The Open Access Israeli Journal of Aquaculture – Bamidgeh

As from **January 2010** The Israeli Journal of Aquaculture - Bamidgeh (IJA) will be published exclusively as **an on-line Open Access (OA)** quarterly accessible by all AquacultureHub (http://www.aquaculturehub.org) members and registered individuals and institutions. Please visit our website (http://siamb.org.il) for free registration form, further information and instructions.

This transformation from a subscription printed version to an on-line OA journal, aims at supporting the concept that scientific peer-reviewed publications should be made available to all, including those with limited resources. The OA IJA does not enforce author or subscription fees and will endeavor to obtain alternative sources of income to support this policy for as long as possible.

Editor-in-Chief
Dan Mires

Editorial Board

Sheenan Harpaz	**Agricultural Research Organization Beit Dagan, Israel**
Zvi Yaron	**Dept. of Zoology Tel Aviv University Tel Aviv, Israel**
Angelo Colorni	**National Center for Mariculture, IOLR Eilat, Israel**
Rina Chakrabarti	**Aqua Research Lab Dept. of Zoology University of Delhi**
Ingrid Lupatsch	**Swansea University Singleton Park, Swansea, UK**
Jaap van Rijn	**The Hebrew University Faculty of Agriculture Israel**
Spencer Malecha	**Dept. of Human Nutrition, Food and Animal Sciences University of Hawaii**
Daniel Golani	**The Hebrew University of Jerusalem Jerusalem, Israel**
Emilio Tibaldi	**Udine University Udine, Italy**

Copy Editor
Ellen Rosenberg

Published under auspices of
The Society of Israeli Aquaculture and Marine Biotechnology (SIAMB),
University of Hawaii at Manoa Library
and
University of Hawaii Aquaculture Program in association with
AquacultureHub
http://www.aquaculturehub.org

ISSN 0792 - 156X

© Israeli Journal of Aquaculture - BAMIGDEH.

PUBLISHER:
Israeli Journal of Aquaculture - BAMIGDEH -
Kibbutz Ein Hamifratz, Mobile Post 25210,
ISRAEL
Phone: + 972 52 3965809
http://siamb.org.il

The Israeli Journal of Aquaculture – Bamidgeh 60(4), 2008, 253-260

Screening Cage Culture Fish Species for Organic Chlorinated Pesticide and Polychlorinated Biphenyl Residues in Turkey

Feride Koc[1]*, Yasemin Gurel[2], Yusuf Yigit[2], Muhammed Atamanalp[3], Yavuz Kursad Das[4] and Fikrullah Kisa[1]

[1] Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ataturk University, Erzurum 25700, Turkey

[2] Central Veterinary Control and Research Institute Toxicology Laboratory, Ankara 06100, Turkey

[3] Department of Fisheries, Faculty of Agriculture, Ataturk University, Erzurum 25240, Turkey

[4] Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, 19 Mayis University, Samsun 11111, Turkey

(Received 31.3.08, Accepted 1.6.08)

Key words: polychlorinated biphenyles (PCB), organic chlorinated, pesticides, residues

Abstract

Organic chlorinated compounds (aldrin, α-endosulfan, β-endosulfan, 2,4'-DDT, and 4,4'-DDE) and polychlorinated biphenyles (PCB28 and PCB52) were screened in fish culture cages off the Aegean Coast of Turkey. Sea bass (*Dicentrarchus labrax)* and sea bream (*Sparus aurata)* were randomly selected from three coastal areas during June-December 2004. Samples of 114 fish were analyzed by gas chromatography (GC) with an electron capture detector and GC-mass spectrometry (GC-MS). No residues exceeding limits established by the European Union Directive were detected. However, 4,4'-DDE was found in 2.63% of the samples. The amounts of residues in all positive samples were lower than the maximum tolerance limits (0.2-1 mg/kg) accepted by the EU Directive. Contamination levels varied with species, ranging 18-200 ng/g wet weight. Other chemicals were not detected in fish samples.

