

# Metals and organochlorine compounds in eel (*Anguilla anguilla*) from the Lesina lagoon, Adriatic Sea (Italy)

M.M. Storelli, G. Barone, R. Garofalo, G.O. Marcotrigiano \*

Pharmacological-Biological Department, Chemistry and Biochemistry Section, Medicine Veterinary Faculty, University of Bari, Strada Prov. le Per Casamassima Km 3, 70010 Valenzano (BA), Italy

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## Abstract

The muscle tissue of eels was analysed for metals (Hg, Cd, Cu and Zn), polychlorinated biphenyls (PCBs) and organochlorine pesticides (DDTs) to ascertain whether the concentrations exceeded the maximum levels fixed by the European Commission. Zinc showed the highest concentrations (mean:  $20.2 \mu\text{g g}^{-1}$  wet wt), followed by copper (mean:  $0.58 \mu\text{g g}^{-1}$  wet wt), mercury (mean:  $0.18 \mu\text{g g}^{-1}$  wet wt) and cadmium (mean:  $0.03 \mu\text{g g}^{-1}$  wet wt). None of the fish samples analysed presented metal concentrations exceeding the proposed limits. Among the organochlorine pesticides, only *p,p'*-DDE and *p,p'*-DDT were found with mean values of 19.2 and  $3.0 \text{ ng g}^{-1}$  wet wt, respectively, while mean concentrations of PCBs were  $94.0 \text{ ng g}^{-1}$  wet wt. With regard to DDT and its metabolites the concentrations were well below the maximum residue limit (MRL), while the mean PCB concentrations, calculated as the sum of the seven “target” congeners indicated by the European Union, exceeded the established limit. From an ecotoxicological point of view, the concentrations of metals and organochlorine compounds reflect a comparatively clean and pollution-free environment. These concentrations may be, thus, considered as useful background levels to which to refer for comparison within the Adriatic Sea.

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**Keywords:** Metals; Eel; PCBs; DDTs

## 1. Introduction

Seafood usually contains residues of toxic trace metals, polychlorinated biphenyls and organochlorine pesticides and it is often considered to be a major source of intake of these contaminants for humans (Falandysz et al., 2004). Reports from the literature suggest that polychlorinated biphenyls, particularly dioxin-like PCBs, have a complex spectrum of toxicological properties, including chloracne, thymic atrophy, liver damage, immunotoxicity and cancers (IARC, 1997; IPCS, 1993), and have, also, been associated with low birth weights and learning and behavioural deficiencies in children of women who consume large quantities of contaminated fish or are occupa-

tionally exposed (Tanabe, Kannan, Subramanian, Watanabe, & Tatsukawa, 1987a). Also metals are responsible for hazardous effects on human health. For example, cadmium and mercury injure the kidneys and cause symptoms of chronic toxicity, including impaired kidney function, poor reproductive capacity, hypertension, tumours and hepatic dysfunction (Luckey & Venugopal, 1977; Waalkes, 2000). Also essential metals, such as copper and zinc can produce toxic effects when their intake is excessively elevated. Excessive intake of copper may lead to liver cirrhosis, dermatitis and neurological disorders, while toxicity due to excessive intake of zinc has been reported to cause electrolyte imbalance, nausea, anaemia and lethargy (Fairweather-Tait, 1988; Prasad, 1984). For these reasons, it is important to determine the chemical quality of seafood, in order to evaluate the possible risks to health.

