AN ABSTRACT OF THE THESIS OF

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Title: Assessment of Chlorinated Hydrocarbons and Trace Metal Contamination of Moroccan Marine Species

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The major aims of this study are to: (1) Assess the relative degree of heavy metal and chlorinated hydrocarbons contamination of fish products collected from several different locations and times from the Mediterranean Sea and Atlantic Ocean; (2) Conduct health risk analysis of high fish consumers; (3) Assess the extent of heavy metal contamination of mussels along the Atlantic Coast of Morocco; and (4) Examine the feasibility of using hepatic mixed function oxidase as a bioindicator of fish exposure to pollutants.

Contamination results of fishery products by metals revealed relatively high levels but they remain within acceptable limits for most of the species. The mean concentration detected in the most consumed fish species are 0.195 $\pm$ 0.103 for Pb, 0.186 $\pm$ 0.118 for Cr, 0.504 $\pm$ 0.138 for total Hg and 0.030 $\pm$ 0.006 mg/kg fresh weight (f.w.) for Cd.

The use of mussels (*Mytilus edulis*) to assess metal contamination in a coastal area shows considerable promise since these invertebrates are sedentary and thus accurately reflect the degree of local contamination. Despite some variations due to size of mussels and seasonal effects, this assay can be optimized by using the whole soft tissue of a composite mussel sample of similar size organisms (5-6 cm).

Contamination of fish by chlorinated hydrocarbon pesticides showed moderate levels of DDTs (pp'DDE, pp'DDD, pp'DDT) and lindane as the predominant compounds in most species with mean concentrations for DDTs varying from 5.6-18 µg/kg f.w. from the Atlantic Ocean and from 3 - 19.2 µg/kg f.w. from the Mediterranean Sea. PCB contamination yielded a mean concentration of 10.8 µg/kg f.w. for Mediterranean fishes and 17.9 µg/kg for Atlantic species.

Chlorinated hydrocarbons, cadmium, lead, and total mercury were selected for human health risk assessment. The risk was evaluated by measuring contaminant concentration in the

most consumed fish species by a population of fishermen with heavy fish consumption; and by calculation of intake based on fish consumption. Risk evaluation was based on the Acceptable Daily Intake (ADI) set by FAO/WHO regulatory policy. This assessment revealed that chlorinated hydrocarbons and PCBs are not likely to cause toxicity problems for the study population. However, lead, cadmium, and mercury intake were respectively 10%, 14%, and 145% of the ADI which is alarming, especially regarding mercury.

The use of mixed function oxidase as an index of exposure of fish to contaminants was difficult to establish in environmental samples. Fish are exposed to a mixture of pollutants and a correlation between chlorinated hydrocarbon or polyaromatic hydrocarbon exposure and the induction of mfo systems becomes rather uncertain.

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LIST OF ABBREVIATIONS

- AAS: Atomic Absorption Spectrometry.
- ADI: Acceptable Daily Intake.
- BNF: β-Naphthoflavone.
- B(a)P: Benzo(a)pyrene.
- b.w.: body weight.
- CRC: Critical Reviews of Chemistry.
- CEC: Commission of the European Communities.
- CERBOM: Centre d'Etudes et de Recherches de Biologie et d'Oceanographie Medicale.
- DDT: Dichlorodiphenyl-trichloroethane.
- DDD: Dichlorodiphenyl-dichloroethane.
- DDE: Dichlorodiphenyl-dichloroethylene.
- d.w.: dry weight.
- ECD: Electron capture detector.
- ECL: Enhanced Chemiluminescence.
- EDTA: Ethylenediamine tetraacetic acid.
- EROD: Ethoxyresorufin O-deethylase.
- FAO: Food Agricultural Organization.
- f.w.: fresh weight.
- GC: Gas chromatography.
- GESAMP: Group of Experts on the Scientific Aspects of Marine Pollution.
- IAEA: International Atomic Energy Agency.
- IARC: International Agency for Research on Cancer.
- IgG: Immunoglobulin G.
- IOC: Intergovernmental Oceanographic Commission.
- IUPAC: International Union of Pure and Applied Chemists.
- JECFA: Join Expert Committee on Food Additives.
- MARA: Ministere de l'Agriculture et de la Reforme Agraire.
- Med Pol: Mediterranean Action Plan Pollution Monitoring Research programme.
- MC: Methylcholanthrene.
- MFO: Mixed Function Oxidase.
- NAS: National Academy of Sciences.
- NCI: National Cancer Institute.
- NIH: National Institute of Health.
- NIOSH: National Institute for Occupational Safety and Health.
- ODI: Office du Developpement Industriel.
- PAHs: Polyaromatic Hydrocarbons.
- PB: Phenobarbital.
- PC: Principal Component
- PBS: Phosphate Buffered Saline.
- PCBs: Polychlorinated Biphenyls.
- PMSF: Phenylmethylsulfonyl fluoride.
- PROD: Pentoxyresorufin O-deethylase.
- PTWI: Provisional Tolerable Weekly Intake.
- USEPA: United States Environmental Protection Agency.
- RfDo: Reference oral dose.
- USFDA: United States Food and Drug Administration.
- UNEP: United Nations Environmental Programme.
- WHO: World Health Organization.

ASSESSMENT OF CHLORINATED HYDROCARBONS AND TRACE METAL CONTAMINATION OF MOROCCAN MARINE SPECIES

CHAPTER I

INTRODUCTION

The introduction by man directly or indirectly of various chemicals into the marine environment can result in deleterious effects to living resources, hazards to human health, and hindrance to human activities including fishing.

The geographical situation of Morocco provides the country with a large marine coast (3,400 km) on both the Atlantic and Mediterranean sides. This marine space constitutes an important biological reserve and contributes significantly to the economy of the country with an annual fish production of 594,000 tons with 179,000 tons for overseas export.

The growing need to increase agricultural and industrial production in Morocco has been associated with an increased use of toxic chemicals including pesticides, heavy metals... etc. In recent years considerable concern has been expressed over the worldwide distribution, the fate and ecotoxicological effects of various persistent chemicals especially the chlorinated pesticides (DDT, HCH, aldrin, dieldrin...), polychlorinated biphenyls (PCBs) and toxic metals (mercury, cadmium, lead, chromium...). Both organic and inorganic pollutants tend to accumulate in various compartments of the marine environment sometimes reaching toxic levels. There is, therefore, a pressing need in Morocco for the development of a monitoring program to insure both human and environmental health and to meet the chemical residue tolerances established by many countries where Moroccan fishery products are being marketed.

The objective of this study is to evaluate the state of contamination by some major contaminants of seafood products consumed by Moroccans. These include, chlorinated hydrocarbons pesticides, polychlorinated biphenyls, and some trace metals such as cadmium, chromium, lead, and mercury. In addition, an assessment of any resultant human health risk originating from the consumption of seafood products will be conducted. Finally, we will examine both the utility of using mussels as indicator of local contamination and the use of mixed function oxidase as bioindicator of fish contamination.

Part I, covers the general facts on the pollutants to be analyzed, their uses, sources and inputs into the marine environment. It also reviews data on levels encountered in marine organisms along with the human health effects and regulatory limits and guidelines adopted by international regulatory organizations.

Part II, reports specific studies that we have carried out dealing with different aspects of marine organisms contamination.

1. Examination of the relative degrees of heavy metals and chlorinated hydrocarbons contamination of Morrocan fishery products collected from several different locations and at several different times from the Mediterranean Sea and Atlantic Ocean.
2. Risk assessment analysis of fishermen families as a segment of the population that seems to be the most exposed to any potential health effects originating from the consumption of contaminated seafood products.
3. Assessment of the extent of heavy metals contamination of mussels along the western coast of Morocco.
4. A tentative study of the feasibility of using fish hepatic mixed function oxidase activities as indices of pollutant exposure.

CHAPTER II

LITERATURE REVIEW

PART I: TRACE METALS

I. INTRODUCTION

Before the exploitation by man of heavy metals for industrial uses, they were present principally in mineral ores. This exploitation has resulted in the wider distribution of heavy metals in the environment. Contamination of organisms by these metals derives partly from metals remaining in the mining waste, partly from refining of ores and partly from the end use of metals. Ores are not pure and metals other than the target metal are discarded into the environment during the refining process. Among the multiple pollutant sources introducing sizeable amounts of trace metals in the marine habitat, the following industrial activities appear to be the most important:

- metal mining industries;
- ferrous and non-ferrous metal industries, including metal plating;
- processing industries producing both organic and inorganic chemical wastes;
- direct discharge and dumping of domestic sewage, sewage sludges and various industrial wastes.

2. CADMIUM

2.1 General Facts on Cadmium and Cadmium Compounds

Cadmium is a relatively rare metal which occurs in nature as a minor component of other, ubiquitous, non-ferrous metal ores such as zinc, lead, and copper; substitution of zinc by cadmium in metal ores (sphalerite) is the most common. The cadmium salts of strong acids are easily soluble in water. At low pH, cadmium compounds are more soluble than under basic conditions. In nature, the predominate oxidation state of cadmium is Cd^{2+}. Cadmium released to the atmosphere is rapidly oxidized to CdO and removed by precipitation and fallout. In soils, cadmium is not very mobile. It is leached from soils into ground water and rivers. Industrial sources are zinc mining activities and cadmium plating. Municipal wastes can contain significant amounts of cadmium (sewage sludge and fallout from incinerators). In fresh water, cadmium is predominantly associated with colloidal and particulate matter. In seawater environments, cadmium is partially desorbed from particles and replaced by chelating substances (UNEP/FAO/WHO, 1989).

Cadmium has no known biological function and in marine organisms it should be associated with natural complexes, metallothionein or other metal-binding proteins which are naturally present in these organisms or induced by exposure to cadmium.

2.2 Sources, Uses and Pollution by Cadmium

Generally we cannot divide sources of cadmium into natural and anthropogenic ones. The fallout of cadmium estimated by Arnold et al. (1983), for example is about 140 metric tons/year/million km^2. This value refers both to natural and anthropogenic cadmium.

Atmospheric transport of trace elements was studied during the Etna and Phycemed cruises in the Western Mediterranean (Arnold et al. 1983). From the anomalous deposition enrichment of Ag, As, Au, Cd, Cr, Hg, Sb, Se, Pb and Zn the authors concluded that the main atmospheric deposition of trace metals originate from the anthropogenic aerosol from the industrial areas of Western Europe.

2.2.1. Natural Sources

Cadmium is one of the rare elements in the earth's crust. The average concentration is about 0.1 mg/kg. It is widely distributed and it is found in shale and igneous rocks, coal, sandstones, limestones, lake and marine sediments, soil, etc. (UNEP/FAO/WHO, 1988). Natural sources therefore are zinc, lead and copper mining areas (Fig. 1). High cadmium concentration in sediment and biota can be expected in these areas. In areas regarded as non-polluted, the cadmium concentration in soil is up to 4 mg/kg. Occasionally much higher values are found (up to 30 mg/kg soil) originating from natural and anthropogenic sources (Aston and Thornton, 1977; Webb et al., 1978). Phosphates contain an average of 15 mg/kg (UNEP/FAO/WHO, 1989).

The marine environment is considered as the final sink for cadmium like other trace metals. Due to weathering, this compound may be enriched by 2 to 3 times in sediments.

2.2.2. Anthropogenic Sources

The main anthropogenic sources of cadmium relate to ore mines, metallurgical industries, and to sewage sludges. Cadmium is generally used in:

- protective plating on steel (anti corrosive);
- nickel-cadmium batteries, (most for consumer use but also in emergency lighting supplies);
- pigments, particularly cadmium sulfides to give yellow-to-orange colors and cadmium sulpho-selenides to give pink-to-red and brownish. Applications of this type are found in traffic paints and, until recently in crates for beer;
- stabilizers for polymers in plastic;
- various alloys.

Cadmium used in the electroplating was estimated to be 34% of the US consumption in 1979, followed by its use in batteries (22%), pigments (13%), plastics (11%) and other purposes (3%). About 80% of all the pigment sales in Europe goes to plastic industry, the corresponding figures for Japan and the United States are 60-80% and 75%, respectively (Nriagu, 1980).

2.3 Levels in Marine Organisms

The concentration of cadmium in biota depends, not only on the degree of contamination of seawater, but also on their food-chain position and in particular, on the chemical species of cadmium to which the organisms are exposed. Various biological species may have different cadmium concentrations. Further, different biological tissues of the same species can have different cadmium concentrations. This means that for a comparison of cadmium concentrations in marine organisms from different locations the same tissue of the same biological species must be considered. For example, Lafaurie et al. (1981) reported that in *Mullus barbatus*, the cadmium concentration in the muscle tissue ranged from nondetectable to about 40 mg/kg d.w. while the corresponding levels for the gonads, kidney, and liver are 20 to 130, 50 to 280 and 500 to 1200 μg/kg d.w., respectively. The muscle contains the lowest concentration and the liver the highest.

Following the "mussel-watch" program, the mussel *Mytilus galloprovincialis* has been used as a pollution indicator in the Mediterranean Action Plan Pollution Monitoring and Research Programme (MED POL). A wide range of concentrations has been observed and background concentrations are difficult to establish because they can reflect only local conditions. The mean of cadmium concentration in *Mytilus* was 120 μg/kg f.w. Cadmium levels in *Mullus barbatus*, another pollution indicator of the MED POL programs, are considerably lower for this contaminant with mean value from different areas of 46 μg/kg f.w. Levels of Atlantic marine organisms are comparable to those of the Mediterranean species (Table. 1).

2.4 Toxicokinetics

Humans are exposed to cadmium from ambient air, drinking water, tobacco and food. On an average, approximately 5% of ingested cadmium is absorbed. Cadmium does not readily penetrate the skin, but it is readily absorbed following inhalation since 96% deposited in the lungs may be absorbed (CEC, 1978).

Cadmium distributes through the body and accumulates in the kidney and liver, where it may reach levels 10 to 100 times higher than those of other tissues (Anonymous, 1989a).

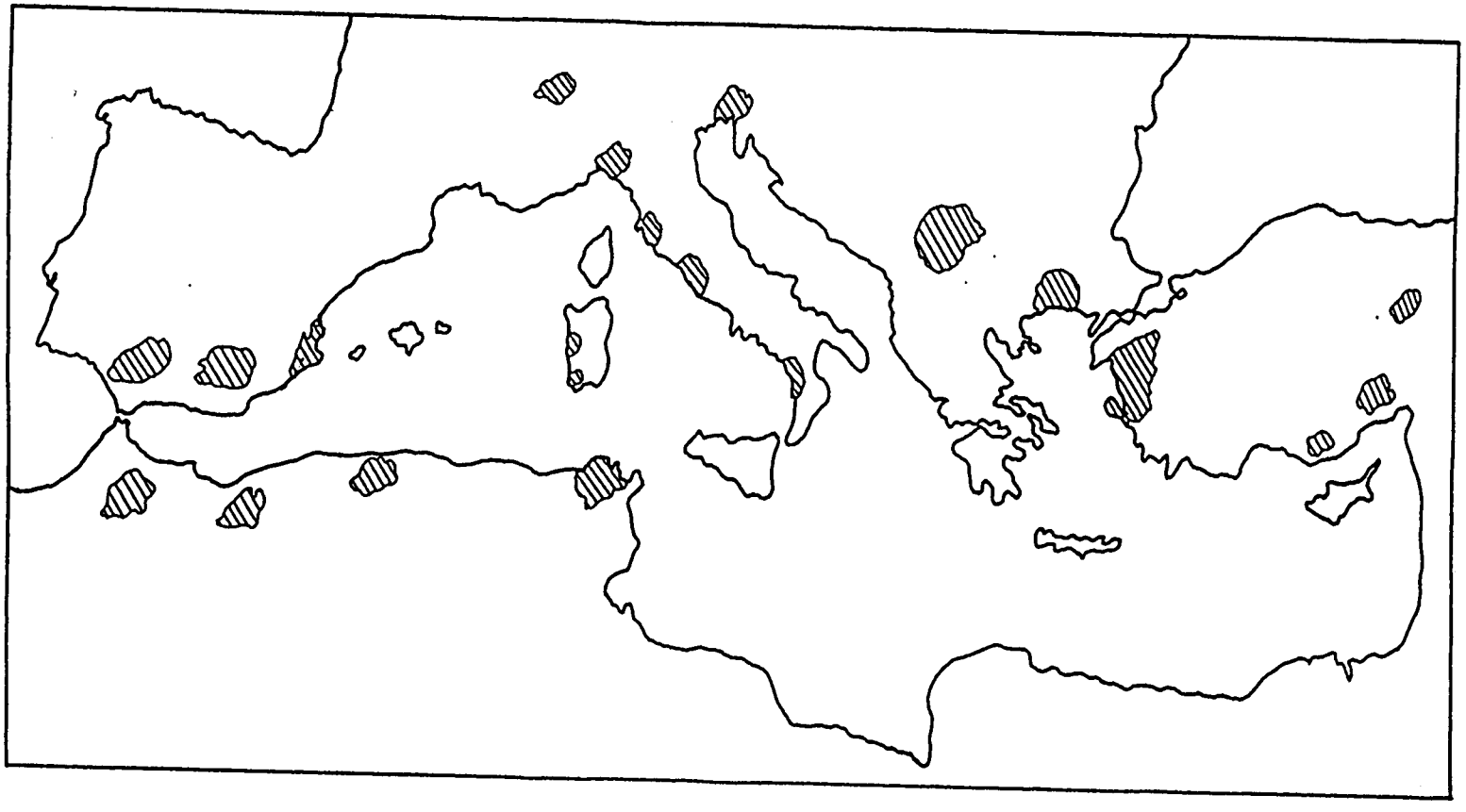

Figure 1. Mediterranean map showing mining area of zinc, copper, and lead (UNEP/FAO/WHO, 1989).

Table 1. Cadmium concentrations in muscle tissue of selected Mediterranean and Atlantic fish species.

Species	Cadmium Conc. (mg/kg f.w.) Range	Location	References
P. acarne	0.115	Mediterranean	UNEP/FAO/WHO, 1989
M. Merluccius	0.063 0.02-0.06	Mediterranean Atlantic	UNEP/FAO/WHO, 1989
S. solea	0.04	Atlantic	VOS and HOVENS, 1986
S. pilchardus	0.15	Mediterranean	C.E.R.B.O.M., 1982
T. trachurus	0.135	Mediterranean	UNEP/FAO/WHO, 1989
M. barbatus	0.046 (0.017-0.05)	Mediterranean	UNEP/FAO, 1986
T. thunnus	0.038	Mediterranean	UNEP/FAO, 1986
M. edulis	(0.05-1.9)	Atlantic Moroccan Coast	MAHYAOUI et al., 1989
	0.12	Atlantic Spain and Portugal	UNEP/FAO/WHO, 1989

Cadmium, unlike other metallic drinking water contaminants, is not metabolized to other compounds. Once in blood, cadmium combines to the low-molecular-weight protein metallothionein (Foulkes, 1982; Whanger, 1985). It is also sequestered in the liver by metallothionein.