Introduction

Industrial pollution and common use of organochlorine pesticides (OC) over the last five decades threaten aquaculture due to polychlorinated biphenyl (PCB) residues (Naso et al., 2005; Coelhan et al., 2006). PCB are dangerous pollutants because of their toxicity, stability, high liposolubility, long biological half-life, and consequent high degree of bioaccumulation (Di Muccio et al., 2002). They are persistent in the environment and

---

* Corresponding author. Tel.: +90-442-631-4193, ext: 1007, fax: +90-442-631-4188, e-mail: feridekoc@yahoo.com

tend to accumulate in aquatic ecosystems (Pastor et al., 1996; Sahagun et al., 1997; Loizeoi et al., 2001ab; Antunes and Gil, 2004; Borga et al., 2004; El Nemr and Abd-Allah, 2004).

The pollutants are transferred to fishery units from the surrounding environment or via diets. The lipid content in fish influences the bioaccumulation process (Pastor et al., 1996; Antunes and Gil, 2004; Coelhan et al., 2006). Thus, consumption of contaminated fish could be a serious risk to humans (Naso et al., 2005; Sun et al., 2006).

While many studies deal with contamination of fish with various chemicals in Turkey, few studies deal with OC and PCB residues in the Aegean Sea where there is intensive fish farming. The most commonly cultivated species in the Aegean are sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*), comprising 28% and 22% of Turkey's total fish production, respectively (Dikel, 2005). The Aegean Sea receives extensive discharges from industrial cites and dumped sewage sludge from highly populated nearby cities. The aim of the present study was to determine the levels of some OC pesticides (aldrin, α-endosulfan, β-endosulfan, 2,4'-DDT, 4,4'-DDE) and PCB (PCB28, PCB52) in cultivated sea bass and sea bream from three areas along the Turkish Aegean coast.

Materials and Methods

Biological materials. Sixty-seven sea bass and 47 sea bream were collected from cage farms off the shores of Izmir (northern Aegean), Aydin (mid-Aegean), and Mugla (southern Aegean) during June-December 2004 (Table 1). The mean weight and length of the sea bass were 276.2 g and 29.4 cm and of the sea bream 318.5 g and 18.7 cm. The samples were stored in deep-freeze (-70°C) for laboratory analyses conducted in 2004 and 2005.

Standard solutions. Standards for OC (aldrin, α-endosulfan, β-endosulfan 2,4'-DDT and 4,4'-DDE) and PCB (PCB28 and PCB52) were obtained from Dr. Ehrenstorfer GmbH (Referans Chemical, Ankara). Six replicates of each calibration standard (at concentrations of 0.1, 0.5, 1, 2, and 4 µg/ml for each substance) were prepared from a reference standard solution (10 ng/µl).

Chromatographic conditions. Qualitative and quantitative data were generated from gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). Analyses were performed in a Hewlett Packard 6890 N gas chromatograph with an electron capture detector (ECD). Separation was performed on a HP-5 capillary column (HP code 19091J-413 HP-5; 5% phenyl methyl siloxane, 30 m x 0.32 mm I.D.). Chromatographic conditions were prepared as outlined by Di Muccio et al. (2002). The column temperature program was 60°C for 2 min, an increase of 10°C/min up to 160°C, and a further increase of 2°C/min up to 250°C at which it was held for 10 min. The carrier gas was helium, supplied at a constant flowrate of 1.5 ml/min. The temperature of the detector was 300°C.

Table 1. Number of samples from cage farms off the Izmir (northern Aegean), Aydin (mid-Aegean), and Mugla (southern Aegean) coasts of Turkey

	Sample collection (2004)						*Species*	
Region	*Jun*	*Jul*	*Aug*	*Sep*	*Oct*	*Dec*	*Sea bass*	*Sea bream*
Northern	5	5	5	4	5	4	16	12
Mid	2	2	3	2	3	3	6	9
Southern	12	10	10	14	12	13	45	26
Total	19	17	18	20	20	20	67	47

Sample preparation. Samples were processed as outlined by Di Muccio et al. (2002). Twenty-five gram samples of homogenized fish were transferred to a 250-ml centrifuge tube, then 100 ml light petroleum-acetone (1/1, v/v) was added. The mixture was homogenized for 2 min, 100 ml of a sodium chloride solution (5%) was added, and the mixture was centrifuged for 15 min at 1500 rpm. The organic phase was transferred to a column of anhydrous sodium sulfate (25 g in a glass tube, 200 x 20 mm I.D.). The solvent was collected into a 250-ml Erlenmeyer flask. Extraction was repeated with 2 x 50-ml portions of light petroleum. The combined solvent phases were concentrated to a small volume (about 1 ml) in a rotary evaporator and dried by manually rotating the flask (bath temperature of 40°C with reduced pressure). After dissolving the residue with 1 ml *n*-hexane, the eluate was transferred to an Extrelut-NT 3 cartridge (Merck, cat. no. 115095). The *n*-hexane solution was allowed to drain into the cartridge and sit for 10 min. The solvent was removed by passing a stream of nitrogen through the cartridge at 0.5 l /min for 30 min, from the bottom to the top.