Lesina lagoon located in the southern Adriatic Sea, internationally known as a breeding area for many migratory

\* Corresponding author. Tel.: +39 080 5443866; fax: +39 080 5443863.  
E-mail address: [g.o.marcotrigiano@veterinaria.uniba.it](mailto:g.o.marcotrigiano@veterinaria.uniba.it) (G.O. Marcotrigiano).

bird species, is a water body where professional fishing is a traditional activity and aquaculture has been developed with greater intensity in recent years. European eel (*Anguilla anguilla*) lives in the brackish water of this lagoon feeding on natural food available in its bottom sediments. Because of its high fat content and benthic feeding behaviour, this species is considered extremely prone to the bioaccumulation of contaminants (Roche, Buet, & Ramade, 2003), especially lipophilic ones (Larsson, 1990). This is a matter of concern from both hygienic and ecotoxicological points of view. These organisms are, in fact, part of the human diet and in addition, because they are employed as bioindicators, can provide useful indications on the state of pollution of the aquatic environment (Falandyz et al., 2004; Mason & Barak, 1990; Pieters, 1991).

In this respect two main objectives are being pursued in this work:

- (a) to determine concentrations, of some metals (Hg, Cd, Cu and Zn) and organochlorine compounds (PCBs and DDTs) in muscle tissue of these organisms in order to ascertain whether the examined fish could be considered suitable for human consumption;
- (b) to compare our results with those from other studies to evaluate the relative significance of the contamination in this lagoon, wetland of international importance.

## 2. Materials and methods

*Anguilla Anguilla* (European eel) specimens (No. 104) were caught from the Lesina lagoon located in the southern Adriatic coast (Apulia-Italy) (Fig. 1). Specimens were grouped into pools according to their size (length 30.1–41.5 cm; weight: 55.0–131.5 g) and from the specimens constituting each pool muscle tissue was taken, homogenised and finally analysed. The analytical methods for metals have been previously described (Storelli, Ceci, Storelli, & Marcotrigiano, 2003a). Briefly, homogenised sub-samples (about 2 g) were digested in a  $\text{HNO}_3\text{--HClO}_4$  mixture for Cd, Cu and Zn determination, and in a  $\text{H}_2\text{SO}_4\text{--HNO}_3$  mixture for Hg. Quantitative determinations of Cd, Cu and Zn were made using an atomic absorption spectrophotometer (Analyst 800 P.E.) equipped with a heated graphite furnace system (THGA-800 P.E.), while Hg was determined by the cold vapour technique after reduction by  $\text{SnCl}_2$  (FIAS-Furnace, P.E.). Precision was checked against standard reference material from National Research Council of Canada (TORT-1 Lobster Hepatopancreas) and was within the range of certified values. Recovery of all metals was over 94%. For PCBs, (IUPAC numbers = 8, 20, 28, 35, 52, 60, 77, 101, 105, 118, 126, 138, 153, 156, 169, 180 and 209) and DDT compounds (DDTs = *p,p'*-DDT, *p,p'*-DDE, *o,p'*-DDT, *p,p'*-DDD and *o,p'*-DDD) the extractive analyt-

