.06<br>0.05±0.01 | 0.06-0.08<br>0.07±0.01 | 0.01-0.02<br>0.02±0.01 | 0.06-0.13<br>0.11±0.04 |
| | Liver | 0.07-0.12<br>0.09±0.03 | 0.28-0.37<br>0.31±0.10 | 0.03-0.08<br>0.05±0.03 | 0.09-0.29<br>0.18±0.10 |
| *M. galloprovincialis* | Whole body | 0.02-0.19<br>0.06±0.04 | ND-0.48<br>0.19±0.09 | 0.15-0.68<br>0.36±0.15 | 0.06-0.71<br>0.27±0.20 |
| ***M. galloprovincialis* (1991)** | **Whole body** | **0.19-0.28<br>0.23** | **0.36-0.60<br>0.48** | **0.09-0.25<br>0.14** | **0.03-0.15<br>0.06** |
| *T. decussata* | Whole body | 0.02-0.06<br>0.04±0.01 | N.D.-0.23<br>0.14±0.06 | 0.15-0.31<br>0.22±0.06 | 0.15-0.31<br>0.22±0.06 |
| Water | ---- | T | T | ND-0.05<br>0.03±0.02 | 0.02-0.05<br>0.03±0.01 |
| **Water (1991)** | ---- | **T** | **T** | **ND-0.05<br>0.03** | **T** |
| Sediments | ---- | 0.03-0.06<br>0.04±0.01 | 0.71-2.22<br>1.60±0.71 | 0.15-0.23<br>0.19±0.03 | 1.21-6.54<br>3.31±2.12 |
| **Sediments (1991)** | ---- | **0.28-0.40<br>0.35** | **4.83-10.87<br>7.98** | **0.22-0.25<br>0.23** | **3.60-6.37<br>5.04** |

1992). Cd comparable concentrations were found in *M. galloprovincialis* from the Sacca di Scardovari (0.22-0.53 mg/kg wet wt.) (Crisetig *et al.*, 1984). *T. decussata* samples from the Venezia Lagoon showed mean Pb levels (0.13 mg/kg wet wt.) (Campanini *et al.*, 1989) similar to those found by us in the same species.
The comparison between the metal concentrations in molluscs and fish showed Hg and Pb similar levels and Cd and Cr levels markedly higher in molluscs (Cd: $p< 0.001$; Cr: $p< 0.001$). The ability of bivalve molluscs, particularly those of the genus *Mytilus*, to concentrate trace metal in their tissues is well known. Mussels are filter-feeding organisms characterised by low biotransformation capacities. They accumulate the bioavailable fraction of the contaminants present in the water column and the metal content of their tissues reflect generally the profile of the pollution they have been exposed to. In fact the only metals present in water, thought of modest amount, were Cd and Cr, while Hg and Pb were below the instrumental detection limit. Concentrations of the same order of magnitude were found in water samples collected in 1991 (Morini *et al.*, 1992).
As for sediments the highest concentrations were for Cr followed by Pb, Cd and Hg. The comparative analysis of the samples of sediments from Laguna di Varano in 1991 showed similar Cr and Cd concentrations while Hg and Pb the values

found in the present survey were inferior than those found in sediment samples sampled in 1991 (Morini *et al.*, 1992). Because sediments serve as a repository for a large number of environmental contaminants, it is comprehensible that benthic animals may accumulate more toxic compounds, given their close contact with sediment particles and interstitial water for extended periods of their life cycle (Traunspurger and Drews, 1996). Fish selected for this study are benthic eurohaline species which inhabit the lagoons and coastal waters and eats mainly benthic invertebrates. This would imply that fish preys, which are generally sediment-dwelling invertebrates, accumulate contaminants to high concentrations, that are then absorbed by fish predators. In fact fish analysed in the present study, showed Pb and Cr highest levels that were the elements present at highest concentrations also in sediments. However, the presence of pollutants in fish and molluscs bivalve is not only determined by the concentrations of metal in sediments and waters, but also by physiological and biochemical processes within the organisms, that are species dependent (Barron, 1990). From the comparison of the results obtained with those found in the previous survey (Morini *et al.*, 1992), it is possible to conclude that the environment quality appears markedly improved, mainly for what regards the pollution by Hg and Pb. For Cr and Cd the pollution loads in water and sediments have remained similar, while in *M. galloprovincialis*, which is considered one of the best biological markers in defining environment pollution, the levels di of these two elements result considerably higher. This observation suggests the need for an increasing effort in controlling sources of pollution in this area.

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