Cadmium is excreted by humans mainly via the urine (USEPA, 1985a). The half-life for elimination has been estimated to be 10 to 33 years (Ellis et al. 1979).

2.5 Human Intake

For non-occupationally exposed populations, food is the major source of cadmium intake. Food contribute to about 80 to 90% of cadmium intake for non-smoker (Stoeppler, 1984). Unlike mercury, where the main source of intake is the consumption of contaminated seafood, the overall food consumption pattern combined with other habits such as smoking are contributing to a significant amount in cadmium intake.

2.6 Health Effects

There has been only one significant incident involving cadmium (Friberg et al. 1974). It occurred in Japan, where in 1947, 44 cases of "rheumatic "disease were recorded in villages on the Jintsu River. These patients presented painful skeletal deformities and, because of severe pain and discomfort, this disease was described as "Itai-Itai" (ouch-ouch) disease. The wastes from a zinc mine were discharged into the Jintsu River and were carried by floods and irrigation into pad fields. The victims were all associated with areas liable to flooding or irrigation. Full treatment was introduced in 1955, which resulted in a rapid decline in the incidence of the disease. Although the disease was attributed to cadmium there has been a continuing controversy on the real etiology. Even the official statements attested that the disease required such aggravating factors as pregnancy, age, or calcium deficiency for it to become manifest, other studies have suggested other dietary deficiencies and possible interactions with other metals such as zinc. In total, approximately 200 people were affected, of whom 50% died. Itai-Itai disease has not been reported from other areas affected by zinc mining or smelting and as shown by the studies at Shiphamin, England, high soil concentrations do not necessarily induce a threat to human health (Sherlock, 1983a).

Cadmium and cadmium compounds have been shown to cause sarcomas at local injection sites (Gunn et al. 1967; Haddow et al. 1964). In addition, exposure of rats to cadmium chloride by aerosol for 18 months resulted in significant increase in lung tumors (Takenaka et al. 1983). These data may not be relevant to oral exposure to cadmium in drinking water (USEPA, 1986). Although cancers of the prostate and lung have been reported in cadmium smelters, evidence

regarding the carcinogenicity of cadmium in human following oral exposure are largely conjectural (USEPA, 1986). Chromosomal aberrations were also reported in several *in vitro* studies, but no strong evidence of mutagenic effects following oral ingestion are available (USEPA, 1986).

2.7 Regulatory Limits and Guidelines

In 1972, a joint FAO/WHO Expert Committee recommended a provisional tolerable weekly intake (PTWI) of 0.4-0.5 mg of cadmium per adult person (70 kg weight) (Table 2) (FAO/WHO, 1972). WHO has not yet issued health criteria for cadmium since there is still a relative lack of information on epidemiological data and data on dose-response relationship due to cadmium intake from various sources (UNEP/FAO/WHO, 1989). However, several countries have established limits for cadmium in fish; in the majority of cases limits range between 0.1 and 1 mg/kg (Table 3).

Table 2. Compilation of acceptable limits for mercury cadmium and lead in fish and fishery products.

Metal	mg/person (70 kg)	mg/kg Body Weight	Reference
Mercury			
PTWI	0.3	0.0042	WHO, 1972
ADI	0.2	0.0028	USEPA, 1980
Methylmercury			
PTWI	0.2	0.0028	WHO, 1972
Cadmium			
PTWI	0.4-0.5	0.0057-0.0071	WHO, 1972
RfDo	0.04	0.0005	UNEP/FAO/WHO, 1988 USEPA, 1988c
Lead			
PTWI (adults)	3.5	0.05	WHO, 1972
PTWI (infants)		0.025	UNEP/FAO/WHO, 1988
ADI*	8.4	0.12	USEPA, 1988b

*For alkyl derivatives of lead.

The reference dose (RfD) is an estimate of an exposure level that would not be expected to cause adverse effects when exposure occurs for a significant portion of life time. The RfD is route-specific and estimates acceptable exposure for either oral (RfDo) or inhalation (RfD_i) with the implicit assumption that exposure by other routes is insignificant (USEPA, 1988c). USEPA (1988c) calculated an RfD of oral exposure to waterborne cadmium of 0.04 mg/day for 70 kg human, or 0.0005 mg/kg/day.

Table 3. Compilation of legal limits for some hazardous metals in fish and fishery products.

Country	Cadmium (mg/kg f.w.)	Chromium (mg/kg f.w.)	Mercury (mg/kg f.w.)	Lead (mg/kg f.w.)
Australia[a]	0.2-5.5		0.5-1.0	1.5-5.5
Brazil			0.5[b]	
Canada			0.5	0.5
Chile	0.5			2.0
Denmark			0.5	
Ecuador			1.0	5.0
Finland			1.0	2.0
France			0.5-0.7	
Germany	0.5		1.0	0.5
Greece			0.7	
Hong Kong	2.0	1.0	0.5	6.0
India			0.5[b]	5.0
Israel			0.5	
Italy			0.7[b]	2.0
Japan			0.3-0.4[b]	
Korea			0.5	
Netherlands	0.05-1.0		1.0[b]	0.5-2.0
New Zealand			0.5[b]	2.0
Philippines			0.5	0.5
Poland				1.0-2.0
Russia			0.2-1.0	
Spain			0.5	
Sweden			1.0[b]	1.0-2.0
Switzerland	0.1		0.5	1.0
Thailand			0.5	1.0
United Kingdom				2.0-10
United States			1.0[b]	
Venezuela	0.1		0.1-0.5	2.0
Zambia			0.2-0.3	0.5-10
Range				
Minimum	0.1	1.0	0.1	0.5
Maximum	5.5	1.0	1.0	10.0

[a] Limits vary among states
[b] Total

Reference: Nauen (1983); USFDA (1982,84)

2.8 Analytical Methods

Atomic Absorption Spectrophotometry (AAS) with flame or electrothermal atomization is the most currently used method for cadmium determination. The sample is prepared and mineralized in presence of nitric acid at 140°C. After appropriate mineralization of the sample, the analysis is performed at a wavelength of 228.8 nm. Absorbance is measured as a function of cadmium concentration. The detection limits of the method will vary with the equipment used. Typical ranges of the detection limits are 0.1-0.2 mg/kg f.w. for flame atomization and 0.0005-0.001 mg/kg f.w. for electrothermal atomization (UNEP/FAO/IAEA/IOC, 1984b).

3. CHROMIUM

3.1 General Facts on Chromium and Chromium Compounds

Chromium is a relatively rare metal in the earth's crust. It occurs in most rocks and minerals at levels of 200 mg/kg. Industries are the major source of chromium release to the environment. However, current data suggest that surface and ground water contamination is the result of naturally occurring chromium leaching from mineral deposits. USEPA (1986b) reported soluble chromium values of 84 μg/l in surface waters and 50 μg/l in ground waters, and established an interim standard value of 50 μg/l in drinking water. Cr^{3+} is considered as an essential nutrient required in trace quantities for normal glucose metabolism. Some forms of chromium may also be important in the metabolism of lipids and other carbohydrates (USEPA, 1986b).

3.2 Uses of Chromium

Chromium and its salts have variety of uses. The hexavalent chromium (Cr^{6+}) compounds are widely used in:

- electroplating and alloy industries;
- metal finishing and corrosion control;
- textile industry as mordants.

Chromium salts are used in:

- cooling waters as anticorrosive agents;
- the leather tanning industry;
- the manufacture of catalysts;
- pigments and paints;
- fungicides and wood preservatives.

3.3 Levels in Marine Organisms

Limited data on chromium concentration levels in marine organisms are available in the literature. Fowler and Oregioni (1979) reported a range value of 0.5-28.8 μg/kg dry weight in *Mytilus* from the North-West Mediterranean (Ligurian Sea). Burns et al. (1982) reported a range of 0.3-3.1 mg/kg dry weight in shellfish tissue and a range of 3.2-3.4 mg/kg dry weight in fish muscle from a coastal area of Oman which is considered unpolluted by heavy metals.

3.4 Toxicokinetics

The metabolism of chromium in mammalian species is not well understood. It is complicated by the two oxidation states, Cr^{3+} and Cr^{6+} (USEPA, 1986b).

In general, Cr^{6+} is more readily absorbed than Cr^{3+}. In both humans and experimental animals, gastrointestinal absorption of inorganic salts of Cr^{3+} is low (from 0.5 to 3%). However, Cr^{6+} and organic complexes of Cr^{3+} are more readily absorbed (approximately 2 to 10% for Cr^{6+} and 10 to 25 % for organic complexes of Cr^{3+}) (USEPA, 1986b).

Cr^{3+} and Cr^{6+} differ in their patterns of distribution. In general, Cr^{3+} crosses membranes much more slowly than Cr^{6+} (USEPA, 1986b). In fact, Hopkins and Schwarz, (1964) showed that Cr^{3+} circulated in the plasma primarily in nondiffusable form and has an affinity for iron-binding protein. The spleen and kidneys were shown to have the highest concentrations of chromium when rats were administered Cr^{3+} as chromium chloride ($CrCl_3$) intravenously (Hopkins, 1965).

The kidney appears to be the principal route of excretion of chromium compounds, but little is known about the form of excretion (Anonymous, 1989b).

3.5 Health Effects

In general, Cr^{6+} is more toxic than Cr^{3+} compounds. The oral LD50 for various salts of Cr^{3+} ranges from 600 to 2,600 mg/kg (Smith et al., 1969) while that of Cr^{6+} (as $Na_2Cr_2O_7$) in rats is 19.8 mg/kg (NIOSH, 1987). Cr^{6+} has been shown to produce liver and kidney damage, internal hemorrhage, dermatitis, and respiratory problems. The immediate symptoms are generally nausea, repeated vomiting and diarrhea (USEPA, 1986b).

The carcinogenicity of Cr^{6+} by inhalation is well established for occupational exposure of humans (Hayes et al., 1979), but there is no adequate evidence to determine whether oral exposure to chromium can lead to cancer.

3.6 Regulatory Limits and Guidelines

The recommended maximum allowable concentration of chromium in air, dusts and mists, in form of CrO_3 is 0.1 mg/m^3 for daily 8 hours exposure (CRC, 1980). No data are available for dietary intake of chromium. The drinking-water standard is set at 0.05 mg/l Cr^{6+} (WHO/UNEP, 1982).

3.7 Analytical Methods

The sample is prepared and mineralized with acid at 140°C. Chromium levels are determined by atomic absorption spectrophotometry with flame atomization or, after appropriate drying and charring, with electrothermal atomization at a wavelength of 357.9 nm. Absorbance is measured as function of chromium concentration. The detection limits of the method will vary with the equipment used, typical ranges of the detection limits in mg/kg fresh weight are 0.05 for flame atomization and 0.001 for electrothermal atomization (Anonymous, 1989b).

4. LEAD

4.1 General Facts on Lead and Lead Compounds

Lead is one of the oldest metals known to man: a leaden figure dating from 3800 B.C. was found at Abydos, and lead was probably used in Egypt as early as 5000-7000 B.C. (Nriagu, 1978). This element is one of the few metals that seems to have only toxic effects but no essential function in the organism. The difference between toxic levels and those normally in the organism is very small. Normal blood levels are in the order of 200 μg/l; subclinical changes are noted in particularly sensitive persons at 400-600 μg/l; and clinical symptoms can be seen above 600 μg/l (Gerber et al. 1980).

In the environment, concentrations of lead differ widely between regions and locations. Background levels of lead in fresh water are in the order of 1-4/L, while those from naturally contaminated areas may reach levels of 150-200 μg/L (Aston and Thornton, 1977). Lead concentrations varied between 25 and 140 mg/kg in sediment from unmineralized areas, and levels of 8500 mg/kg have been published for sediments from areas with metalliferous mineralizations (Aston and Thornton, 1977).

4.2 Sources, Uses, and Pollution by Lead

Lead is ubiquitous in the natural environment; the most important lead mineral contains the metal as sulphide (galena), carbonate (cerrusite) or sulphate (anglesite).

This metal found extensive application in antiquity for water pipes, tiles, storage containers, paints, etc. The usage of lead culminated in Roman times to such an extent that nearly the

same amount of lead *per capita* was used as today. About four 10^9 kg of lead are now manufactured yearly (Nriagu, 1978).

Lead is used in:

- storage batteries;
- metal products such as ammunition and soldier, the manufacture of sound proofing material (both as sheets and composition paneling);
- in gasoline as an anti-knocking agent (as tetraethyl lead);
- in paints as pigments (as red lead and lead chromates);
- other uses include: various alloys, radiation shielding against gamma rays.

Lead, as a gasoline additive and in batteries, represented 75% of total consumption in 1975 in the U.S.A (Nriagu, 1979). In 1979, a total of 123,000 tons were emitted in Europe, about 60 percent of this amount was accounted for gasoline combustion alone (Pacyna, 1986).

4.3 Trends in Lead Levels in Marine Organisms

Lead is one of the most frequently monitored contaminants in food. An assessment of lead contamination of food showed that in general, molluscs and crustaceans are usually highly contaminated by this metal followed by fish and shellfish while moderate levels were recorded in milk, meat, cereals, vegetables and fruits (UNEP/FAO/WHO, 1988).

The mean of all lead levels in *Mytilus galloprovincialis* from the Mediterranean "mussel-watch" is reported to be 0.800 mg/kg f.w. (UNEP/FAO, 1986). Levels in *Mullus barbatus* are considerably lower; the mean for all samples in several areas of the Mediterranean is about 0.070 mg/kg f.w. (UNEP, 1989). Several other marine organisms have been analyzed from both the Mediterranean sea and the Atlantic Ocean. According to fish species and sampling locations average lead levels varied from 0.04 for *Platessa platessa* (plaice) to 1.17 mg/kg for *Thunnus thunnus*, which is the most contaminated species (Table 4).

4.4 Toxicokinetics

Lead can be absorbed via the oral route (food or drink) or by inhalation (Mahaffey, 1978). Oral uptake is the predominant route under most conditions. About 200-300 μg of lead are taken daily with food; about 5-10 % is absorbed by adults and up to 50 % by children (Rabinowitz et al., 1976).

Table 4. Lead concentrations (mg/kg f.w.) in some marine species.

Species	Lead concentration Average (Range) mg/kg f.w.	Location	References
S. solea	0.07 (0.02-0.260)	Netherlands	VOS and HOVENS, 1986
C. ahrengus	0.03-0.041	North Sea	VOS and HOVENS, 1986
P. platessa	0.04 (0.02-0.260)	Irish Sea	VOS and HOVENS, 1986
M. galloprovincialis	0.8 (0.60-1.80)	Mediterranean	UNEP/FAO, 1986
M. barbatus	0.07 (0.06-0.37)	Mediterranean	UNEP/FAO, 1986
T. Thunnus	1.17	Mediterranean	UNEP/FAO, 1986
All species	0.18* (0.14-0.90)	Oman, Persian Gulf	IAEA, 1987

*Calculated value: 4 µg/g dry weight is equivalent to 1 µg/g fresh weight.

Gerber et al., (1980) reviewed lead metabolism and noted that lead retention is greatly influenced by dietary factors such as vitamin E and D. They also reported that lead toxicity is enhanced when food is deficient in calcium, protein, phosphate, iron or zinc.

4.5 Human Exposure

Exposure to lead occurs by inhalation and by ingestion of food and water. Exposure from drinking water can be appreciable where lead pipes and lead-lined storage tanks are used. In addition children can be exposed by eating non-food item such as lead ingestion from dust or soil by hand-to-mouth activities (WHO, 1977). Respiratory exposure by polluted air is of great concern for human health because inhaled lead is 50% more efficiently absorbed than ingested lead (Chamberlain et al., 1975).

4.6 Health Effects

Lead is classified as a cumulative poison. It produces various intoxication effects that were reviewed by Gerber et al., (1980) which involve the following signs:

a) The hematopoietic system (microcytic anemia from an abnormal and impaired production and an increase destruction of erythrocytes).
b) The nervous system (encephalopathy, mainly in children, peripheral neuropathy, mainly in adults).
c) The kidney (tubular damage in acute intoxication, interstitial glomerular fibrosis and tubular atrophy in chronic intoxications).
d) The gastrointestinal tract (colic, alternating constipation and diarrhea).
e) Various effects on bone, liver, defense mechanisms of the body, hormonal secretion, etc.

4.7 Regulatory Limits and Guidelines

The Joint Expert Committee on Food Additives (JECFA) has established a Provisional Tolerable Weekly Intake (PTWI) of 50 μg/kg of body weight, applicable to adults only (WHO, 1972) (Table 2). In 1986, the committee evaluated the health risks of lead to infants and children and established a PTWI of 25 μg/kg body weight for this group of population, considered more sensitive than adult to lead exposure because of metabolic and behavioral differences (WHO, 1987).

4.8 Analytical Methods

The sample is prepared and mineralized in presence of nitric acid at 140°C. Lead levels are determined by atomic absorption spectrophotometry with flame atomization or, after appropriate drying and charring, with electrothermal atomization at a wavelength of 283.3 nm. Absorbance is measured as function of lead concentration. The detection limits of the method will vary with the equipments used, typical ranges of the detection limits are 5-8 mg/kg fresh weight for flame atomization and 0.01-0.05 mg/kg fresh weight for electrothermal atomization (UNEP/FAO/IAEA/IOC, 1984).

5. MERCURY

5.1 General Facts on Mercury and Mercuric Compounds

Mercury is a silver-white metal, liquid at room temperature, highly volatile and can exist at three different oxidation states: elemental mercury (Hg^0) mercurous ion (Hg_2^{2+}), and mercuric ion (Hg^{2+}). It is known that all ionic mercury entering the body binds to the thiol groups in proteins and other biological molecules (Clarkson, 1972) causing their inhibition or inactivation and ultimately leading to mitotic disturbances (Das et al., 1982; Elhassani, 1983).