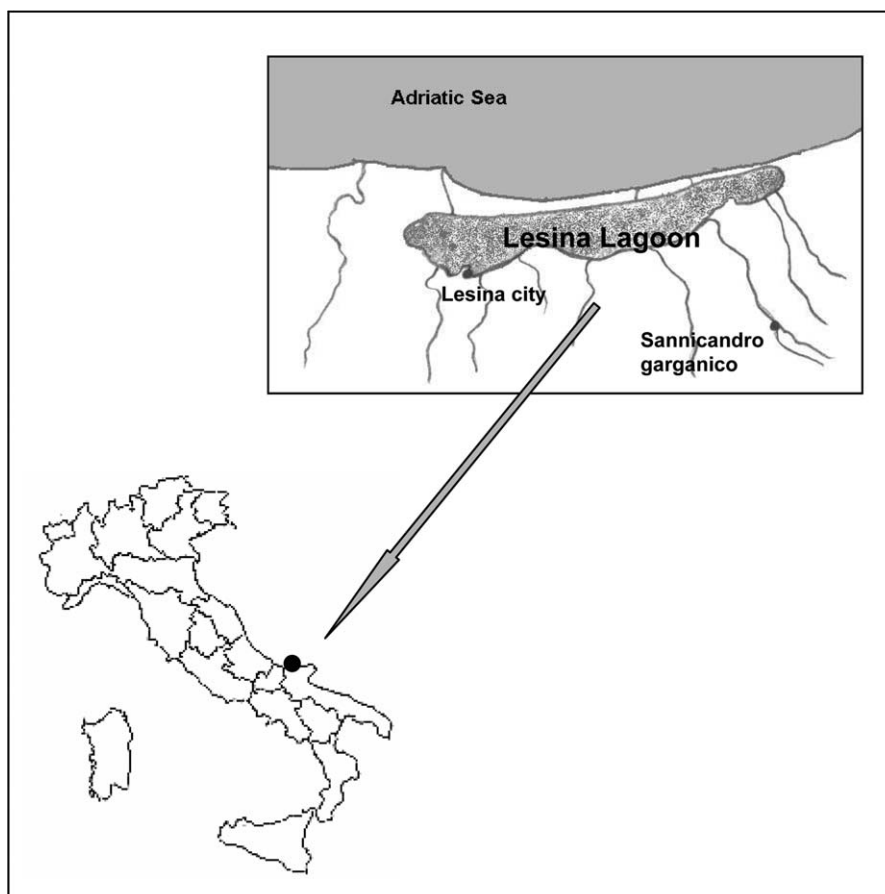


Fig. 1. Location of the sampling site.

ical procedure and the instrumental conditions for identifying the peaks and for quantitative analysis have been described in detail elsewhere (Storelli, Giacomini-Stuffler, Storelli, & Marcotrigiano, 2003b; Storelli, Storelli, & Marcotrigiano, 2004). Briefly, aliquots (2 g) of the homogenised samples were ground with anhydrous sodium sulphate in a mortar. The mixture was extracted with petroleum ether according to Erney's procedure (Erney, 1983). The extracts were then concentrated and subsamples were taken in order to determine the tissue fat content by gravimetry. An aliquot of the remaining extract was dissolved in hexane (5 ml) and mixed with conc.  $\text{H}_2\text{SO}_4$  for the clean up, following the procedure described by Murphy (1972). After centrifugation, the hexane solution was concentrated (about 1 ml) and transferred on a glass column (i.d. 5 mm) filled with 1 g of florisil (activated at 120 °C for 16 h) for the separation of PCBs from other organochlorine compounds. The first fraction eluted with hexane (12 ml), contained PCBs and some DDTs, whereas the second fraction, eluted with 10 ml of 15% ethylether in hexane, contained the remaining DDTs and other organochlorine compounds. For the separation of non-ortho PCB congeners, 3,3', 4,4'-T<sub>4</sub>CB, (IUPAC 77), 3,3',4,4', 5-P<sub>5</sub>CB (IUPAC 126), and 3,3',4,4',5,5'-H<sub>6</sub>CB (IUPAC 169) from other PCBs, the method reported by Tanabe, Kannan, Wakimoto, and Tatsukawa (1987b) was used. The individual PCB congeners were determined against the corresponding individual standards obtained from ULTRA Scientific, Inc. (chemical purity 99%). The reference material employed was CRM 349 for PCBs and CRM 598 for DDTs (cod liver oil). The recovery for each PCB (28, 52, 101, 118, 153, 180 and 138) and DDT (*p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD and *o,p'*-DDD) quantified in the certified material ranged from 91% to 102%. The recoveries for the other PCB congeners and *o,p'*-DDT, varying between 90% and 110%, were determined by adding known amounts of PCB and *o,p'*-DDT standards (at three levels of concentrations) to empty samples before extraction (method of additions). Residues in 100% of the samples were confirmed by gas–liquid chromatography–mass spectrometry (Fisons MD 800). Concentrations of metals and organochlorinated compounds, means of duplicate measurements, are presented as  $\mu\text{g g}^{-1}$  and  $\text{ng g}^{-1}$  on a wet weight basis, respectively.