After the above procedure, about 1 cm filling material remained in the Extrelut-NT 1 cartridge (Merck, cat. no. 115094), located under the Extrelut-NT 3. C18 material (0.36 g) was added and the cartridge was filled with filling material. The eluate was obtained with acetonitrile (4 x 5 ml) in an Erlenmeyer flask and evaporated at a bath temperature of 40°C under reduced pressure. The dissolved extract (in 1 ml *n*-hexane) was transferred to a glass column filled with activated florisil and anhydrous sodium sulfate. The column was washed with *n*-hexane.

The eluate was obtained at three fractions. DDE, DDD, and PCB were collected from the column with 30 ml *n*-hexane (6 x 5 ml) in the first fraction. DDT was collected with 25 ml *n*-hexane/toluene (v/v, 80/20, 5 x 5 ml) in the second fraction. Aldrin and endosulfan were collected with 30 ml *n*-hexane/toluene/ethyl-acetate (v/v/v, 180/19/1, 6 x 5 ml) in the third fraction. All the extracts were mixed together and 1 ml isooctane was added to the extract as a protective. The extract was evaporated by an evaporatory (reduced pressure and bath temperature of 50°C). The extract (1 µl injection volume) was injected into GC-MS with an ECD.

Validation. To validate our methods, the following parameters were determined: limit of detection (LOD), limit of quantification (LOQ), linearity, recovery, precision, and accuracy.

LOD (ng/g) was calculated on the basis of a signal-to-noise ratio of 3 by spiking the fish samples at low concentrations. This ratio in LOQ calculations was roughly 10. For linearity (correlation of coefficients; r^2**)**, a five-level (0.1-4 µg/ml) calibration series with three analyses at each concentration level were determined and a calibration curve was automatically calculated. Recovery (%) was calculated as the percentage using the extraction process after spiking (fortifying) by adding 50, 100, or 200 ng/g OC or PCB (n = 6) on a HP-5 capillary column. Precision (relative standard deviations; RSD; %) and accuracy (relative standard errors; %) were determined on spiked pesticide-free fish samples at three concentrations with a calibration curve (n = 6). All samples were freshly prepared from standard solutions (1 ng/µl). Accuracy was calculated as (concentration measured - concentration known)/concentration known x 100.

Chromatograms of blank fish samples and OC and PCB mix standards (1 ng/µl) on a HP-5 capillary column are shown in Fig. 1. Retention times of OC pesticides were 25.73, 31.08, 33.43, 35.49, and 36.76 min for aldrin, α-endosulfan, 4,4'-DDE, β-endosulfan and 2,4'-DDT, respectively. Retention times for PCB28 and PCB52 were 22.93 and 24.94 min, respectively. Calibration graphs were established within the range of 0.1-4 µg/ml.

Results

Among the organochlorinated compounds, only 4,4'-DDE was detected in the present study and it was detected in both fish species (Table 2). Of 67 sea bass, only two (both from the southern area) contained 4,4'-DDE. The 4,4'-DDE levels in these two samples were 18 and 25 µg/kg, below the maximum residue limit (MRL) of the European Union Directive