All mercury discharged into rivers, bays or estuaries as elemental (metallic) mercury, inorganic divalent mercury, phenylmercury, or alkoxyalkyl mercury can be converted into methylmercury compounds by biological or chemical processes, or both (Beijer and Jernelov, 1979) (Fig. 2). The methylated form is the most hazardous mercury species due to its high stability, its lipid solubility, and its ionic properties that facilitates membrane penetration in living organisms (Beijer and Jernelov, 1979).

Mercury, although a relatively rare element, is ubiquitous in the earth's crust, occurring at level from 10 to 500 μg/kg as sulfide, chloride or oxide. Cinnabar (mercuric sulfide) is the most important ore of mercury, it has been mined continuously since 415 BC (Clarkson and Marsh, 1982).

The ecotoxicology of mercury has been recently reviewed and summarized in six points by Eisler (1987):

1. Hg and its compounds have no known biological function and its presence in living organisms is undesirable and potentially hazardous.

2. Forms of mercury with relatively low toxicity can be transformed into forms with very high toxicity through biological and other processes.

3. Methylmercury can be bioconcentrated in organisms and biomagnified through food chains returning mercury directly to man and other upper trophic level consumers in concentrated forms.

4. Mercury is a mutagen, teratogen, and carcinogen, and causes embryolethal cytochemical and pathological effects.

5. High body burden are normally encountered in some species of fish and wildlife.

6. The anthropogenic use of mercury should be curtailed, because the difference between tolerable natural background levels of mercury and harmful effects in the environment are exceptionally small.

5.2 Sources, Uses, and Pollution by Mercury

The global cycle of mercury involves degassing of the element from the earth's crust and evaporation from natural bodies of waters, atmospheric transport, and deposition back into land and water. Regions with higher average mercury concentrations are near mining sites of this metal (Fig. 3 and 4). These mining sites contribute largely in increasing levels of mercury in both sediment and biota from the Mediterranean sea (UNEP/FAO/WHO, 1987).

In addition, several human activities using mercury account for the major source of the global input of this element to the environment. Mercury is involved in several activities of industrial, medical and agricultural sectors. The most important are listed below:

- Chloralkali process
- Electrical apparatus
- Antifouling and mildew resistant paints
- Control devices
- Dental preparations
- Catalysts
- Agriculture as antifungal
- Laboratory (thermometers, barometers...)
- Pharmaceuticals
- Pulp and paper mill
- Metal amalgamation

The major use of mercury has been as cathode in the electrolytic preparation of chlorine and caustic. For example, in 1968 this use accounted for about 33% of the total U.S. needs (USEPA, 1980). In a recent U.S. survey on mercury consumption, electrical apparatus accounted for about 27%, industrial and control instruments, such as switches, thermometers, and barometers, and general laboratory appliances, 14% followed by its use in paints (12%), Hg formulations to control fungal diseases of seeds, bulbs, and vegetables (5%), and dental amalgams, pulp and paper manufacturers, pharmaceuticals, metallurgy and mining, and catalysts, (9%) (USEPA, 1980).

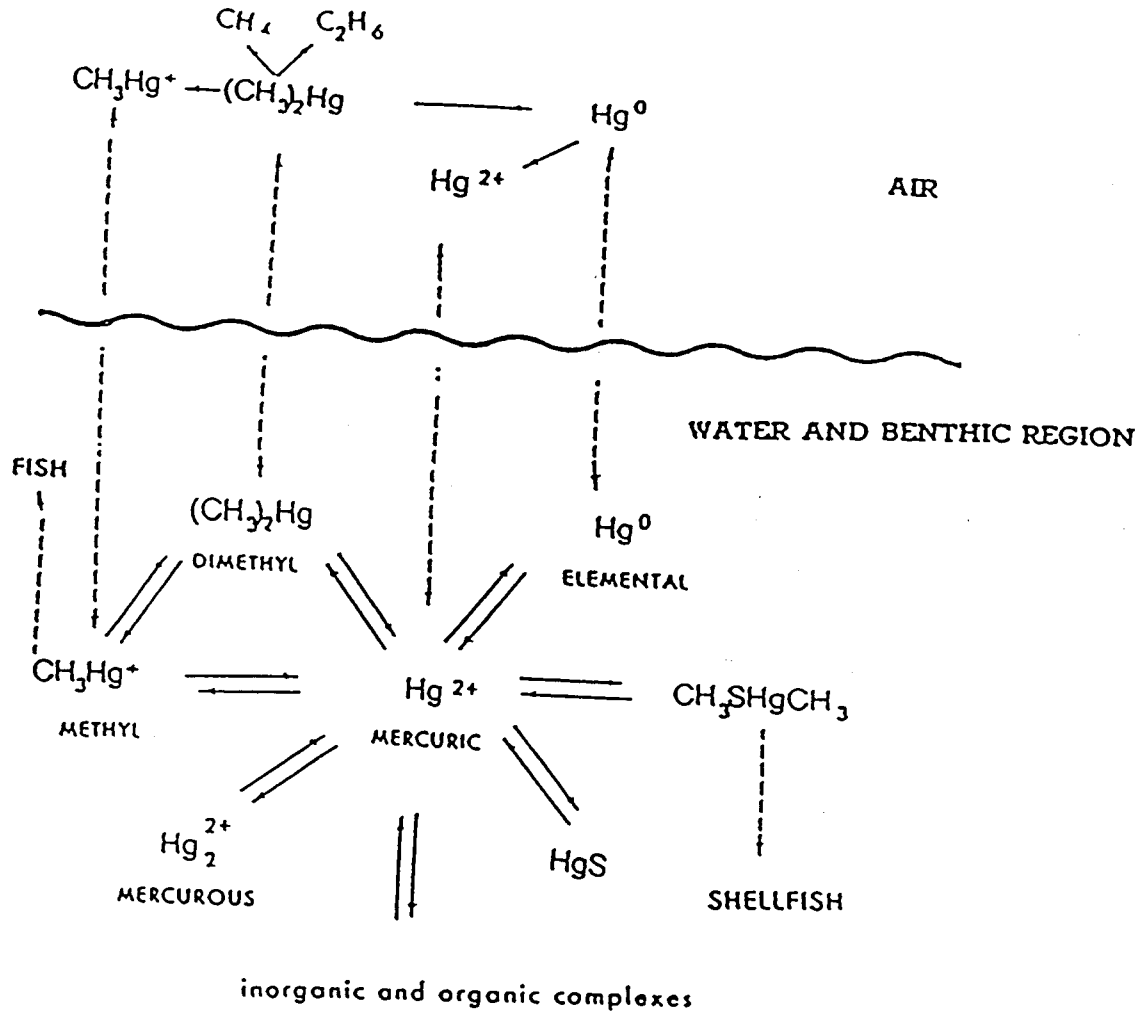

Figure 2. Major transformations of mercury in the environment (Eisler, 1987).

5.3 Trends in Mercury Levels in Marine Organisms

Assessment of chemical contaminant in food, prepared by UNEP/FAO/WHO (1988), showed that in contaminated freshwater areas mercury levels range between 500 and 700 μg/kg of mercury. In uncontaminated waters, the concentrations in freshwater fish are around 200 μg/kg. Most oceanic species have average mercury levels of about 150 μg/kg or less. However, the large carnivorous species (shark, swordfish, and tuna) usually have mercury levels in the range of 200-1500 μg/kg f.w. while levels recorded in molluscs and crustaceans seldom exceed 100 μg/kg.

Special attention has been given to mercury in the Mediterranean Sea and a large amount of data in fish has been collected under the UNEP coordinated Mediterranean Pollution Monitoring and Research Programme (UNEP/FAO/WHO, 1983) (Table 5). It should be noted that active mining sites account for 50% of the world production of mercury (Fig. 3). High mercury levels (up to 1,200 μg/kg f.w.) were observed in *Mullus barbatus* from coastal regions near the mercury bearing area of Monte Amiato, northern Tyrrhenian coast of Italy but, a more representative mercury concentration was 160 μg/kg f.w. It was observed that fish caught along the coast of Tuscany have considerably higher mercury levels than fish caught near the strait of Gibraltar (UNEP/FAO/WHO, 1988).

Several reports indicate that, in general, levels of mercury were higher in fish from the Mediterranean sea than from the Atlantic, Pacific or Indian oceans. However, a more comprehensive assessment of mercury in these regions would be necessary before a more definite relationship could be expressed (UNEP/FAO/WHO, 1988).

5.4 Toxicokinetics

About 7 to 15% of orally administered inorganic mercury is absorbed by humans (Task Group on Metal Accumulation, 1973). Mercury is distributed in the body primarily to kidney, liver, skin, and muscle. Other tissues contained lesser percentages of the administered dose (Rothstein and Hayes, 1960; Jugo, 1976). It is known that nearly all ionic mercury entering the body binds to the thiol groups in proteins and other biological molecules (Clarkson, 1972), but no information is available on the biotransformation of inorganic mercury in the literature (Anonymous, 1989c). The excretion of mercury was studied by Rothstein and Hayes (1960) in rats; the clearance occurred in three phases: a rapid phase lasting a few days and eliminating 35% of the dose, a slow phase with half-life of 30 d eliminating 50% of the dose, and a slower phase (half-life of approximately 100 d) eliminating the remaining 15%.

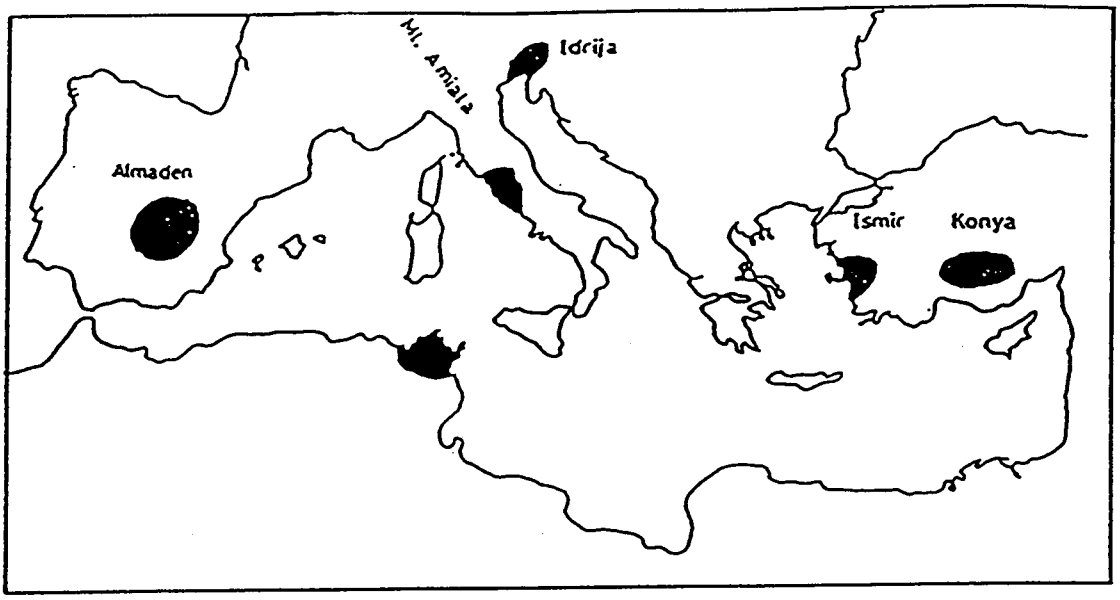

Figure 3. Mediterranean map showing mining area of mercury (UNEP/FAO/WHO, 1987).

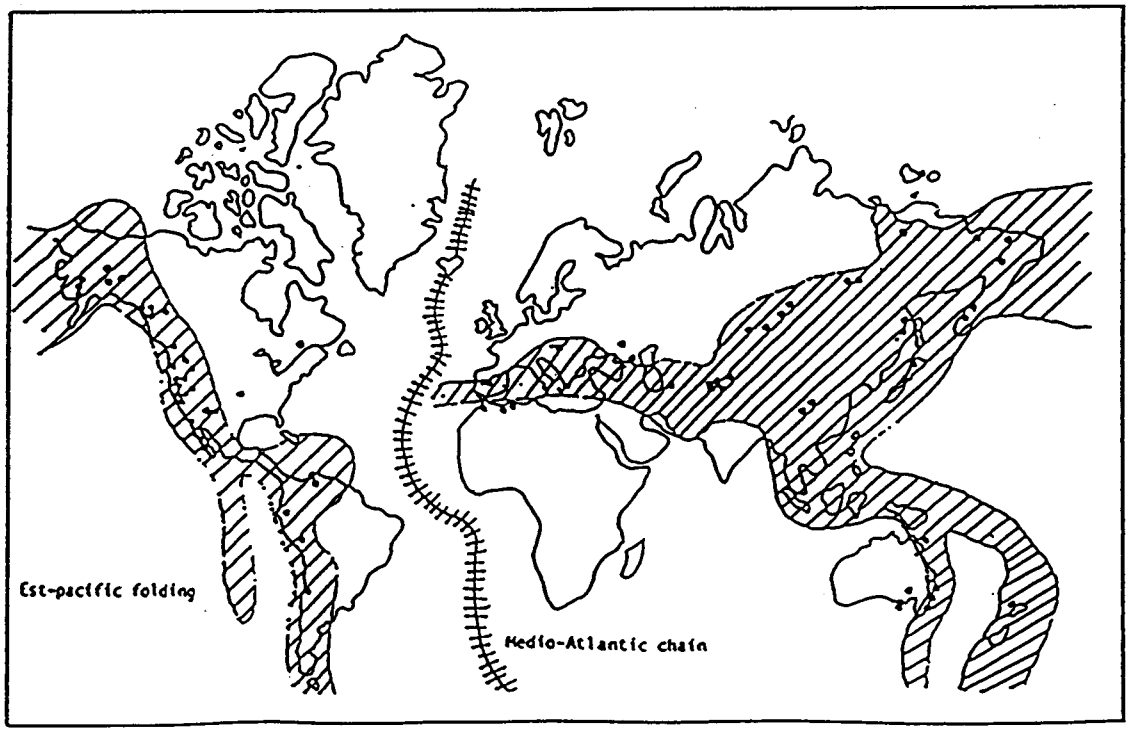

Figure 4. Mercury belt of the earth (Vuillement, 1974).

Table 5. Mercury concentrations in muscle of selected Mediterranean and Atlantic marine organisms.

Species	Mercury Concentrations Average (Range) mg/kg f.w.	Location	References
P. acarne	0.170 (0.030-0.337	Mediterranean Sea	UNEP/FAO/WHO, 1987
	1.38	Mediterranean Sea	C.E.R.B.O.M., 1982
M. merluccius	0.62	Mediterranean Sea	C.E.R.B.O.M., 1982
	0.131 (0.031-0.258) 0.232 (0.025-0.850	Mediterranean Sea	FAO/UNEP/WHO/IOC/IAEA, 1986 UNEP/FAO/WHO, 1987
	0.09 (0.03-0.130)	Atlantic	UNEP/FAO/WHO, 1987
	0.05	Atlantic	FAO/UNEP/WHO/IOC/IAEA
S. solea	0.15 (0.05-0.320)	Atlantic	UNEP/FAO/WHO, 1987
S. pilchardus	0.159 (0.070-0.380)	Mediterranean Sea	UNEP/FAO/WHO, 1987
	0.235 (0.06-0.08)	Mediterranean Sea Atlantic	FAO/UNEP/WHO/IOC/IAEA, 1986
B. boops	0.128 (0.020-0.432) (0.127-0.137)	Mediterranean Sea	UNEP/FAO/WHO, 1987 FAO/UNEP/WHO/IOC/IAEA, 1986
T. trachurus	0.36 (0.08-0.848)	Mediterranean Sea	UNEP/FAO/WHO, 1987
Mytilus sp.	0.232 (0.004-7.00) 0.04	Mediterranean Sea North Atlantic	UNEP/FAO/WHO, 1983 Vos and Hovens, 1986
T. thunnus	1.05 (0.02-6.30)	Mediterranean Sea	UNEP/FAO/WHO, 1983

5.5 Human Intake

The dietary intake of mercury depends primarily on the concentration in fish and the amount and frequency of consumption, the duration of the total exposure period, and the results of interaction with other contaminants. As fish are the major source of mercury intake (Clarkson, 1989), several countries have established limits for mercury in their seafood products (Nauen, 1983; USFDA, 1982). In the majority of cases limits have been set for total mercury rather than methylmercury, with 0.5 mg/kg f.w. as the most prevalent value (Table 3).

5.6 Health Effects

One of the earliest and well documented examples of mercury poisoning is that of Minamata Bay in Japan (Fujiki 1963, 1980; NAS 1978; Nishimura and Kumagai, 1983). The bay constituted a semi-enclosed area and was receiving the waste discharged from an acetaldehyde plant were mercuric oxide was used as a catalyst. The factory began using mercury in 1932 and continued until 1968.

In 1950's the town of Minamata had a population of 50,000. Fish were the main component of the diet (286 g/day in winter and 410 g/day in summer per person). In 1953 severe neurological disorders were recognized in several inhabitants and the disease reached an epidemic character. One hundred and eleven cases of poisoning were reported by the end of 1960 and 41 deaths by 1965. In 1982, there were 1,800 verified human victims of mercury poisoning. Symptoms included sensory impairment, constrictions of visual fields, hearing loss, ataxia, and speech disturbances.

"Minamata disease" resulted from the human consumption of fish and shellfish which have been heavily contaminated by methylmercury through several routes of exposure including bioconcentration and food chain biomagnification.

In 1964 a separate incident happened in Nigata (Japan). Here again the river fish and seafood were an important component of the diet which confirmed the etiology of Minamata disease.

Further outbreaks of mercury poisoning have been reported in Pakistan, Guatemala, Ghana, Yugoslavia, and Iraq (Clarkson and Marsh, 1982; Das et al., 1982; Elhassani, 1983; Greener and Kochen, 1983). In 1972, for example, in northern Iraq farmers were acutely poisoned after eating bread from seed wheat treated with a methylmercury fungicide. There were 6,500 recorded hospital admissions within a 18 months period and 459 hospital deaths among these farmers (Eisler, 1987).

5.7 Regulatory Limits and Guidelines

The JECFA has established a PTWI of 300 μg of total mercury per person, of which no more than 200 μg should be present as methylmercury (WHO, 1972). In 1988 the committee reassessed the PTWI and confirmed the previously recommended value of 200 μg (3.3 μg/kg body weight) methylmercury for the general population but noted that pregnant women and nursing mothers are likely to be at greater risk to adverse effects of methylmercury. The data were insufficient to recommend a specific tolerable weekly intake for this segment of the population (UNEP/FAO/WHO, 1988).