### 3. Results and discussion

The concentrations of the metals analysed in this study are given in Table 1, while organochlorine pesticide and polychlorinated biphenyl concentrations are reported in Table 2. All metals presented detectable levels in the samples analysed. Between essential metals, zinc showed the highest concentrations with values ranging from 17.9 to 24.6  $\mu\text{g g}^{-1}$  (20.20  $\mu\text{g g}^{-1}$  wet wt), whereas copper presented much lower concentrations ranging from 0.39 to 1.13  $\mu\text{g g}^{-1}$  (0.58  $\mu\text{g g}^{-1}$  wet wt). The considerable difference in concentrations between the two metals is not

Table 1

Mercury, cadmium, copper and zinc concentrations ( $\mu\text{g g}^{-1}$  wet wt) in muscle tissue of eel

|    | Minimum | Maximum | Mean | Standard deviation |
|----|---------|---------|------|--------------------|
| Hg | 0.13    | 0.24    | 0.18 | 0.04               |
| Cd | 0.02    | 0.04    | 0.03 | 0.01               |
| Cu | 0.39    | 1.13    | 0.58 | 0.32               |
| Zn | 17.9    | 24.6    | 20.2 | 2.57               |

Table 2

DDTs and PCBs concentrations ( $\text{ng g}^{-1}$  wet wt) in muscle tissue of eel

|                          | Minimum | Maximum | Mean  | Standard deviation |
|--------------------------|---------|---------|-------|--------------------|
| Fat (%)                  | 25.1    | 30.7    | 27.7  | 2.0                |
| <i>p,p'</i> -DDE         | 12.0    | 28.0    | 19.2  | 6.1                |
| <i>p,p'</i> -DDT         | 2.0     | 6.0     | 3.0   | 1.7                |
| $\sum$ DDT               | 14.0    | 34.0    | 22.2  | 7.5                |
| PCB 52                   | 2.0     | 4.0     | 2.8   | 0.8                |
| PCB 60                   | 3.0     | 6.0     | 4.0   | 1.2                |
| PCB 77 <sup>a</sup>      | 2.0     | 4.0     | 3.0   | 1.0                |
| PCB 101                  | 12.0    | 15.0    | 13.8  | 1.3                |
| PCB 105                  | 10.0    | 13.0    | 11.0  | 1.4                |
| PCB 118                  | 12.0    | 16.0    | 14.4  | 1.5                |
| PCB 126 <sup>a</sup>     | 2.0     | 4.0     | 3.0   | 1.0                |
| PCB 138                  | 14.0    | 20.0    | 17.8  | 2.5                |
| PCB 153                  | 14.0    | 21.0    | 18.6  | 2.9                |
| PCB 180                  | 10.0    | 13.0    | 11.6  | 1.1                |
| $\sum$ PCB               | 78.0    | 104.0   | 94.0  | 10.2               |
| Target PCBs <sup>b</sup> | 259.0   | 313.0   | 284.0 | 20.2               |

<sup>a</sup>  $\text{pg g}^{-1}$ .<sup>b</sup> Sum of the PCB 52, PCB 101, PCB 118, PCB 138, PCB 153 and PCB 180 concentrations ( $\text{ng g}^{-1}$  lipid wt).

unique to the species studied here, but it is part of a general picture that suggests that muscle tissue is not considered to be a specific physiological site for copper (Zia & Khan, 1989). Between non-essential metals the highest concentrations were recorded for mercury with values between 0.13 and 0.24  $\mu\text{g g}^{-1}$  (0.18  $\mu\text{g g}^{-1}$  wet wt), while cadmium registered the lowest values between 0.02 and 0.04  $\mu\text{g g}^{-1}$  (0.03  $\mu\text{g g}^{-1}$  wet wt). A comparison with data in the literature showed that results obtained in fish samples in this work were in good agreement with values found in the muscle tissues of eels from the Portuguese coast (Pérez Cid, Boia, Pombo, & Rebelo, 2001), Mediterranean coast of Spain (Bordajandi et al., 2003) and from Belgian river basins (Maes et al., 2005).