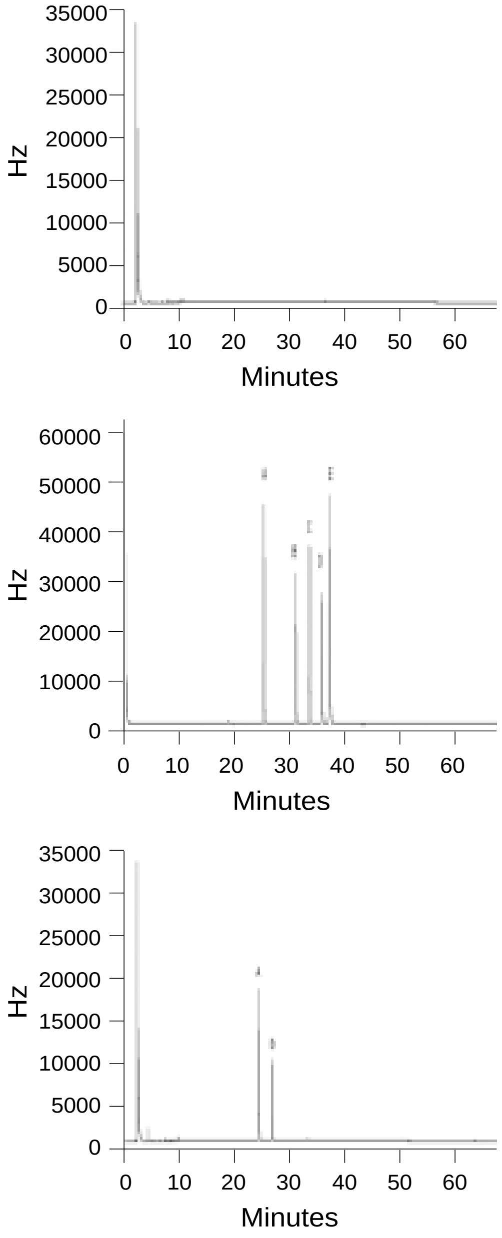

Fig. 1. Chromatograms of (a) blank fish samples, (b) organic chlorinated compound (OC) standards (A = aldrin peak, B = α-endosulfan peak, C = 4,4'-DDE peak, D = β-endosulfan peak, E = 2,4'-DDT peak), and (c) polychlorinated biphenyle (PCB) standards (A = PCB28 peak, B = PCB52 peak).

(1986). Of 47 sea bream, only one sample (also from the southern area) had 4,4'-DDE residue and it contained 200 µg/kg, also below the MRL. Recovery was higher for OC than PCB. Chromatograms of spiked samples (100 ng/g) for OC and PCB are shown in Fig. 2. The results of OC pesticides and PCB contaminations are shown in Table 3. Analyses of the three fish containing 4,4'-DDE residues are given in Fig. 3.

Table 2. Mean residue levels (ppb) of organic chlorinated compounds (OC) and polychlorinated biphenyles (PCB) in fish cultured in cages in the Aegean Sea off the coast of Turkey in 2004.

Compound	*Sea bass*			*Sea bream*		
	Northern	*Mid*	*Southern*	*Northern*	*Mid*	*Southern*
	n = 16	*n = 6*	*n = 45*	*n = 12*	*n = 9*	*n = 26*
OC						
Aldrin	*	*	*	*	*	*
α-endosulfan	*	*	*	*	*	*
β-endosulfan	*	*	*	*	*	*
2,4'-DDT	*	*	*	*	*	*
4,4'-DDE	*	*	21.5	*	*	200
PCB						
PCB28	**	**	**	**	**	**
PCB52	**	**	**	**	**	**

* Detection <0.5 ppb ** Detection <2.5 ppb

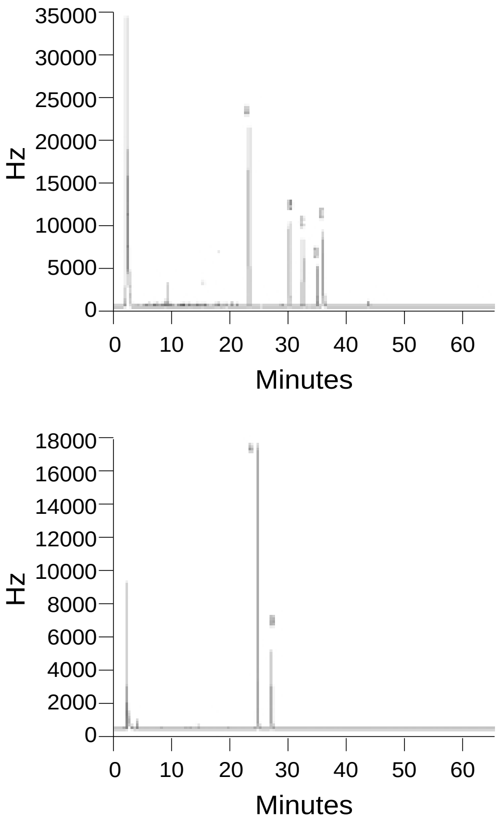

Fig. 2. Chromatograms of spiked samples (100 ng/g) of (a) organic chlorinated compounds (OC; A = aldrin peak, B = α-endosulfan peak, C = 4,4'-DDE peak, D = β-endosulfan peak, E = 2,4'-DDT peak) and (b) polychlorinated biphenyles (PCB; A = PCB28 peak, B = PCB52 peak) on HP-5 capillary columns.