5.8 Analytical Methods

Mercury is determined by flameless atomic absorption. This procedure is a physical method based on radiation at 273.7 nm by mercury vapor. The mercury is reduced to the elemental state and aerated from solution in closed system. The mercury vapor passes through a cell positioned in the light path of an atomic absorption spectrophotometer. Absorbance is measured as a function of mercury concentration (UNEP/FAO/IAEA/IOC, 1984b).

6. CONTROL AND MONITORING

Control must be undertaken after identification of an acceptable rate of each metal in terms of avoiding both environmental and economical consequences. How this control is to be determined dictates the practical requirements for knowledge of toxicity for metals.

Metals may be measured in the water itself, in the underlying sediment or in permanent resident biota. Analysis of the water is complicated by the low concentrations involved and the consequent sophistication of the techniques to be employed. Therefore, additional complications may be caused by variable composition of water (water hardness and pH) and the chemical form of metal which is actually involved. A number of studies of chemistry of aquatic metals have considered the detail chemical speciation of metals in the water; however, in practice such techniques are not easily transferable to routine monitoring at present.

In contrast, sediment and biota contain higher concentrations of metals, because the chemical extractions involved are presented in standard medium for analysis. Although both sediment and biota analysis provide useful information in relation to special and temporal trends and possible hazard levels (e.g. critical body burden) there are at present no reliable methods for translating tissue or sediment concentrations into equivalent water concentrations. This is necessary to enable direct estimation of the acceptable rate of effluent discharges.

Effluents may be controlled in two ways. One option is to require all discharges to be treated as well as possible, but this may be very expensive. The alternative is to base control

on the known toxicity of each metal in water and to ensure that environmental concentrations never reach critical concentration. This requires reliable toxicity information, preferably supported by well documented field studies.

The metal contamination of aquatic organisms may be employed in monitoring for three main purposes. First and most important is the protection of human health, and, for organisms harvested as food, direct analysis is the only guarantee of protection. This type of monitoring is comparable with that of water concentrations where the concentration is judged against predetermined acceptable standards. In the case of contamination of food organisms these standards are normally based upon the concept of maximum acceptable intake over a period of time such as day or week (Sherlock, 1983b).

Apart from the obvious need to safeguard human health, monitoring of heavy metal contamination has two uses of practical importance: spatial and temporal monitoring. Spatial monitoring has two additional purposes: at a macro-scale, there is the identification of potentially unknown areas of elevated contamination, and at micro-scale there is the need to assess the extent of the zone of contamination. Temporal monitoring is used to identify the trend of contamination, especially near effluents, in order to identify stability, improvement or deterioration. These spatial and temporal monitoring information is used to assess the risk to top predators and consumers in particular situations.

PART II: CHLORINATED HYDROCARBONS

1. INTRODUCTION

Chlorinated hydrocarbons are a group of organic compounds which are substituted with chlorine (Figure 5). In contrast to the other contaminant groups, all of the below-mentioned chlorinated hydrocarbons are exclusively of anthropogenic origin. They are commonly used for agricultural and industrial purposes.

1.1. DDT and Its Metabolites

DDT is the abbreviation for dichloro-diphenyl-trichloroethane. Out of the chlorinated pesticides used for agricultural purposes, DDT is one of the most widespread. However, because of its toxicity, it has been banned for many years in most countries. In the environment it can be degraded by solar radiation or metabolized in organisms. Dehydrochlorination gives the metabolites DDE and the dechlorination the metabolite DDD. The main component in technical formulations is the isomer p,p'DDT which yields the corresponding metabolites p,p'DDE and p,p'DDD. The o,p'DDT isomer occurs in formulations in low concentrations as an impurity.

1.2. Hexachlorocyclohexane (HCH)

This fully chlorinated alicyclic compound, was also known (incorrectly but commonly) as benzene hexachloride (BHC). HCH can exist as seven stereoisomers but the technical product contains only 5 of these, the most common being alpha, beta, gamma, and delta. The gamma-isomer known as lindane is the one normally used as an agricultural pesticide because it is 100 to 1000 times more efficient than the others. HCH is the more water-soluble and it is considered as one of the less persistent organochlorine pesticides (UNEP/FAO/WHO/IAEA, 1990).

1.3. Hexachlorobenzene (HCB)

HCB is a fully chlorinated compound formed when all the hydrogen atoms are substituted by chlorine atoms. It is generally accepted to be stable and persistent in the environment. It is used as a general fumigant and fungicide in grain storage. It also occurs in many products as an impurity and can be found in the environment as lindane metabolite.

1.4. Aldrin - Endrin - Dieldrin

Aldrin is an alicyclic chlorinated hydrocarbon and is therefore less resistant to oxidation than the aromatics. It is rapidly converted to the epoxide, dieldrin, which is also used as a pesticide. Further degradation of the dieldrin occurs but much more slowly. Endrin is a stereoisomer of dieldrin and is one of the most toxic of the chlorinated pesticides. The use of aldrin as broad spectrum insecticide has been restricted or banned in many countries. One of its continuing uses is in the treatment of soil around buildings for termite control.

1.5. Polychlorinated Biphenyls (PCBs)

PCBs are a group of aromatic organochlorines which are used only as industrial products. The commercial production of PCBs began in the 1930s. These are produced by the chlorination of biphenyl with anhydrous chlorine. The mixture obtained is purified and, during this process, hydroxylated biphenyls and chlorinated dibenzofurans can be formed.

Commonly used technical mixtures of PCBs are Aroclor, Clophen, Phenochlor, Pyralene, Kanechlor, Santhotherm and Fenchlor. Aroclors are classified as 1200 series (in which 12 as the two digits indicate a PCB mixture and the third indicate the percentage of chlorination). For example, both Aroclor 1260 and Clophen A60, are technical PCB mixtures of 60% chlorination, although their exact composition are slightly different. Depending on the methods applied for their synthesis, these technical mixtures can consist of up to 209 individual compounds distributed among the 10 levels of chlorination (Figure 5). The term congener is applied to any of the 209 possible PCBs. Isomers are PCBs that have the same number of chlorine atoms with different arrangements of the chlorines on the biphenyl rings. All congeners were given code numbers by Ballschmiter and Zell (1980) and this system has been adopted by the International Union of Pure and Applied Chemists: for this reason, the numbers are also called IUPAC numbers. The current availability of the high resolution capillary column gas chromatography makes possible the separation of many of these compounds from each other and thus to be analyzed as individual compounds.

2. SOURCES AND INPUTS OF CHLORINATED HYDROCARBONS INTO THE MARINE ENVIRONMENT

2.1 Production and Use

2.1.1. Chlorinated Pesticides

Before the mid-1940s the primary pesticides in use were either botanical in origin or compounds of heavy metals. Subsequently, there has been a marked increase in use of

synthetic organic pesticides. In the mid-1960s there was a slight shift in the type of pesticides used, from the organochlorine to the less stable organophosphate and carbamate classes. Shifts in uses of the major classes reflect the restrictions that have been placed on the use of organochlorine compounds since the early 1970s because of their persistence in the environment and their accumulation in biologic as well as non-biologic media. In Morocco the use of these pesticides has been restricted since 1984 (M.A.R.A., 1985).

2.1.2. Polychlorinated Biphenyls

PCBs have been produced industrially since the 1930s and were or are manufactured in many industrial countries. Because of their good thermal and chemical stability and dielectric properties, PCBs are used as coolants, as dielectric fluids in transformers and capacitors, as heat transfer fluids, and as coatings to reduce the flammability of wood products. These products are used in the formulation of lubricating and cutting oils, and as plasticizers in paints, copying paper, adhesives, sealants and plastics (WHO, 1976; Geyer et al., 1984; Alford-Stevens, 1986).

2.2. Input into the Marine Environment

2.2.1. Atmospheric Inputs

Exchange of organochlorine and other chemicals across the air-sea interface can occur in variety of ways; downward gaseous transport by incorporation of contaminants in precipitation and by direct transfer across the air-sea interface, and from heavy rains and washout through particulate transport. Upward transport contributes to this input by molecular evaporation from surface and purging by bubbles (GESAMP, 1980).

Comparison of atmospheric and riverine input rates of organohalogen compounds to the oceans made recently by GESAMP (1989) shows that pollution of the marine environment by these contaminants through the atmosphere is more important than through river discharges (Table 6).

2.2.2. Other Discharges

Agricultural run-off and discharge of industrial and municipal wastes into rivers constitute an important input of chlorinated hydrocarbons to the sea (Figure 6). It was estimated that a total load of about 90 tons per year (range 50-200) of organochlorine pesticides enter the Mediterranean sea carried by surface run-off or through rivers (UNEP/FAO/WHO/IAEA, 1990). Mediterranean coast of Morocco (Kessabi et al., 1988) has shown that high concentrations of these pollutants are discharged into the marine environment. Similar studies have been

conducted in other Mediterranean countries; for example on the French coast, Elder (1976), has shown that high concentrations of chlorinated hydrocarbons occurs near the mouth of the Rhine river and similar results were obtained by Burns and Villeneuve (1982) working off the Var river estuary, near Nice.

Table 6. Comparison of atmospheric and riverine input rates of organochlorine compounds to the world oceans.

Compound	Atmospheric ($\mu g/m^2/Yr$)	Riverine ($\mu g/m^2/Yr$)	% Atmospheric of Total Input to the Ocean
HCH	143	0.1-0.2	99
PCBs	0.72	0.1-0.2	78
DDTs	0.49	0.01	98
Chlordane	0.066	0.01	87
Dieldrin	0.13	0.01	93
HCB	0.23	0.01	96

Table extracted from UNEP/FAO/WHO/IAEA, 1990.

3. LEVELS AND TRENDS IN MARINE ORGANISMS

Mean of PCBs found in marine organisms from the Mediterranean sea ranges from 1.5 to 815 $\mu g/kg$ f.w. More numerous data are available only for the mussel and the red mullet. Both of these species appear to have the highest concentrations (Table 7). The high maximum and low mean values observed in some areas are explained by the existence of "hot spots" (UNEP, 1989).

Means of DDT range from 0.1 to 343 $\mu g/kg$ f.w. with *Thunnus thunnus* having the highest contamination levels. Again high maxima are due to "hot spot", p,p'DDD levels range from 0.4 to 325 $\mu g/kg$ f.w. and p,p'DDE from 1.5 to 600 $\mu g/kg$ f.w. Dieldrin mean values range from 0.4 to 6.2 $\mu g/kg$ f.w., and aldrin mean values from 0.2 to 2 $\mu g/kg$ f.w., HCB from 0.7 to 20 $\mu g/kg$ f.w. and lindane from 0.4 to 19 $\mu g/kg$ f.w. (UNEP, 1989).

4. HEALTH EFFECT

4.1. Chlorinated Pesticides

DDT was used extensively during World War II in control of lice and other insects by direct application to humans. There is no evidence of harmful effects to these people resulting from this direct application. However, some acute, nonfatal poisoning occurred as a result of suicide attempts.

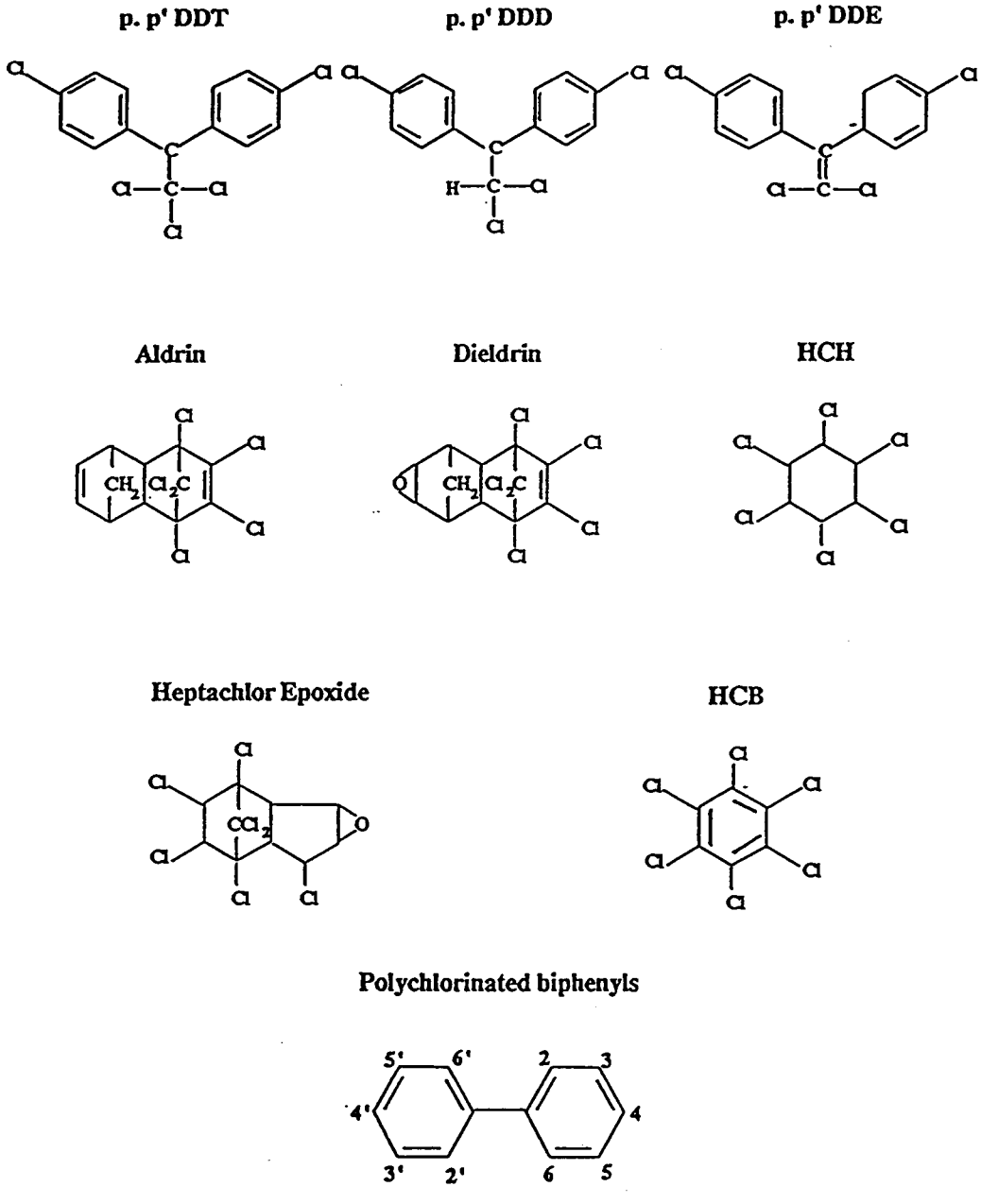

Figure 5. Structural formula of some chlorinated pesticides and PCBs.

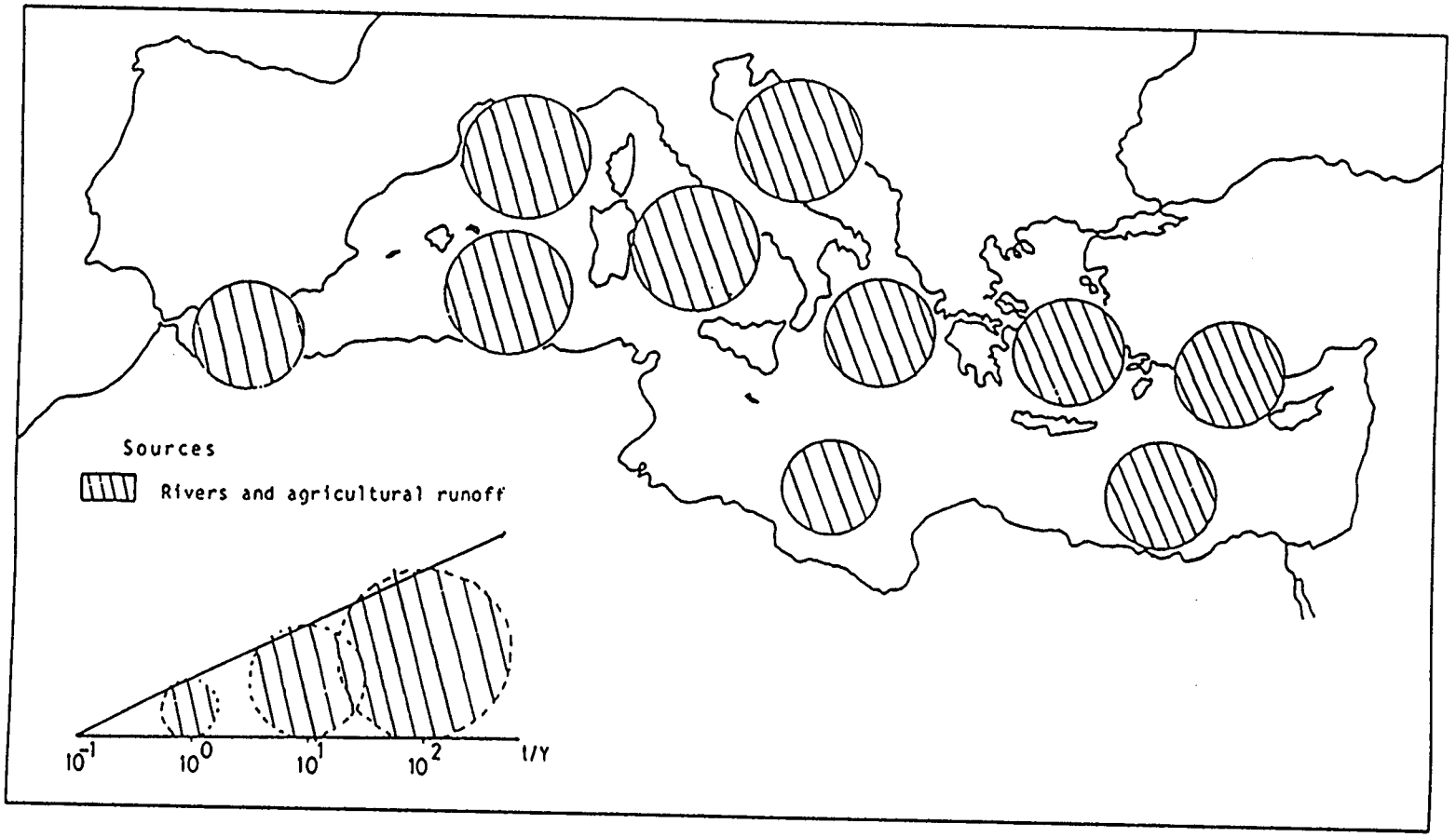

Figure 6. Regional contribution of organochlorine pesticides to the Mediterranean Sea (UNEP/FAO/WHO/IAEA, 1990).