Regards to DDT component pattern, only *p,p'*-DDE and *p,p'*-DDT were found with values ranging from 12.0 to 28.0  $\text{ng g}^{-1}$  wet wt (mean: 19.2  $\text{ng g}^{-1}$  wet wt) and 2.0 to 6.0  $\text{ng/g}$  wet wt (3.0  $\text{ng g}^{-1}$  wet wt), respectively. The high proportion of DDE found in the samples analyzed compared to DDT is consistent with a long period, since this compound was banned in Italy. Among PCBs, only PCB 52, PCB 60, PCB 77, PCB 101, PCB 105, PCB 118, PCB 126, PCB 138, PCB 153 and PCB 180 were found in all of the samples analyzed. The concentrations of these congeners were 78.0–104.0  $\text{ng g}^{-1}$  wet wt, with a mean concentration of 94.0  $\text{ng g}^{-1}$  wet wt. Polychlorinated

biphenyl congener profile in eels showed that hexachlorobiphenyls PCB 138 and 153 were predominant accounting for 18.9% and 19.8% of the total PCB concentrations, respectively. The other congeners were in the following order: PCB 118 (15.3%) > PCB 101 (14.7%) > PCB 180 (12.3%) > PCB 105 (11.7%). The PCB bioconcentration in aquatic organisms correlates with the degree of chlorination, the stereochemistry and lipophilicity (Fox, Zauke, & Butte, 1994). Generally, congeners with high chlorination grade are more difficult to metabolise and eliminate than less chlorinated congeners. The data in the fish in question fit this general picture well being low chlorinated congeners (PCB 8, 20, 28, 35) below the detection limit, though the contribution of other low chlorinated PCB, such as PCB 52 and PCB 60, was not small (7.2%). However, in general, bioaccumulation patterns in these organisms were similar to those of other fish from the Adriatic Sea, being that PCB 138, 153 and 180 were the prevailing congeners (Bayarri, Baldassarri, Iacovella, Ferrara, & Di Domenico, 2001; Storelli et al., 2003b). The chlorination pattern of the PCBs is important for the toxicity of the substance. A number of PCB congeners show, in fact, “dioxin-like” toxicity. These PCBs have no or only one chlorine atom at the *ortho*-position. In our case, referring to non-*ortho* substituted PCBs, the congener PCB 169 was below the detection limit in all samples, while PCB 77 and PCB 126, present in all samples, made up together a small percentage of the total PCB residue (0.006%). Regarding mono-*ortho*, PCB 118 and PCB 105 contributed together to the total PCB burden in a consistent percentage (27%), while PCB 156 was absent in all samples.

The PCB levels found in the samples here analyzed were lower than those reported for the same species living in areas with a wide industrial presence, such as the Berlin rivers (1049 ng g<sup>-1</sup> wet wt) (Fromme, Otto, Pilz, & Neugebauer, 1999), and River Po delta (265 ng g<sup>-1</sup> wet wt) (Bressa, Sisti, & Cima, 1997). They were also lower than the levels found in Scottish rivers (82–910 ng g<sup>-1</sup> wet wt) (Weatherley, Davies, & Ellery, 1997) and in moderately polluted areas, such as the river Vanajavesi in Finland (852–1742 ng g<sup>-1</sup> wet wt) (Tulonen & Vuorinen, 1996). Comparable levels were reported by (Bordajandi et al., 2003) for eels caught along the river Turia basin in Spain. Regarding DDTs concentrations, samples analysed in this study presented levels of the same order of magnitude of those reported for eels from Welsh rivers, Delta Po river, and from river Turia basin (Bordajandi et al., 2003; Fromme et al., 1999; Weatherley et al., 1997).