Table 3. Limit of detection (LOD), limit of quantification (LOQ), correlation, recovery, relative standard deviations (RSD), and accuracy (n = 6).

Compound	*LOD (ng/g)*	*LOQ (ng/g)*	*Correlation (r^2)*	*Recovery (%)*	*RSD (%)*	*Accuracy (%)*
OC						
Aldrin	0.5	2.0	0.9998	80.85	7.28	- 19.15
α-endosulfan	0.5	2.0	0.9999	77.12	5.88	- 12.88
β-endosulfan	0.5	2.0	0.9999	79.15	6.11	- 19.85
2,4'-DDT	0.5	2.0	0.9965	75.91	9.10	- 24.09
4,4'-DDE	0.5	2.0	0.9999	82.17	6.45	- 17.83
PCB						
PCB28	2.5	9.0	0.9998	72.35	8.23	- 27.65
PCB52	2.5	9.0	0.9997	68.11	7.90	- 31.99

Discussion

In general, the DDE values detected in this study are consistent with previous results. 4,4'-DDE was found in greater concentrations than any other OC or PCB and ranged 18-200 µg/kg fish. In *Mullus barbatus* from the eastern Aegean Sea, 4,4'-DDE ranged 10-18 µg/kg (Kucuksezgin et al., 2001) and in trout from the Kaweah River (California), p,p'-DDE ranged 40.1-65.7 ppb and was higher than the PCB level (Datta et al., 1998). In a few samples of Italian mullet, DDE compounds ranged 2.44-25.90 µg/kg (below the MRL) while no appreciable PCB residues were detected (Licata et al., 2003). DDT was more common than PCB and hexachlorobenzene (HCB) in sharks from the Italian Mediterranean (Storelli and Marcotrigiano, 2001) while DDE levels were higher in mackerel, anchovy, and red mullet, which have high lipid contents (<0.01-19.88 ng/g wet wt), than in other aquatic

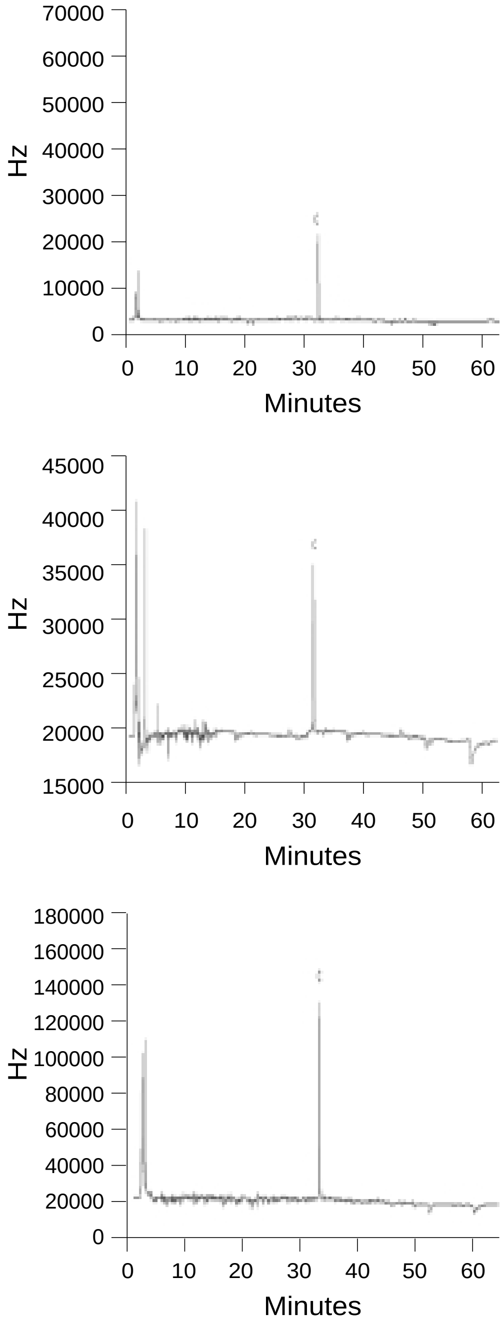

Fig. 3. Chromatograms of fish samples contaminated by 4,4'-DDE: (a) sea bass (18 µg/kg), (b) sea bass (25 µg/kg), and (c) sea bream (200 µg/kg) on a HP-5 capillary column (C = 4,4'-DDE peak).