The toxicity of DDT has been reviewed by various expert bodies (IARC, 1974; WHO, 1979). The only demonstrated impact on the general population is the accumulation of residues of DDT and some of its derivatives in body tissues, followed by excretion in urine and milk (WHO, 1979). The health significance of these residues is not currently apparent and remains to be further evaluated. There is also convincing evidence that DDT and metabolites accumulate in natural food chains. NCI reviewed the results of twenty-five laboratory animal carcinogenicity assays for DDT. All of these studies showed sufficient evidence of carcinogenicity except one which was conducted in Osborne-Mendel rats exposed to dietary doses of 25-40 mg/kg/day for 78 weeks gave no indication of DDT tumorigenicity (NCI, 1978a). Reviews of animal carcinogenicity data indicate observation of tumors (generally of the liver in seven studies in various strains of mice and rats, but no adequate epidemiological studies in human carcinogenicity of DDT have been conducted; thus DDT has been classified as a probable human carcinogen (USEPA, 1987a).

Unlike the situation with DDT, there have been a number of fatalities of acute poisoning by the cyclodiene insecticides. Davies and Lewis (1956) reported that 49 cases of endrin poisoning resulting from eating bakery foods that had been prepared with endrin contaminated flour. Several human fatalities related to drinking endrin emulsions were also mentioned by Hayes (1963).

In addition, Hodge et al. (1967) have reviewed the acute, subacute, and chronic toxicity studies of aldrin and dieldrin. For 12 animal species the acute lethal doses for both compounds ranged between 20 and 70 mg/kg. The lowest dietary levels that resulted in some mortality in feeding studies of several mammal species were: monkeys (5 mg/kg of dieldrin); mice (10 mg/kg of aldrin or dieldrin); rats (100 to 150 mg/kg of aldrin or dieldrin); dogs (10 mg/kg of aldrin, 25 mg/kg of dieldrin). The most apparent effect of poisoning is the increase of liver/body weight ratios and pathological changes of the liver.

Increased incidence of liver tumors in mice fed aldrin has been observed in chronic feeding studies in three different strains of mice (David and Fitzhugh, 1962; Epstein, 1975; NCI, 1978b). Tumor induction has been observed for many structurally related chemicals, including its metabolite dieldrin.

Dieldrin was classified as a carcinogen in seven strains of mice when administered orally (USEPA, 1988b). Technical hexachlorocyclohexane was found to induce liver cell tumors in mice when fed in the diet at high concentrations (IARC, 1974).

4.2 Polychlorinated Biphenyls

Contamination of rice oil with PCBs has led to a large-scale intoxication incident in Japan (Yusho, 1968) and Taiwan in 1979 (WHO, 1976; WHO, 1987b). The most predominant signs were hypersecretion from the eyes, pigmentation and acneiform eruptions of the skin, and disturbances of the respiratory system. The smallest dose of PCBs calculated to cause an effect was approximately 0.5 g over about 120 days. As the rice oil was also contaminated by chlorinated dibenzofurans (concentration around 5 mg/kg) and by PCBs (2,000-3,000 mg/kg), it is not certain that the symptoms were due to the PCBs only (WHO, 1976).

Limited evidence on carcinogenicity for humans has been obtained from an incident in Japan as well as from several occupationally exposed populations in the USA (Bahn et al., 1976; Brown and Jones, 1981) and in Italy (Bertazzi et al., 1987). However, several animal studies in three strains of rats and two strains of mice showed sufficient evidence of carcinogenicity in experimental animals, and as such it is prudent to consider PCBs as potential carcinogens for humans (Kimbrough et al., 1975; NCI., 1978c; Norback and Weltman, 1985; Ito et al., 1973; Schaffer et al., 1984).

A risk assessment for PCBs was made in fish. The estimation indicates that, based on a nonthreshold model, an increased risk of cancer can be caused by the consumption of fish containing high levels of PCBs (Cordle et al., 1982).

5. REGULATORY LIMITS AND GUIDELINES

5.1 Chlorinated Pesticides

The FAO/WHO recommended acceptable daily intake (ADI), converted to 70 kg body weight, is: dieldrin plus aldrin, 7 μg; heptachlor, 350 μg (FAO/WHO, 1972); DDT, 350 μg; HCH, 700 μg (FAO/WHO, 1979). No ADI has been established for HCB since the conditional ADI of 420 μg was withdrawn in 1978 because it was considered too high (FAO/WHO, 1979).

5.2 Polychlorinated Biphenyls

No tolerable intake levels for man or limits for PCBs in food have been established by the FAO/WHO Codex Alimentarius Commission. The United States Food and Drug Administration has suggested a maximum consumption of 1 μg/kg body weight per day for adults (Swain, 1988). Some countries have established maximum levels of PCBs in several food items and these are summarized in Table 8.

Table 7. Chlorinated hydrocarbons in western Mediterranean* marine organisms.			
Chlorinated Hydrocarbons	Species	Mean Concentration (µg/kg f.w.)	Range (µg/kg f.w.)
PCB	*Mytilus galloprovincialis*	307	22-1200
	Mullus barbatus	813	30-8,000
	Parapenaeus longirostris	12.3	0-51
p,p'DDT	*Mytilus galloprovincialis*	22	3-150
	Mullus barbatus	28	8-170
	Parapenaeus longirostris	4.2	0.3-9
	Thunnus thunnus	343	25-1,401
p,p'DDD	*Mytilus galloprovincialis*	15	5-125
	Mullus barbatus	38	0-180
	Thunnus thunnus	107	5-117
p,p'DDE	*Mytilus galloprovincialis*	13	2.2-42
	Mullus barbatus	29	11-70
	Thunnus thunnus	352	23-1,582
Lindane	*Mytilus galloprovincialis*	4.8	0.5-20
	Mullus barbatus	19	2-36
Dieldrin	*Mytilus galloprovincialis*	3.5	1-6
	Mullus barbatus	6.2	0.5-19
Aldrin	*Mullus barbatus*	0.5	0.5-0.5

Table extracted from UNEP, 1989
* Spanish and French Coasts

Table 8. Compilation of legal limits for chlorinated hydrocarbons in fish and fishery products (mg/kg f.w.).

Countries	PCB	HCB	Aldrin Dieldrin	DDT	DDE	DDD	DDTs	Endrin	Lindane
Canada	2		0.1	5	5	5	5	0.1	0.1
Denmark				2-5					
Germany		0.5	0.5-1.0				2-5	0.01	2
Iceland									0.5
Netherlands	5								0.2
Sweden	2-5	0.2	0.1				5		0.2
Thailand		0.1-0.3		5				0.3	0.5
U.S.A.	5		0.3	5	5	5	5	0.3	
Range Minimum Maximum	 2 5	 0.2 0.5	 0.1 1	 2 5	 5 5	 5 5	 5 2	 0.01 0.3	 0.1 2

Reference: Nauen (1983); USFDA (1982, 1984).

6. ANALYTICAL METHODS

For the determination of chlorinated hydrocarbon pesticides, gas chromatography (GC) with the electron capture detector (ECD) is most commonly used. However, it is first necessary to employ a suitable and elaborate extraction and cleanup procedure depending on the matrices to be analyzed. Most PCB determinations are performed either by an ECD or with mass spectrometry (MS).

ECD determination is used to identify and quantify PCBs in terms of commercial mixture based on the GC retention time of the standard of interest analyzed under the same conditions as the sample. Of the 209 PCBs synthesized (Mullin et al., 1984), only about 90 are commercially available for use in the laboratory to perform PCB determinations (Alford Stevens, 1986). Therefore, aroclors have been used to determine PCBs appearance in sample chromatograms. Although Aroclor mixtures vary somewhat from one batch to another and the samples frequently do not contain all of the PCB congeners, no alternative standards are available. In using ECD to identify PCBs, analyses are performed by matching an arbitrary number of peaks in the sample chromatogram with those of the nearest commercially available Aroclor formulation and measuring the height or area of the different PCB peaks.

The most widely used PCB measurement procedure was developed in 1973 by Webb and McCall. They determined the weight percent of the major components of each GC peak observed from packed-column separation of the PCBs. The Webb-McCall procedures involve determination of every sample component peak corresponding to an Aroclor peak but analysts frequently select only a few GC peaks and relate the heights or areas in samples to those in Aroclor standards.

McFarland and Clarke (1989) reviewing the recent and theoretical experimental studies of environmental occurrence, abundance, and potential toxicity of PCB congeners, have indicated that the biological properties of PCB isomers are significantly influenced by the number and the position of the chlorine atoms. Hence, for a thorough analysis of PCB in environmental samples, it requires single component identification as well as the structural aspects of their degradation and toxicity. The identification of single components in technical PCB mixtures by capillary gas chromatography has been done for Aroclor (Monsanto, USA) and Clophen A (Bayer, Germany) by Ballschmiter and Zell (1980). Similar work has been done by Capel et al. (1985) using capillary column separation of the three aroclor mixtures: 1242, 1254, and 1260 to identify the single components along with the congener percentages in technical preparations.

MS determination, a mass spectrometer provides, in addition to all of the information obtained with an ECD, the molecular weight and the number of chlorine atoms in the PCBs. This information confirms the PCB identification.

PART III. MIXED FUNCTION OXIDASE

1. INTRODUCTION

In the past decade, considerable attention has been given to the concept that biochemical and physiological characteristics can serve as sensitive biomarkers of contaminant exposure and environmental quality. Induction of mixed-function oxidase (MFO) enzymes is one such parameter that has been proposed as an indicator for identifying environmental contaminant (Rattner et al., 1989). The use of MFO for monitoring contaminant exposure in invertebrates and fish has been reviewed in depth by Payne and coworkers (1987). They concluded that a variety of organic contaminants can result in MFO induction and that the extreme sensitivity of this response can be of value in addressing concerns about the toxicological significance of "high-profile" chemicals (and potent inducers) such as polycyclic aromatic hydrocarbons and organochlorines.

Recently MFO use to monitor contaminant exposure in wildlife has been reviewed by Rattner et al. (1989). In their review they emphasized the utility of MFO of wildlife for predicting exposure, toxicological hazard and environmental quality. However when using such indicators in birds and mammals, age, sex and season are a source of variation that requires consideration when field trials are conducted.

2. MIXED-FUNCTION OXIDASE SYSTEMS

MFO, also referred to as monooxygenases, or multienzyme system principally localized in the smooth endoplasmic reticulum. Components of this system include cytochrome P450 (a group of heme-containing isoenzyme, each catalyzing a limited overlapping spectrum of biotransformation), NADPH-cytochrome P450 reductase (a flavoprotein capable of transferring one or two electron to cytochrome P450) and phospholipids (Lee et al., 1980). MFO enzymes play a critical role in detoxification by carrying out a series of oxidation reactions. The relatively insoluble organic compounds are converted into water-soluble metabolites which may be further conjugated and excreted in urine or bile (Bend and James, 1978; Lech et al., 1982; Payne, 1984). In these oxidation reactions one atom of molecular oxygen is reduced to water while the other is incorporated into an enzyme substrate. Substrates include foreign compounds (e.g. many drugs, pesticides and polycyclic aromatic hydrocarbons and endogenous substrates such as steroid hormones, vitamins and bile acids). Although, MFO is considered as a detoxifying system, in some cases the intermediate product formed is more toxic than the parent compound (e.g. epoxidation of aldrin to dieldrin, desulfuration of parathion to paraoxon and activation of some polycyclic aromatic hydrocarbons (PAHs) to proximate carcinogens.

2.1. Induction of MFO Enzymes and Toxicological Implications

Treatment or exposure of animals to various drugs, pesticides and industrial chemicals has been known to induce mfo activity (Conney, 1967; Khan, 1984). Many xenobiotics of major environmental interest including polycyclic aromatic hydrocarbons (PAHs) (Gerhart and Carlson, 1978), polychlorinated biphenyls (PCBs) (Addison et al., 1982), polybrominated biphenyls (PBBs) (Franklin et al., 1982), and petroleum hydrocarbons (Payne and Penrose, 1975; Chambers, 1979; Collodi et al., 1984). All of these compounds are quite effective in inducing MFO in fish. Historically, inducers were divided into two major classes based on the morphological and biochemical responses they evoked in laboratory rodents. One class included barbiturates, insecticides and some industrial chemicals, (e.g. DDT, o-polychlorinated biphenyls, and o-polybrominated biphenyls) that cause slight increase in liver weight, and increase synthesis of proteins and induction of some cytochrome P450 isoenzyme: (referred to as phenobarbital (PB)-type inducers) (Khan, 1984; Rattner et al. 1989). The second class includes some PAHs [e.g.3-methylcholanthrene (MC), benzo(a)pyrene B(a)P, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), mono-ortho-PCBs and PBBs] that cause slight increases in liver weight, an increase of specific group of cytochrome isoenzymes (cytochrome P448 e.g. aryl hydrocarbons hydroxylase) referred to as MC-type inducers (Khan, 1984).

2.2. Field Investigations Using MFO as Biochemical Monitor of Contaminant Exposure in Fish.

Several approaches have been used to study MFO as potential indicators of contaminant exposure in fish. These include: (a) examining the association between contaminant burdens and MFO activity, (b) comparing MFO activities between animals living at polluted sites and those collected from relatively unpolluted sites, and (c) monitoring changes in MFO activity at various intervals following a pollution event.

2.2.1. Field Trials Associated with Hydrocarbon Pollution

Several field investigations have shown an elevated MFO enzyme levels in fish in association with hydrocarbon pollution. The earliest studies were carried out in the early 1970s in Newfoundland, where it was shown that brown trout (*Salmo trutta*) taken from a small urban lake with a history of hydrocarbon contamination had elevated benzo(a)pyrene (B(a)P) hydroxylase levels in liver tissue (Payne and Penrose, 1975). In another study, near the site of a large oil refinery in Placentia Bay, Newfoundland, liver and gill tissue of cunners were observed to have elevated MFO enzyme levels (Payne, 1976). Similar field studies were carried out in the Northern Adriatic Sea have also shown that blennies (*Blennius pavo*) collected near

the area of the oil spill displayed a marked level of enzyme induction. This activity level remained elevated for 2-3 weeks period (Kurelec et al., 1977). Fish collected in a natural petroleum seep in the Pacific Ocean have been shown to contain elevated B(a)P hydroxylase levels (Spies et al., 1980; Spies et al., 1982). Davies et al. (1984) have also reported elevated MFO enzyme levels in codfish (*Gadus morhua*), haddock (*Melanogrammus aeglefinnus*), and whiting (*Merlangius merlangus*) around oil rigs in the North Sea. A relatively recent study was carried out in Finland in perch (*Perca fluviatilis*) collected at the site of an oil spill in the Vassa Archipelago. The perch were observed to have elevated MFO enzyme levels in comparison with fish taken from control sites (Lindstrom-Seppe et al., 1985). In this study, enzyme activities were still detectable after 4 months and returned to levels found at control sites after 9 months. All these field studies with a freshwater and marine species confirm the value of the MFO enzyme system as a sensitive monitoring tool for point sources of petroleum hydrocarbons as well as establishing "recovery" from the effects of oil pollution.

2.2.2. Field Trials Associated with Mixed Organic Pollution

A number of field studies have now been carried out linking induction with organic mixed pollution. Mixed organic wastes could contain trace levels of potent inducers such as selected organohalogens, dioxins and aromatic hydrocarbons. However, a depressed, rather than elevated, MFO enzyme levels have been found in fish taken from a lake in Finland receiving high concentrations of pulp mill effluents (Ahokas et al., 1976). It has been speculated that high concentrations of strong inducing agents such as sulphite, which is used in some pulp bleaching processes, may inhibit MFO enzyme levels through cytochrome reduction and inactivation (Payne et al., 1987).

The induction of MFO has also been evaluated in an experimental trial on ocean incineration of hazardous waste. The enzymes catalase and adenosine triphosphatase as well as cytochrome P450 were measured in mummichogs held in cages near the sea-incineration trials of organochlorines in the Gulf of Mexico in 1977 (Kamlet et al., 1981). Of these, only P450 showed a significant increase.

All of these trials carried out around sewage outfalls or marine dumpsites demonstrate that MFO induction can be a useful index for assessing water quality in these point sources of mixed domestic/industrial waste.

Other studies have focused on non-point sources of organic pollution. An investigation has been carried out in the Sava, a large river in Yugoslavia which flows through an area heavily polluted with domestic and industrial wastes. A number of fish species, including barbel (*Barbatus barbatus*), chub (*Leuciscus cephalus*) and nase (*Chondrostroma nasus*) taken from

the heavily polluted stretch of the river, showed elevated MFO levels compared with fish taken from less polluted tributaries of the river (Kezic et al.,1983).

Lindstrom-Seppe et al. (1985) reported that the liver tissues of fish taken downstream from a fish hatchery had MFO enzyme levels comparable with fish taken upstream which had lower body burden of PCB. However the PCB concentrations in the fish taken downstream were still relatively low and according to laboratory experiments with trout, they could be below the threshold levels required for induction (Addison et al., 1978; 1981). Tukey et al. (1982) suggested that concentrations of PCB (or other inducers) found in water, food or sediment may often be so low that they can be sequestered into cellular lipids and not be available in sufficient threshold concentrations for binding to the specific cytosol receptors to initiate the induction process.

2.3. Variability of MFO Enzymes

Factors such as sex and state of gonadal maturity influence biochemical, physiological and histological parameters in fish and induce variations in MFO responses (Payne et al., 1987). These variations are related to hormonal status such as steroid and thyroxine levels. Seasonal abundance of chemicals or dietary inducers could also play a role. Thus, when sampling, comparable groups of animals should be collected from experimental and reference sites at similar times of the year to minimize this variability.

2.4. Sensitivity of MFO Enzyme Induction

Most biochemical changes are secondary in nature and appear in association with cellular damage, nutritional deficiencies, etc., but MFO enzyme induction is a primary detoxification response. Thus, its use as an environmental monitor can serve as an early warning of possibly serious pathologies, especially when potent chemical inducers such as PCBs, dioxins and PAHs are considered. Payne et al. (1987) and Nikunen (1985) carried out a field study to compare the sensitivity of MFO enzyme with a large variety of other parameters. This field study was carried out near a large petrochemical complex in Parov on the southern coast of Finland. Experiments were conducted on rainbow trout held in cages near the waste water discharge as well as at a reference site 10 km away. A large number of parameters including serum and liver enzymes as well as condition indices, levels of plasma ions, liver lipids, glycogen and protein were investigated after a 21-day of exposure. The only clear differences among the 25 parameters measured in both groups were an increase in activity of, the detoxification enzymes, MFO and UDP glucuronyltransferase.