When considering the metal content in marine organisms suitable for human consumption, the most important aspect is their toxicity to humans. In this way, to safeguard public health, concentration standards in seafood for mercury and cadmium have been established in various countries. In Europe, the limit value for total mercury in seafood, proposed by the European Commission (Commission of the European Communities, 2001), is 0.5 µg g<sup>-1</sup> wet weight, increased to 1.0 µg g<sup>-1</sup> wet weight for the edible

parts of some listed species, which for physiological and ecological reasons, concentrate mercury more easily than others. Generally the species listed are either high trophic level predators, such as tuna and swordfish, or species that have a close relationship with sediment. Eel, typically a benthic organism, is included in this list and consequently total mercury concentration should not exceed 1.0 µg g<sup>-1</sup> wet weight. Also for cadmium the European Commission (2001) has proposed a limit value in the edible part of these fish of 0.1 µg g<sup>-1</sup> wet weight. In this context, none of the fish samples analysed presented concentrations exceeding the proposed limits by the European Directive, moreover they were far below the permissible limits for human consumption. With regards to copper and zinc, the European Community has not established guidelines on acceptable levels in the edible parts of fish. However, comparisons with the Canadian food standards (Cu: 100 µg g<sup>-1</sup>; Zn: 100 µg g<sup>-1</sup>), Hungarian standards (Cu: 60 µg g<sup>-1</sup>; Zn: 80 µg g<sup>-1</sup>) and Australian accepted limits (Cu: 10 µg g<sup>-1</sup>; Zn: 150 µg g<sup>-1</sup>), demonstrate that the content of these metals in the edible part of the examined fish is lower than the guidelines above mentioned.

With regard to DDT and its metabolites, a maximum residue limit (MRL) for fish is not yet established, while in some food products of animal origin (meat, milk and eggs) the European Union (UE) has recommended a tolerance limit of 1000 ng g<sup>-1</sup> on lipid basis, relative to the sum of *p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD and *o,p'*-DDD (Decreto Ministeriale 19/05/2000). The sum of *p,p'*-DDE and *p,p'*-DDT concentrations in fish here analysed (80 ng g<sup>-1</sup> on lipid basis) were well below this value. Also for PCBs the European Union has set, with the directive 1999/788/CE, a maximum content of 200 ng g<sup>-1</sup> lipid basis, calculated as the sum of the concentrations of the seven “target” congeners (PCB 28, PCB 52, PCB 101, PCB 118, PCB 138, PCB 153 and PCB 180) in meat, poultry and derived products (Commission of the European Communities, 1999), but not in fish. In our case, as shown in Table 2, the mean PCB concentrations expressed on lipid basis and as the sum of the seven “target” congeners indicated by the EU, exceeded the above-mentioned limit.

The results obtained in this work allow us to conclude that the concentration levels of the metals appear to be below the permissible limits for human consumption, and therefore give no indication of important health risks associated with consumption of these fish. Also DDT concentrations are very far from the maximum residue limit established by legislation, and consequently, for what concerns possible effects of the studied organochlorine pesticides, the consumption of these fish by humans should be perfectly safe. Regarding PCBs, levels exceeded the maximum limit of 200 ng g<sup>-1</sup> lipid basis, set by European Union. However, it should be pointed out that being such limit specific for terrestrial edible animals do not allow an appropriate evaluation of the significance of the contamination in seafood. In fact, referring to the only international limit relative to the maximum PCB content in fish

(2  $\mu\text{g g}^{-1}$ ) set by the US FDA (2001), our values are much lower. On this basis the most important problem is, as soon as possible, to set limits for PCBs in seafood, which are the main source of human exposure to these toxic compounds within the European Union (Commission of the European Communities, 2004). The concentrations of the metals and organochlorine compounds are generally amongst the lowest values reported in the literature and reflect a comparatively clean and pollution-free environment. These concentrations may be, thus, considered as useful background levels to which to refer for comparison within the Adriatic Sea.

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