species from the coast of Italy (Di Muccio et al., 2002). In fish from Egypt, p,p'-DDE dominated all other p,p'-isomers (El Nemr and Abd-Allah, 2004). In salmonids from Lake Tahoe (USA), p,p'-DDE contamination ranged 5-85 ppb and PCB 18-430 ppb (Datta et al., 1999). In fish from the USA/Mexican border area, the most commonly found DDT compound was p,p'-DDE at a high concentration of 10 µg/g wet wt (Mora et al., 2001).

In our study, 3% of the sea bass and 2% of the sea bream were contaminated by 4,4'-DDE, with a higher accumulation in sea bream than in sea bass. This can be attributed to differences in their lipid content, the most important factor in bioaccumulation of OC (Bayarri et al., 2001; Kucuksezgin et al., 2001; Antunes and Gil, 2004). In addition, feeding, habitat, fish size/age, and temperature play a role in pollutant accumulation (Kannan et al., 1995; Pastor et al., 1996; Kucuksezgin et al., 2001; Loizeoi et al., 2001ab; Mora et al., 2001; Pandit et al., 2001; Hoekstra et al., 2003).

In samples from the Marmara Sea, DDT pesticides composed most of organochlorine. Levels in the Marmara were lower than in the Black Sea but higher that in the Aegean and Mediterranean Seas. Thus, inflow from the Black Sea might be the source of pesticide contamination in the Marmara. On the other hand, total PCB concentration was lower than in the Aegean and Mediterranean (Coelhan et al., 2006). In our study, the southern area was more contaminated than the northern and mid areas, indicating that the southern area is still under the influence of OC. Indeed, the main reason for OC contamination can be related to the persistence of OC pesticides that were widely used in agriculture before 1985 in Turkey. The illegal use of some OC pesticides such as DDT continued beyond that year in the Goksu Delta of Turkey (Ayas et al., 1997).

In conclusion, the only OC or PCB detected in samples of sea bass and sea bream from the Aegean coast of Turkey was 4,4'-DDE, and that was detected at a level lower than the maximum residue level determined by the EU Directive. This positive finding for Turkish aquaculture on the Aegean coast may be the result of legislation and enforcement of the ban on use of all OC pesticides and toxic chemicals in 1985.

References

Antunes P. and O. Gil, 2004. PCB and DDT contamination in cultivated and wild sea bass from Ria de Aveiro, Portugal. *Chemosphere*, 54:1503-1507.

Ayas Z., Barlas N.E. and D. Kolankaya, 1997. Determination of organochlorine pesticide residues in various environments and organisms in Goksu Delta, Turkey. *Aquat. Toxicol.*, 39:171-181.

Bayarri B., Baldassarri L.T., Iacovella N., Ferrara F. and A. Di Domenico, 2001. PCDDs, PCDFs, PCBs and DDE in edible marine species from the Adriatic Sea. *Chemosphere*, 43:601-610.

Borga K., Gabrielsen G.W. and J.U. Skaare, 2004. Biomagnification of organochlorines along a Barents Sea food chain. *Environ. Pollut.*, 113:187-198.

Coelhan M., Strohmeier J. and H. Barlas, 2006. Organochlorine levels in edible fish from the Marmara Sea, Turkey. *Environ. Int.*, 32:775-780.

Datta S., Hansen L., McConnell L., Baker J., LeNoir J. and N.J. Seiber, 1998. Pesticides and PCB contaminants in fish and tadpoles from the Kaweah River Basin, California *Bull. Environ. Contam. Toxicol.*, 60:829-836.

Datta S., Ohyama K., Dunlap D.Y. and F. Matsumura, 1999. Evidence for organochlorine contamination in tissues of salmonids in Lake Tahoe. *Ecotox. Environ. Safe.*, 42:94-101.