3. CONCLUSION

In the review of Payne and coworkers (1987), compelling data of laboratory and field studies demonstrate that induction of MFO activity is a sensitive response for monitoring petroleum hydrocarbons and mixed organic pollution in aquatic invertebrates and fish. Variations due to factors such as hormonal status and seasonal effects have been examined to some degree as well. Effects of factors cytochrome P450 isoenzymes in aquatic forms have been well characterized, and there is a considerable documentation of induction by petroleum hydrocarbons. Cytochrome isoenzyme P4501A1 is induced by pollutants such as PCBs, dioxins and PAHs, etc.., and has been used as an index of environmental contamination in fish. One major problem with this approach is the fact that both PAHs and halogenated compounds (PCBs, TCDD, etc.) induce P4501A1. Fishes metabolize PAHs very rapidly and one of the only ways of showing environmental exposure is to look for its "fingerprint", i.e. P450 induction. By contrast PCBs, TCDD, etc. are not readily metabolized and remain as residues in fish.

CHAPTER III

CADMIUM, LEAD AND MERCURY IN MOROCCAN FISHERY PRODUCTS FROM THE MEDITERRANEAN SEA

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ABSTRACT

Contamination of seafood products collected near Moroccan coasts of the Mediterranean Sea, by cadmium, lead, and mercury, has been evaluated using atomic absorption spectrophotometry on 82 fishes of 17 species.

Results are given for each species. Ranges of contamination for edible species varied between 0.005 and 0.150 mg/kg fresh weight for cadmium; between 0.019 and 0.450 mg/kg f.w for lead and between 0.13 and 0.79 mg/kg f.w. for mercury.

These levels are compared to those already published in other countries. This comparison demonstrates that concentrations found in Morocco are slightly higher; however, they are under the acceptable limits established by several countries where legislation about residues is more exigent.

INTRODUCTION

Contamination of the marine environment by heavy metals could result in serious problems as for example Minamata disease in Japan. In contrast to organic pollutants, heavy metals are not subject to either chemical or biological degradation and thus can accumulate in the marine food chain, reaching sometimes toxic concentrations (Dorn, 1979; Cumont, 1984; Eilser, 1985; 1987).

The aquatic environment receives most of these pollutants which could lead to changes in behavior and affects the health of marine organisms. Moreover, it can induce human health risks through the consumption of contaminated seafood products especially among the high fish consumers.

This investigation was carried out in order to determine contamination levels in fish products for lead, cadmium and mercury. The samples were collected in the occidental part of Mediterranean Sea, near Moroccan coasts, to evaluate the extent of this contamination.

MATERIAL AND METHODS

Seventeen fish species were obtained by trawling (Figure 7). All samples were stored in plastic bags and deeply frozen at -18°C until analyses. In the laboratory, every fish was dissected and lateral muscle samples were mixed, then ground and homogenized. Aliquot fractions weighing 1 g were prepared for digestion (UNEP/FAO/IAEA/IOC; 1984a).

Lead and cadmium, digestion was carried out by dry ashing with 1 mL of saturated solution of sulfuric acid at 700°C for 4 hours in a Thermolyne 1500 Furnace. Ashes were dissolved with saturated solution of nitric acid. Measurements of concentrations of both

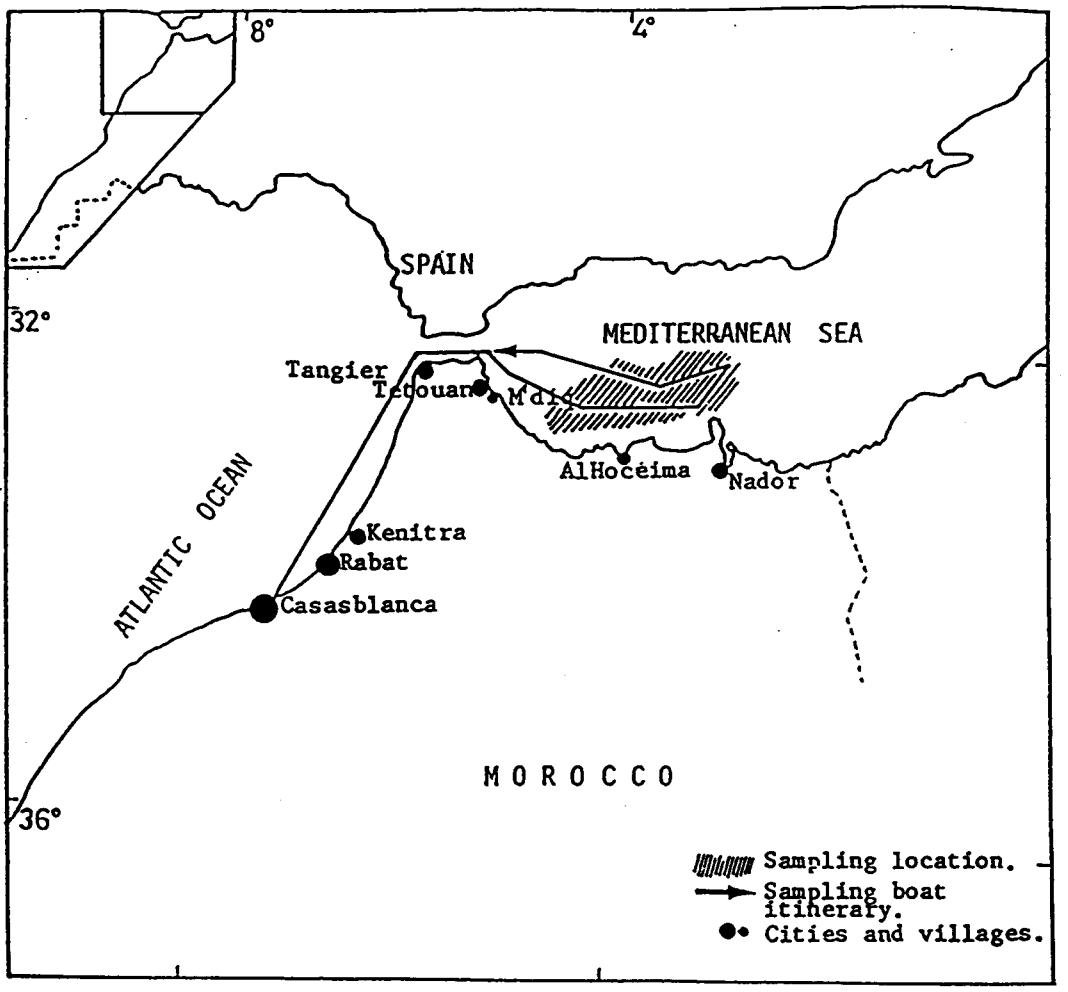

Figure 7. Map showing the sampling location of marine organisms.

elements were obtained on a graphite furnace (HGA 700) atomic absorption spectrophotometer (Perkin-Elmer 1100B) (UNEP/FAO/IAEA/IOC, 1984b).

Sample digestion for mercury analysis was carried out in 5 ml of concentrated nitric acid at room temperature overnight for predigestion and completed in a water bath at 90°C for 3 hours. Concentrations were measured using a Colman mercury analyzer MAS B50 (Lazzlo, 1972; UNEP/FAO/IAEA/IOC, 1984c).

To avoid any contamination, all glassware used was systematically soaked in diluted nitric acid solutions and rinsed several times with demineralized water before use. Blank samples and certified reference materials (International Atomic Energy Agency) were analyzed with every set of 20-40 samples to check the accuracy of determinations. Average percent recover ranged from 85 to 92%.

RESULTS AND DISCUSSION

Contamination levels of seafoods are given in Table 9 for cadmium, lead and mercury.

CADMIUM

Cadmium was detectable in the 82 fish samples analyzed. Concentrations were rather low. They were found to range between 0.005 and 0.150 mg/kg. These concentrations are similar to those found in the same species in other Mediterranean locations; for example, the average concentrations for *Mullus barbatus*, a pollution indicator of the Mediterranean Pollution Programmes (MED POL) was 0.046 mg/kg f.w. for Mediterranean regions (UNEP/FAO/WHO, 1989).

These concentrations are slightly higher than those found in fishes collected on Holland coasts and the North Sea coasts. In these two situations, cadmium concentrations were found to be between 0.002 and 0.006 mg/kg (Vos and Hovens, 1986) and 0.005 mg/kg in soles (Vyncke et al., 1984).

Several countries have established legal limits in their fish and fishery products; in the majority of cases limits range between 0.1 and 0.5 mg/kg (Nauen, 1983). The levels detected in this study are all acceptable according to these established limits.

LEAD

Our data demonstrate that concentrations of lead are between 0.019 mg/kg for ray (*Raja marginata*) which is the less contaminated species and 0.45 mg/kg for longnose spurdof (*Squalus blainvillei*). In all cases, these concentrations were found to be slightly higher than those published for fishes collected in the North Sea and in the Ireland Sea. Vos and Hovens

(1986) reported concentrations of 0.04 mg/kg for ray, plaice and cod and 0.006 mg/kg for herring. Vynke et al. (1984) described concentrations of 0.03 and 0.14 mg/kg in rays collected in North Sea and Ireland Sea.

Several countries have decided acceptable levels for cadmium in seafoods. In Holland, this threshold is 0.5 mg/kg for fishes and shrimps and 2 mg/kg for mussel (Vos and Hovens, 1986). When comparing the results obtained on Moroccan coasts, all the concentrations are acceptable according to this threshold.

MERCURY

Mercury was found in all samples. The concentrations were found to range from 0.13 and 0.79 mg/kg for most edible fish species. These concentrations are rather high. However, they are still under the acceptable concentrations for human consumption since several countries have established limits for mercury in their seafood products (Nauen, 1983; USFDA, 1982) and in the majority of cases limits have been set between 0.5 and 1 mg/kg as total mercury.

Of 82 fishes which were considered in this study, only one (1.22%) was found to present higher concentrations (1.268 mg/kg). This is not surprising since longnose spurdof (*Squalus blainvillei)* is predator fish and this species is not used for human consumption, so that there is no risk at all for human health. In all cases, this data confirms that mercury contamination in the Mediterranean Sea must be considered by authorities since rather high concentrations are detected in some fish species.

CONCLUSIONS

This first report about heavy metal contamination of seafood products of Morocco demonstrated that concentrations which were measured on 82 fishes from 17 species are rather similar to those found in other countries. No problems are likely to occur with respect to human consumption of fishery products since the levels are below the acceptable thresholds established by different countries. In order to support such data, further investigations should be carried out to check the geographical and seasonal variations of these concentrations. Such data will be helpful to be combined with a survey of daily intake of seafood products in Morocco in order to establish the risk assessment for human consumers.

Table 9. Cadmium, lead and mercury concentrations in marine organisms from the western Mediterranean Sea (Mean and Range).

Scientific name	Common name	Samples	Concentrations (mg/kg f.w.)		
			Cd	Pb	Hg
Trachurus trachurus	Horse mackerel	4	0.013 (0.005-0.02)	0.092 (0.027-0.137)	0.494 (0.24-0.54)
Boops boops	Bogue	6	0.065 (0.01-0.12)	0.239 (0.023-0.565)	0.641 (0.35-0.72)
Pagellus acarne	Axielary seabream	4	0.023 (0.012-0.04)	0.291 (0.013-1.04)	0.347 (0.10-0.55)
Beryx decadactylus	Alfonsino	4	0.029 (0.007-0.05)	0.038 (0.033-0.042)	0.719 (0.21-0.8)
Solea solea	Sole	4	0.032 (0.035-0.03)	0.042 (0.013-0.085)	0.335 (0.15-0.55)
Mullus barbatus	Red mullet	6	0.010 (0.005-0.015)	0.019 (0.012-0.03)	0.719 (0.32-0.83)
Raja marginata	Ray	4	0.006 (0.005-0.007)	0.038 (0.033-0.042)	0.572 (0.24-0.68)
Octopus vulgaris	Common octopus	4	0.005 (0.003-0.006)	0.172 (0.013-0.215)	0.415 (0.28-0.54)
Lophius budegassa	European anglerfish	14	0.150 (0.005-0.3)	0.316 (0.011-0.595)	0.797 (0.51-0.89)
Loligo forbesi	Forbe squid	5	0.007 (0.005-0.014)	0.036 (0.012-0.05)	0.415 (0.22-0.65)
Chronia nodifera	Knobby triton	8	0.086 (0.045-0.12)	0.300 (0.27-0.35)	0.412 (0.24-0.66)
Merluccius merluccius	European bake	6	0.120 (0.04-0.13)	0.075 (0.01-0.12)	0.327 (0.18-0.52)
Merluccius senegalensis	Senegalese bake	4	0.075 (0.01-0.12)	0.120 (0.04-0.13)	0.366 (0.15-0.41)
Nephrops norvegicus	Norway lobster	2	0.075 (0.030-0.09)	0.120 (0.09-0.15)	0.131 (0.07-0.21)
Parapenaeus longirostris	Deep wach roses-shrimp	2	0.014 (0.008-0.04)	0.150 (0.01-0.175)	0.592 (0.35-0.71)
Sepia officinalis	Common cuttle fish	4	0.014 (0.009-0.05)	0.095 (0.065-0.14)	0.616 (0.34-0.75)
Squalus blainvillei	Longnose spurdog	1	0.097	0.450	1.268

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CHAPTER IV

CONTAMINATION OF MOROCCAN FISHERY PRODUCTS BY CADMIUM, CHROMIUM, LEAD, AND MERCURY

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ABSTRACT

Cadmium, chromium, mercury and lead contamination of seafood products has been evaluated near the Moroccan coast on the Mediterranean Sea, on 504 fish samples representing 60 species, collected near Tanger, Tetouan, Al Hoceima and Nador in Morocco. Contamination levels are slightly higher than those published in others countries but they are still under the threshold concentrations for human consumption. Moreover, no significant differences were observed on sample collected in different geographical ares and on species collected in different seasons.

INTRODUCTION

Contamination of the marine environment by heavy metals constitutes one of the major problems of environmental toxicology. In contrast to organic pollutants, heavy metals are not subjected to either biological or chemical degradation and hence can accumulate in food chain reaching sometimes toxic levels (Dorn, and Miller, 1975; Neanthery, 1979; Cumont, 1984) The characterization of biotransformation reactions of mercury, principally through the methylation process by microorganisms in natural conditions leading to the production of methylmercury. This form of mercury accumulated in fish and shellfish and was in the origin of large scale intoxication described for the first time in Japan and known as Minamata disease. Aware of the potential problem presented by heavy metals contamination several countries have established tolerable limits in their fishery products and are conducting a continuous monitoring for these products (Sharma and Street, 1980, Cumont, 1984; Vyncke et al., 1984; UNEP, 1989). A preliminary investigation in Morocco, during an oceanographic campaign in the Mediterranean sea, interested analysis of 82 samples representing 17 species and showed that low contamination by cadmium and moderate contamination by lead and mercury.

This work aims to complete the previous data by considering more representative sampling size and to examine if is there any seasonal and sampling site variations. Four metals are considered here because of their potential toxicity and their occurrence in relatively high concentration in marine organisms.

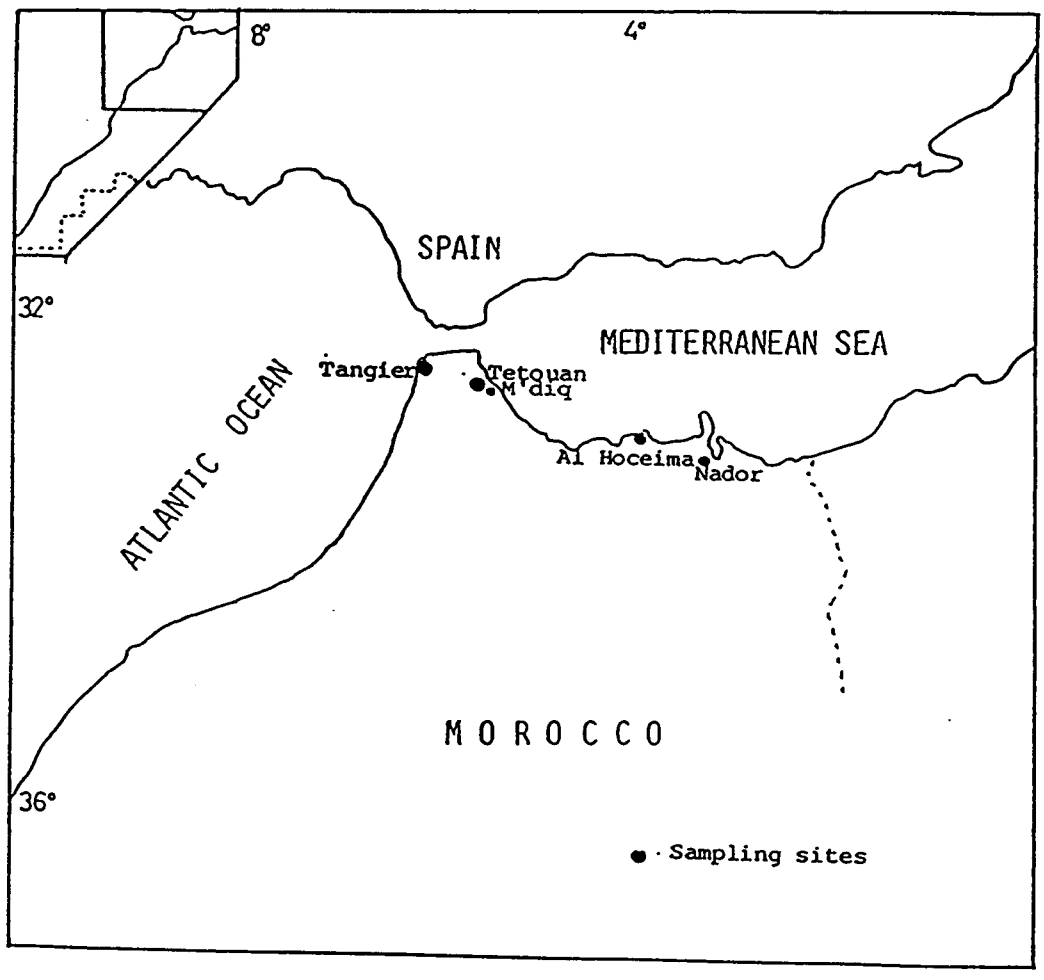

Figure 8. Map of northern Morocco showing the sampling sites of marine organisms.

MATERIAL AND METHODS

1. Sampling

A total of 504 fish samples were collected from Tanger, M'diq; Tetouan; Al Hoceima and Nador in Morocco. Fish chilled in ice were collected from the port immediately after the return of fishing boats which are fishing within 30 km in the Moroccan territorial waters (Figure 8). After being identified, the weight and the length recorded, the fish were dissected and stored in freezer at -18°C until analysis (UNEP/FAO/IAEA/IOC,1984a).

2. Sample preparation

Fish lateral muscle tissues and soft tissues of cephalopods, crustaceans and mussels were taken for analysis after the organisms being dissected. Tissues were then ground and homogenized and an aliquot fraction of 1g was taken for analysis.

3. Sample analysis

A wet procedure was used for digesting tissue to minimize any possible contamination or losses. One g of wet tissue was placed into screw-top culture tubes (175 mm x 22 mm) and 5 ml of concentrated HNO_3 was added (reagent grade). The tubes were left at room temperature overnight for predigestion and the following day were placed on heat block at 140°C for 4 hours for cadmium, chromium, and lead and in water bath at 90°C for 3 h in the case of mercury. To avoid any contamination, the tissue digest was then made up to a volume of 10 ml with 4% of HNO_3 with redistilled water.

Electrothermal atomic absorption (Perkin Elmer 5000) was used to determine cadmium, chromium, and lead concentrations (UNEP/FAO/IAEA/IOC, 1984b). Mercury was measured by flameless atomic absorption spectrophotometry using a Colman mercury analyzer (MAS B 50) (UNEP/FAO/IAEA/IOC, 1984c). Metal recovery was assessed by analysis of IAEA reference material (MA-B-3/TM) and blanks were run with every set of samples.

Species contamination, sampling sites, and seasonal variations are tested by the multivariate analysis: Principal Component Analysis (PCA) technique (Muirhead, 1982). This method focuses on the relationship within a single set of variables and describes the subjects in terms of smaller number of variables. The "new variables" are derived from the original ones by finding linear combinations of those variable that explain most of the variability. The Principal Components procedure allows the finding of the principal component given a correlation or covariable matrix, or the original variable. The percentage of variance for each component is tabulated and plots of the data for the two first principal components with one data point for each variable can be obtained to facilitate analysis of the data.

Table 10. Mercury, lead, chromium, and cadmium concentrations (mg/kg f.w.) in marine organisms from the Mediterranean coast of Morocco (Mean and Range).

Scientific name*	Number Samples	Concentrations (mg/kg fresh weight)			
		Hg	Pb	Cr	Cd
Auxis thazard	4	0.385 (0.203-0.450)	0.087 (0.047-0.100)	0.045 (0.023-0.100)	0.036 (0.022-0.038)
Belone Svetovidovi	4	0.41 (0.323-0.450)	0.38 (0.310-0.450)	–	0.024 (0.018-0.027)
Boops boops	10	0.56 (0.190-1.254)	0.195 (0.120-0.205)	0.205 (0.039-0.850)	0.032 (0.013-0.042)
Chelidonichtys cuculus	5	0.41 (0.220-0.740)	0.092 (0.045-0.175)	–	0.032 (0.018-0.037)
Chelidonichtys lucerna	5	0.657 (0.459-0.856)	0.052 (0.025-0.092	0.263 (0.185-0.300)	0.025 (0.015-0.044)
Chelidonichtys trigloporus	5	0.506 (0.456-0.205)	0.095 (0.026-0.280)	0.451 (0.024-0.568)	0.035 (0.026-0.057)
Citharus linguatula	8	0.108 (0.050-0.205)	0.349 (0.256-0.505)	0.181 (0.120-0.396	0.032 (0.016-0.050)
Dentex macrophtalmus	5	0.513 (0.500-0.570)	0.157 (0.125-0.216)	0.419 (0.3385-0.495)	0.03 (0.014-0.057)
Dentex maroccanus	5	0.315 (0.215-0.335)	0.255 (0.157-0.319)	0.565 (0.121-0.825)	0.024 (0.019-0.027)
Diplodus puntazzo	5	0.285 (0.150-0.607)	0.385 (0.250-0.890)	0.22 (0.120-0.420)	0.034 (0.016-0.067)
Diplodus vulgaris	5	0.583 (0.486-0.656)	0.104 (0.060-0.148)	0.103 (0.039-0.167)	0.026 (0.009-0.032)
Eledone cirrosa	4	0.592 (0.320-0.719)	0.16 (0.104-0.334)	0.125 (0.150-0.405)	0.021 (0.017-0.033)
Helicolenus dactylopterus	4	0.15 (0.090-0.250)	0.33 (0.124-0.560)	0.091 (0.035-0.205)	0.024 (0.018-0.040)
Illex coindettii	5	1012 (0.867-1.450)	0.22 (0.123-0.560)	0.056 (0.032-0.140)	0.029 (0.013-0.039)
Lepidotrigla dieuzeidei	5	0.517 (0.312-0.600)	0.095 (0.038-0.138)	0.159 (0.045-0.321)	0.019 (0.009-0.023)
Leligo forbesi	5	0.535 (0.248-0.717)	0.39 (0.174-0.902)	0.35 (0.180-0.520)	0.015 (0.009-0.019)
Lophius budegassa	8	0.887 (0.725-1.049)	0.132 (0.082-0.586)	0.433 (0.088-0.600)	0.041 (0.017-0.048)
Merliccius merluccius	10	0.55 (0.177-1.247)	0.166 (0.022-0.586)	0.189 (0.042-0.320)	0.040 (0.032-0.050)
Merluccius senegalensis	10	0.784 (0.628-0.817)	0.062 (0.017-0.234	0.158 (0.119-0.197)	0.032 (0.011-0.041)
Mullus surmulletus	10	0.58 (0.215-1.730)	0.118 (0.040-0.219)	0.246 (0.025-0.075)	0.032 (0.012-0.038)

Table 10. Mercury, lead, chromium, and cadmium concentrations (mg/kg f.w.) in marine organisms from the Mediterranean coast of Morocco (Mean and Range).

Scientific name*	Number Samples	Concentrations (mg/kg fresh weight)			
		Hg	Pb	Cr	Cd
Mullus barbatus	10	0.758 (0.709-0.846)	0.350 (0.031-1.037)	0.125 (0.025-0.507)	0.046 (0.022-0.054)
Mytilus galloprovincialis	40	0.605 (0.110-0.750)	0.261 (0.123-0.321)	0.36 (0.196-0.402)	–
Micromesistus poutassou	8	0.13 (0.070-0.091)	0.380 (0.110-1.050)	0.23 (0.182-0.269)	0.041 (0.017-0.047)
Micochirus theophila	5	0.41 (0.120-1.230)	0.159 (0.120-0.400)	0.023 (ND-0.100)	0.024 (0.009-0.028)
Panaeus melicertus	10	0.175 (0.115-0.215)	0.28 (0.021-0.400)	0.128 (0.075-0.273)	0.012 (0.007-0.016)
Octopus macropus	5	0.594 (0.324-0.803)	0.124 (0.014-0.324)	0.149 (0.019-0.511)	0.03 (0.016-0.047)
Octopus vulgaris	5	0.605 (0.213-0.987)	0.285 (0.035-0.560)	0.24 (0.070-0.500)	0.016 (0.013-0.022)
Pagellus acarne	10	0.605 (0.315-0.98)	0.185 (0.035-0.695)	0.319 (0.030-1.246)	0.027 (0.018-0.040)
Pagellus bogaraveo	5	0.676 (0.120-1.049)	0.167 (0.011-0.0345)	0.37 (0.050-0.940)	0.036 (0.014-0.047)
Pagellus bellottii	10	0.754 (0.120-1.23)	0.07 (0.024-0.115)	0.224 (0.1500.297)	0.037 (0.013-0.045)
Pagellus erythrynus	10	0.438 (0.121-1.02)	0.215 (0.097-0.456)	0.044 (ND-0.230)	0.032 (0.015-0.05)
Pagellus mormyrus	10	0.503 (0.342-0.670)	0.154 (0.110-0.200)	0.164 (0.118-0.205)	0.031 (0.012-0.047)
Parapenaeus longirostris	30	0.675 0.208-1.189)	0.195 (0.120-0.200)	0.092 (0.067-0.130)	0.02 (0.007-0.028)
Parapenaeus narval	20	0.23 (0.120-0.433)	0.09 (0.017-0.169)	0.185 (0.076-0.315)	0.013 (0.008-0.023)
Peristedion cataphractum	10	0.34 (0.210-0.406)	0.076 (0.060-0.140)	0.530 (0.500-0.620)	0.025 (0.013-0.033)
Phycis blennoïdes	5	0.340 (0.30-0.406)	0.03 (0.010-0.103)	0.029 (0.038-0.120)	0.035 (0.021-0.052)
Pomadasys incisus	5	0.68 (0.508-0.856)	0.019 (0.012-0.025)	0.136 (0.054-0.215)	0.041 (0.017-0.046)
Psetta maxima	5	0.32 (0.139-0.410)	0.02 (0.013-0.043)	0.037 (ND-0.074)	0.03 (0.013-0.052)
Raja montagui	6	0.484 (0.145-0.678)	0.154 (0.080-0.654)	0.168 (0.100-0.430)	0.022 (0.019-0.027)
Raja naevus	8	0.403 (0.103-0.506)	0.182 (0.056-0.346)	0.157 (0.122-0.205)	0.025 (0.017-0.047)

Table 10. Mercury, lead, chromium, and cadmium concentrations (mg/kg f.w.) in marine organisms from the Mediterranean coast of Morocco (Mean and Range).

Scientific name*	Number Samples	Concentrations (mg/kg fresh weight)			
		Hg	Pb	Cr	Cd
Sardina pilchardus	20	0.19 (0134-0.250)	0.078 (0.015-0.138)	0.13 (0.022-0.680)	0.024 (0.008-0.029)
Sardinella aurita	10	0.68 (0.220-1.23)	0.032 (ND-0.078)	0.075 (0.033-0.099)	0.034 (0.013-0.048)
Scomber japonicus	5	0.609 (0.205-1.07)	0.061 (ND-0.120)	0.043 (0.010-0.058)	0.03 (0.023-0.038)
Scorpaena scrofa	5	0.41 (0.334-0.506)	0.225 (0.160-0.290)	0.228 (0.080-0.641)	0.011 (0.007-0.021)
Scyliorhinus canicula	2	0.912 (0.705-1.120)	–	0.16 (0.089-0.201)	0.03 (0.012-0.048)
Sepia officinalis	10	0.718 (0.315-1.336)	0.26 (0.134-0.330)	0.235 (0.038-0.450)	0.036 (0.015-0.046)
Sepia orbigyna	10	0.648 (0.345-0.806)	0.152 (0.035-0.214)	0.2 (0.148-0.428)	0.037 (0.013-0.045)
Serranus cabrilla	10	0.552 (0.405-0.603)	0.152 (0.010-0.180)	0.2 (0.060-0.204)	0.028 (0.015-0.05)
Serranus scriba	10	0.457 (0.132-0.705)	0.04 (0.023-0.089)	0.05 (0.013-0.090)	0.036 (0.013-0.042)
Solea cuneata	10	0.785 (0.287-1.150)	0.25 (0.082-0.630)	0.05 (0.021-0.086)	0.031 (0.015-0.0038)
Sparus caeryleostictus	5	0.315 (0.215-0.617)	0.013 (ND-0.460)	0.129 (0.076-0.315)	0.008 (0.006-0.017)
Sparus pagrus	5	0.503 (0.400-0.700)	0.45 (0.350-0.509)	0.083 (0.076-0.129)	0.025 0.011-0.034)
Sphyraena Sphyraena	5	0.693 (0.405-0.789)	0.216 (0.105-0.512)	–	0.027 (0.021-0.052)
Squalus blainvillei	2	0.460 (0.220-0.705)	0.613 (0.337-0.89)	0.134 (0.054-0.215)	0.021 (0.017-0.026)
Todarodes sagittatus	5	0.508 (0.258-0.846)	0.18 (ND-0.462)	0.14 (0.097-0.450)	0.037 (0.013-0.058)
Todaropsis eblanae	5	0.778 (0.560-0.887)	0.17 (0.087-0.234)	0.125 (0.100-0.405)	0.024 (0.019-0.027)
Trachinus draco	6	0.662 (0.398-0.852)	0.122 (0.033-0.210)	0.199 (ND-0.337)	0.036 (0.017-0.052)
Trachurus mediterraneus	10	0.215 (0.100-0.287)	0.114 (0.036-0.630)	0.246 (0.23-0.442)	0.015 (0.008-0.026)
Trachurus trachurus	10	0.58 (0.216-0.905)	0.163 (ND-0.386)	0.194 (ND-0.589)	0.051 (0.016-0.058)
Zeus faber	5	0.68 (0.233-0.900)	0.2 (0.033-0.040)	0.067 (0.025-0.075)	0.034 (0.014-0.056)

Fish Species

Boops:	B
Chelidonichthy:	G
Dentex:	D
Diplodus:	S
Merluccius:	M
Mullus:	R
Octopus:	P
Pagellus:	PA
Raja:	RA
Sardina:	SA
Scomber:	MA
Sepia:	SE
Solea:	SO
Pagrus:	SG
Trachurus:	C

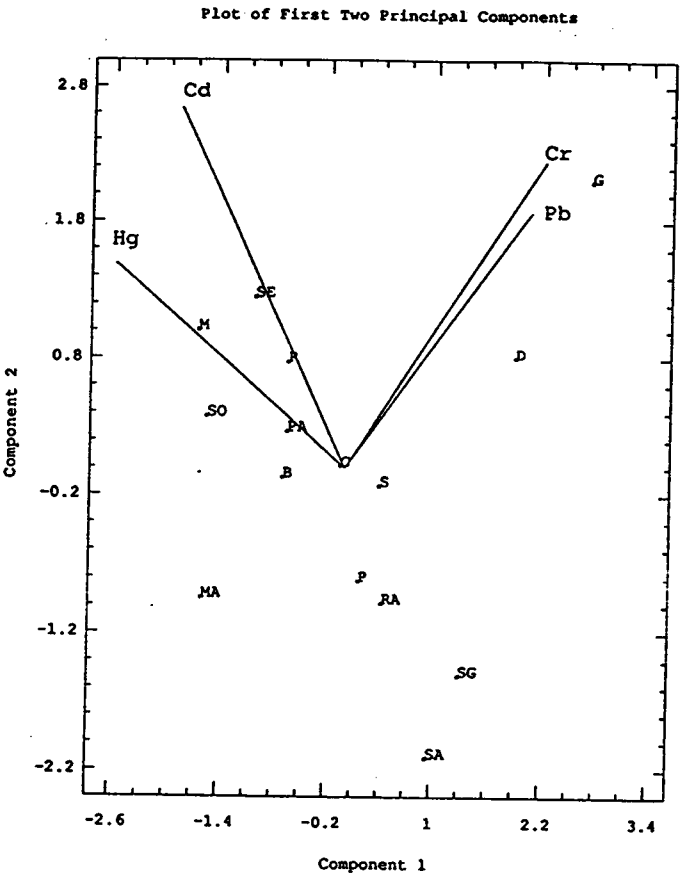

Figure 9. Plot of first two Principal Components (PCs) for species contamination by Hg, Cd, Cr, and Pb.

First PC: percent variability = 43%
Second PC: percent variability = 37.1%

Variability contribution to PCs:

First PC: Cr = 66%
Pb = 61%

Second PC: Cd = 70%
Cr = 60%

Table 11. Lead, Chromium, Mercury, and Cadmium Concentrations (mg/kg f.w.) in the most consumed species.

Scientific Name	Number of Samples	Concentrations (mg/kg fresh weight)			
		Pb	Cr	Hg	Cd
Boops	10	0.195 (0.120-0.205)	0.105 (0.039-0.850)	0.560 (0.19-1.254)	0.032 (0.013-0.042)
Chelidonichthys	15	0.524 (0.022-0.856)	0.415 (0.024-0.451)	0.391 (0.220-0.856)	0.031 (0.015-0.057)
Dentex	10	0.206 (0.125-0.319)	0.492 (0.121-0.825)	0.407 (0.215-0.570)	0.027 (0.014-0.057)
Diplodus	10	0.244 (0.017-0.586	0.161 (0.039-0.420)	0.434 (0.150-0.656)	0.030 (0.009-0.067)
Merlucclus	20	0.114 (0.017-0.586)	0.173 (0.042-0.320)	0.618 (0.42-0.817)	0.042 (0.011-0.050)
Mullus	20	0.184 (0.031-1.037)	0.185 (0.025-0.507)	0.505 (0.373-0.592)	0.039 (0.012-0.054)
Octopus	14	0.168 (0.014-0.95)	0.171 (0.019-0.511)	0.532 (0.213-0.803)	0.023 (0.013-0.047)
Pagellus	40	0.156 (0.024-0.685)	0.187 (ND-1.246)	0.575 (0.120-1.230)	0.033 (0.012-0.047)
Raja	14	0.168 (0.056-0.654)	0.162 (0.100-0.430)	0.443 (0.103-0.678)	0.024 (0.017-0.047)
Sardina	20	0.078 (0.015-0.120)	0.130 (0.022-0.680)	0.190 (0.134-0.250)	0.024 (0.008-0.029)
Scomber	5	0.061 (ND-0.120)	0.043 (0.010-0.058)	0.609 (0.205-1.070)	0.030 (0.023-0.038)
Sepia	10	0.206 (0.035-0.330)	0.217 (0.038-0.450)	0.683 (0.315-1.336)	0.037 (0.013-0.046)
Solea	10	0.250 (0.082-0.630)	0.050 (0.021-0.086)	0.785 (0.287-1.150)	0.031 (0.004-0.015)
Sparus	10	0.310 (ND-0.509)	0.083 (0.076-0.129)	0.409 (0.215-0.700)	0.017 (0.006-0.034)
Trachurus	20	0.139 (ND-0.630)	0.220 (ND-0.589)	0.433 (0.100-0.905)	0.033 (0.008-0.058)
MEAN $\pm$ S.D.		0.195$\pm$0.103	0.186$\pm$0.118	0.504$\pm$0.138	0.030$\pm$0.006

RESULTS AND DISCUSSION

The results are presented at first in form of global results and then by considering different factors that may influence metal content of the organisms such as species, sampling sites and seasonal variations

1. Global analysis of results

The range and the mean concentration levels of the 504 samples are presented in Table 10.