Dikel S. (ed.), 2005. *Cage Fisheries.* Cukurova Univ., Faculty of Fish. Publ. 18, Adana, Turkey. 212 pp.

Di Muccio A., Stefanelli P., Funari E., Attard Barbini D., Generali T., Pelosi P., Girolimetti S., Amendola G., Vanni F. and S. Di Muccio, 2002. Organochlorine pesticides and polychlorinated biphenyls in 12 edible marine organisms from the Adriatic Sea, Italy, spring 1997. *Food Addit. Contam.*, 9(12):1148-1161.

El Nemr A. and A.M.A. Abd-Allah, 2004. Organochlorine contamination in some marketable fish in Egypt. *Chemosphere,* 54:1401-1406.

European Union Commission Directive, 1986. pp. 43-47. In: 86/363/EEC, OJ, L 221.

Hoekstra P.F., O'Hara T.M., Fisk A.T., Borga K., Solomon K.R. and D.C.G. Muir, 2003. Trophic transfer of persistent organochlorine contaminants (OCs) within an Arctic marine food web from the southern Beaufort-Chukchi Seas. *Environ. Pollut.,* 124:509-522.

Kannan K., Tanabe S. and R., Tatsukawa, 1995. Geographical distribution and accumulation features of organochlorine residues in fish in tropical Asia and Oceania. *Environ. Sci. Technol.,* 29:2673-2683.

Kucuksezgin F., Altay O., Uluturhan E. and A. Kontas, 2001. Trace metal and organochlorine residue levels in red mullet *(Mullus barbatus)* from the eastern Aegean, Turkey. *Water Res.,* 35(9):2327-2332.

Licata P., Di Bella G., Dugo G. and F. Naccari, 2003. Organochlorine pesticides, PCBs and heavy metals in tissues of the mullet *Liza aurata* in lake Ganzirri and Straits of Messina (Sicily, Italy). *Chemosphere,* 52:231-238.

Loizeoi V., Abarnou A. and A. Menesguen, 2001a. A steady-state model of PCB bioaccumulation in the sea bass (*Dicentrarchus labrax*) food web from Seine estuary, France. *Estuaries,* 24:1074-1087.

Loizeoi V., Abarnou A., Cugier P., Jaouen-Madoulet A., Le Guellec A.M. and A. Menesguen, 2001b. A model of PCB bioaccumulation in the sea bass food web from the Seine estuary (eastern English Channel). *Mar. Pollut. Bull.,* 3:242-255.

Mora M.A., Papoulias D., Nava I. and D.R. Buckler, 2001. A comparative assessment of contaminants in fish from four resacas of the Texas, USA-Tamaulipas, Mexico border region. *Environ. Int.,* 27:15-20.

Naso B., Perrone D., Ferrante M.C., Bilancione M. and A. Lucisano, 2005. Persistent organic pollutants in edible marine species from the Gulf of Naples, southern Italy. *Sci. Total. Environ.,* 343:83-95.

Pandit G.G., Mohan Rao A.M., Jha S.K.,

Krishnamoorthy T.M., Kale S.P., Raghu K. and N.B.K. Murthy, 2001. Monitoring of organochlorine pesticide residues in the Indian marine environment. *Chemosphere,* 42:301-305.

Pastor D., Boix J., Fernandez V. and J. Albaiges, 1996. Bioaccumulation of organochlorinated contaminants in three estuarine fish species (*Mullus barbatus, Mugil cephalus* and *Dicentrarchus labrax*). *Mar. Pollut. Bull.,* 32:257-262.

Sahagun A.M., Teran M.T., Garcia J.J., Sierra M., Fernandez N. and M.J. Diez, 1997. Organochlorine pesticide residues in rainbow trout, *Oncorhynchus mykiss*, taken from four fish farms in Leon, Spain. *Bull. Environ. Contam. Toxicol.,* 58:779-786.

Storelli M.M. and G.O. Marcotrigiano, 2001. Persistent organochlorine residues and toxic evaluation of polychlorinated biphenyls in sharks from the Mediterranean Sea (Italy). *Mar. Pollut. Bull.,* 42:1323-1329.

Sun F., Wong S.S., Li G.C. and S.N. Chen, 2006. A preliminary assessment of consumer’s exposure to pesticide residues in fisheries products. *Chemosphere,* 62:674-680.