1.1- Cadmium

Cadmium was detectable in all the samples analyzed. Concentrations range from 0.012 to 0.051 mg/kg fresh weight.

1.2- Chromium

Mean concentrations of chromium in all the samples range from 0.023 to 0.568 mg/kg fresh weight.

1.3- Mercury

Mean concentrations of mercury were found to range from 0.108 to 1.012 mg/kg fresh weight.

1.4- Lead

Mean concentrations of lead in marine organisms analyzed varied between 0.013 ± 0.770 mg/kg fresh weight.

2. Factors of Contamination Variation

Three main factors selected for variation analysis include the species effects, by considering only the most consumed species in Morocco, and the seasonal and sampling site effects on metal contents of various species collected.

2.1. Metal concentrations in the most consumed species.

Results in Table 11 show that mean concentrations of the four metals analyzed are about 0.030 ± 0.006 mg/kg f.w. for cadmium; 0.186± 0.118 mg/kg f.w. for chromium; 0.504 ± 0.138 mg/kg f.w. for mercury and 0.195 ± 0.103 mg/kg f.w. for lead. Specie variations in metallic contamination were tested by PCA. The variability on the first PC was driven by Cr (66%) and Pb (61%). On the second PC the Cd (70%) was the main variable. No significant pattern relative to metallic contamination was seen between the different fish species (Figure 9).

Table 12. Mercury Concentrations (mg/kg f.w.) in Species Collected from the Three Sampling Sites.

Scientific Name	Hg (mg/kg f.w.)		
	Tanger	Tetouan	Nador
Boops boops	0.721 (0.582-0.954)	0.635 (0.190-0.815)	0.446 (0.190-0.631)
Chelidonichthys lucerna	--	0.407 (0.353-0.459)	0.120 (0.080-0.200)
Dentex maroccanus	0.513 (0.351-0.935)	0.935 (0.870-1.110)	--
Diplodus vulgaris	--	0.486 (0.350-0.705)	0.453 (0.299-0.607)
Merluccius merluccius	0.404 (0.229-0.562)	0.235 (0.200-0.330)	0.177 (0.158-0.197)
Mullus barbatus	0.215 (0.123-0.234)	0.522 (0.317-0.709)	0.320 (0.025-0.507)
Octopus vulgaris	--	0.803 (0.725-0.830)	0.434 (0.324-0.545)
Pagellus acarne	0.827 (0.315-0.987)	0.365 (0.334-0.398)	0.790 (0.631-0.987)
Raja montagui	--	0.422 (0.380-0.450)	0.484 (0:145-0.675)
Sardina pilchardus	0.701 (0.422-0.856)	1.061 (0.890-1.250)	0.410 (0.234-0.586)
Scomber japonicus	--	--	0.533 (0.205-1.070)
Sepia officinalis	0.724 (0.611-0.815)	0.558 (0.486-0.631)	0.487 (0.315-0.660)
Solea cuneata	0.846 (0.287-1.150)	0.287 (0.134-0.345)	--
Sparus pagrus	--	--	0.580 (0.420-0.700)
Trachurus trachurus	0.450 (0.251-0.720)	0.485 (0.263-0.779)	0.491 (0.248-0.717)

Table 13. Lead Concentrations (mg/kg f.w.) in Species Collected from the Three Sampling Sites.

Scientific Name	Pb (mg/kg f.w.)		
	Tanger	Tetouan	Nador
Boops boops	0.483 (0.220-0.880)	0.126 (0.080-0.250)	0.364 (0.150-0.693)
Chelidonichthys lucerna	--	0.063 (0.030-0.096)	0.122 (0.120-0.125)
Dentex maroccanus	0.309 (0.253-0.365)	0.160 (0.120-0.200)	0.206 (0.125-0.319)
Diplodus vulgaris	--	0.060 (0.045-0.075)	0.264 (0.120-0.420)
Merluccius merluccius	0.214 (0.104-0.285)	0.070 (0.030-0.090)	0.228 (0.180-0.267)
Mullus barbatus	0.180 (0.110-0.250)	0.402 (0.040-1.037)	0.190 (0.135-0.245)
Octopus vulgaris	0.126 (0.026-0.175)	0.026 (0.015-0.053)	0.172 (0.096-0.238)
Pagellus acarne	0.215 (0.200-0.230)	0.152 (0.015-0.353)	0.159 (0.045-0.250)
Raja montagui	--	0.321 (0.220-0.422)	--
Sardina pilchardus	0.060 (0.029-0.114)	0.071 (ND-0.148)	0.091 (0.032-0.150)
Scomber japonicus	--	0.120 (0.080-0.160)	0.243 (0.091-0.393)
Sepia officinalis	0.175 (0.034-0.280)	0.220 (0.190-0.250)	0.253 (0.175-0.330)
Solea cuneata	0.180 (0.100-0.245)	0.175 (0.093-0.257)	0.082 (0.015-0.120)
Sparus pagrus	--	0.315 (0.100-0.540)	0.230 (0.200-0.260)
Trachurus trachurus	0.231 (0.115-0.253)	0.072 (ND-0.225)	0.170 (0.120-0.200)

Table 14. Chromium Concentrations (mg/kg f.w.) in Species Collected from the Three Sampling Sites.

Scientific Name	Cr (mg/kg f.w.)		
	Tanger	Tetouan	Nador
Boops boops	0.139 (0.075-0.223)	0.690 (0.052-1.850)	0.415 (0.066-0.960)
Chelidonichthys lucerna	0.353 (0.300-0.406)	0.097 (0.091-0.110)	0.060 (ND-0.077)
Dentex maroccanus	0.121 (0.110-0.200)	0.409 (0.068-0.750)	--
Diplodus vulgaris	--	0.102 (0.068-0.136)	0.157 (0.025-0.500)
Merluccius merluccius	0.263 (0.237-0.289)	0.250 (0.119-0.390)	0.060 (0.090-0.120)
Mullus barbatus	0.039 (0.025-0.100)	0.550 (0.235-0.876)	0.026 (0.018-0.089)
Octopus vulgaris	--	0.056 (0.014-0.750)	0.162 (0.123-0.560)
Pagellus acarne	0.702 (0.140-1.264)	0.335 (0.146-0.750)0	0.182 (0.062-0.560)
Raja montagui	--	0.168 (0.132-0.300)	--
Sardina pilchardus	0.142 (0.119-0.178)	0.087 (0.070-0.104)	0.220 (0.215-0.300)
Scomber japonicus	--	0.670 (0.042-0.910)	0.043 (0.035-0.090)
Sepia officinalis	0.079 (0.028-0.108)	0.283 (0.027-0.540)	0.051 (0.042-0.061)
Solea cuneata	0.076 (0.055-0.100)	0.021 (0.017-0.034)	0.120 (0.075-0.157)
Sparus pagrus	--	ND	0.085 (0.060-0.134)
Trachurus trachurus	0.560 (0.135-0.715)	0.340 (0.019-0.924)	0.341 (0.023-1.340)

Sampling Sites

Tanger = T
Tetouan = M
Nador = N

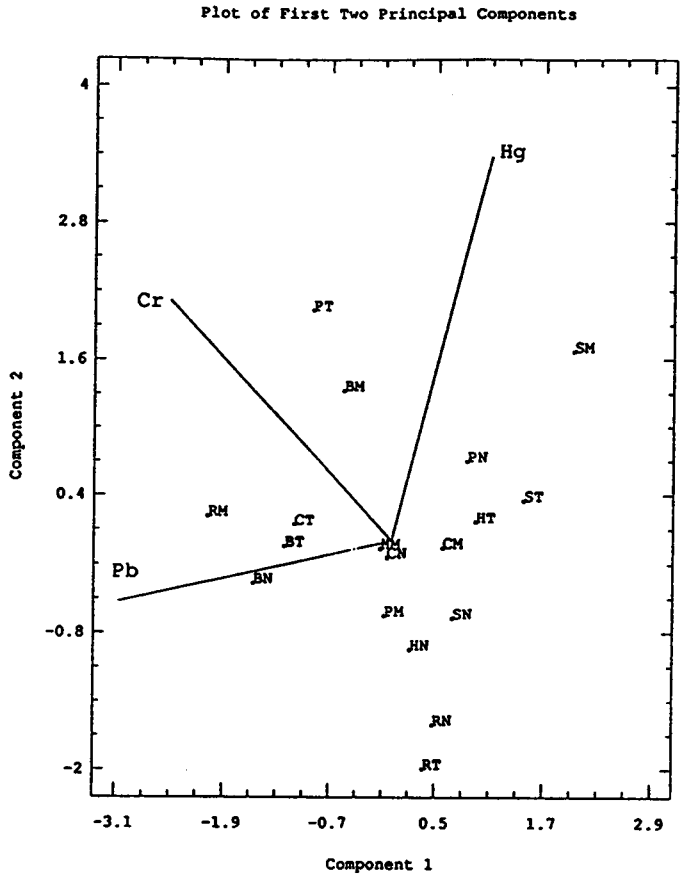

Figure 10. Plot of the first two Principal Components (PCs) for sampling site variability.

First PC percent variability = 39%
Second PC percent variability = 35%
Third PC percent variability = 27%

Variables contribution to PCs.

First PC: Pb = 80%
Cr = 65%

Second PC: Hg = 86%

Table 15. Concentrations of Lead, Chromium, and Mercury During the Sampling Seasons.

Scientific Name	Spring			Summer		
	Pb	Cr	Hg	Pb	Cr	Hg
Boops boops	0.126 (0.080-0.250)	0.110 (0.080-0.147)	0.635 (0.190-0.815)	0.038 (0.020-0.085)	0.690 (0.052-1.850)	0.550 (0.190-1.254)
Chelidonichthys lucerna	0.063 (0.050-0.076)	0.225 (0.100-0.267)	0.407 (0.353-0.459)	0.076 (0.026-0.132)	0.097 (0.091-0.110)	0.539 (0.353-0.856)
Dentex maroccanus	0.160 (0.015-0.200)	0.825 (0.700-1.100)	0.935 (0.857-1.110)	0.201 (0.116-0.286)	0.409 (0.068-0.750)	0.572 (0.450-0.664)
Diplodus vulgaris	0.060 (0.025-0.123)	0.157 (0.080-0.245)	0.486 (0.350-0.705)	0.158 (0.116-0.200)	0.102 (0.068-0.136)	0.517 (0.619-0.415)
Merluccius merluccius	--	--	0.235 (0.200-0.330)	0.081 (0.070-0.092)	0.250 (0.119-0.390)	0.618 (0.420-0.817)
Mullus barbatus	0.402 (0.040-1.037)	0.050 (0.025-0.075)	0.522 (0.317-0.709)	0.145 (0.058-0.219)	0.550 (0.235-0.876)	0.505 (0.373-0.592)
Octopus vulgaris	0.026 (0.010-0.125)	0.255 (N.D.-0.511)	0.803 (0.725-0.830)	0.146 (0.014-0.304)	0.056 (0.014-0.135)	0.571 (0.415-0.729)
Pagellus acarne	0.152 (0.015-0.353)	0.138 (0.044-0.297)	0.365 (0.334-0.398)	0.118 (0.035-0.280)	0.335 (0.146-0.750)	0.594 (0.315-0.821)
Sardina pilchardus	0.071 (0.036-0.167)	0.078 (0.034-0.140)	1.061 (0.850-1.250)	0.190 (0.130-0.220)	0.087 (0.065-0.120)	1.346 (0.870-1.822)
Sepia officinalis	0.220 (0.190-0.250)	0.820 (0.320-1.319)	0.558 (0.486-0.631)	0.093 (0.035-0.150)	0.283 (0.027-0.540)	0.593 (0.248-0.938)
Solea cuneata	0.175 (0.120-0.240)	0.016 (N.D.-0.031)	0.287 (0.125-0.358)	0.156 (0.056-0.300)	0.021 (N.D.-0.085)	0.877 (0.550-1.204)
Trachurus trachurus	0.144 (0.036-0.226	0.110 (0.013-0.120)	0.485 (0.263-0.779)	0.194 (0.116-0.275)	0.340 (0.019-0.921)	0.537 (0.386-0.754)

Seasons

Spring = 1
Summer = 2

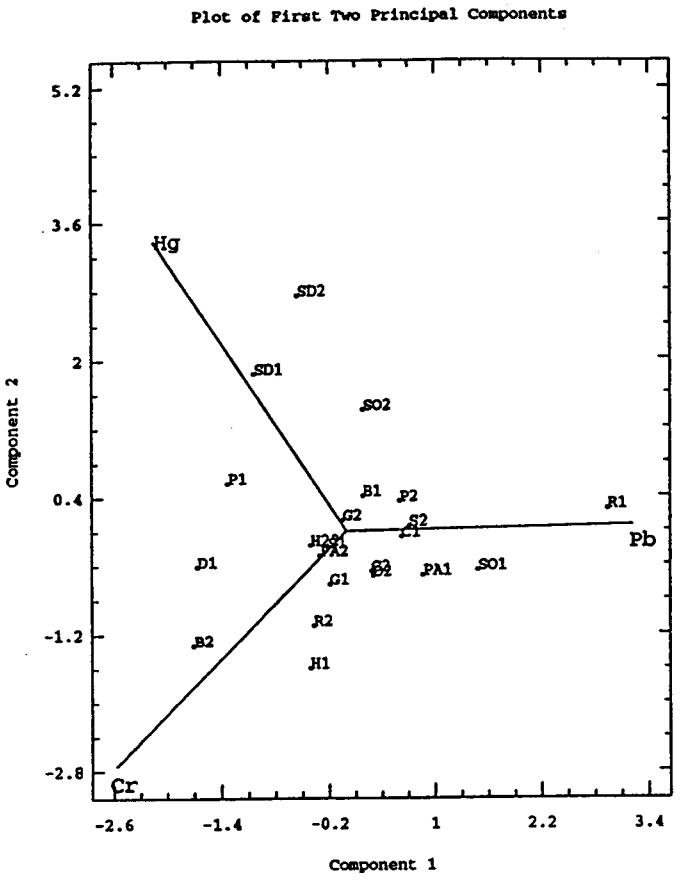

Figure 11. Plot of the first two Principal Components (PCs) for seasonal species contamination.

First PC percent variability = 35%
Second PC percent variability = 33%

Variable contribution to PCs:

First PC: Pb = 71%
Second PC: Hg = 77%
Cr = 63%

In general cadmium concentrations were found to be low in comparison with levels found in other regions of the Mediterranean sea where the mean concentrations reported in *Mullus barbatus* species, considered as a pollution indicator in the Mediterranean Action Plan Pollution Monitoring and Research Programme (MED POL)is about 0.046 mg/kg fresh weight (UNEP, 1989).

There is very limited data on chromium concentration levels in marine organisms in the literature. A study by Luten et al. (1986) has shown that sole, plaice, and herring sampled from North Sea near Denmark coasts contained levels of chromium less than 0.030 mg/kg f.w. while concentrations of 0.430 mg/kg f.w. have been found in mussels from North Sea.

High levels of mercury were found in most of the species, however, the levels were within acceptable limits of those considered dangerous for human consumption. Acceptable limits in most countries vary between 0.5 and 1 mg/kg of total mercury (Nauen, 1983).

Lead concentrations are slightly higher as compared to the published data. Vos et Hovens (1986) reported levels of 0.040 mg/kg f.w. in the plaice; 0.060 mg/kg f.w. for herring sampled from North Sea and concentrations of 0.030 to 0.014 mg/kg f.w. have been found in sole collected from the Ireland Sea.

2.2. Sampling Site Variations

Tables 12, 13, and 14 summarize metal concentration in the Tanger, Tetouan, and Nador sampling sites. Analysis of data for metal contamination in the three sites was tested by PCA. The variability on the first PC was mainly driven by Pb (30%) and chromium (65%). On the second variable the Hg was the main variable (86%). Figure 10 shows the plot of data points of different species according to the three variables (Pb, Hg, and Cr). The graph does not demonstrate any significant pattern relative to the metallic contamination by mercury, lead, and chromium.

2.3. Seasonal variations

The results of samples with metal contamination collected during spring and summer season are given in Table 15 and Figure 11. The data were analyzed by the PC technique. The variability on the first PC was mainly driven by Pb (71%). On the second PC the Hg (77%) and Cr (63%) were the main variables. No significant pattern relative to the metallic distribution was observed between the sampling seasons (Figure 11).

CONCLUSION

Trace metal contamination of Moroccan seafood products are high but they remain within the acceptable limits. These levels are comparable with those found in the other Mediterranean countries and to some other locations of the world.

Mercury is of special importance for the Mediterranean because several fish species analyzed in this study exceed the limits set by several Mediterranean countries. Therefore, the high mercury levels in certain seafood species present a legal and possibly a sanitary problem in addition to any effects these levels may have on marine organisms and to the marine environment.

However even if metal concentrations for most of element remain reasonably low there is a need in Morocco to monitor these pollutants to limits their discharge into the marine environment.

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CHAPTER V

THE USE OF MUSSELS TO DETERMINE THE EXTENT OF TRACE METAL CONTAMINATION ALONG MOROCCAN ATLANTIC COAST

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ABSTRACT

The mussel *Mytilus edulis* was used to evaluate the extent of cadmium, lead and mercury contamination along the Atlantic coast of Morocco between Sale and Mohammadia. Concentrations are given for each sampling site. They varied from 0.222 to 0.375 mg/kg f.w.for cadmium; from 0.473 to 0.682 mg/kg f.w. for lead and from 0.528 to 0.713 μg/kg f.w. for mercury.

There was a significant variation ($P < 0.05$) between sampling sites for the three trace metals analyzed. In general, Mohammadia showed the highest contamination levels followed by Rabat. Spring season yielded the highest contamination levels followed by autumn and summer seasons.

INTRODUCTION

Bivalve mollusks, particularly mussels and oysters, are valuable organisms for monitoring pollution in marine coastal waters, because of their sedentary mode of life and wide distribution. *Mytilus edulis* is widely used for monitoring heavy metal contamination in coastal areas (Phillips, 1977; Davies and Pirie, 1980; Brix and Lyngby, 1985) and it has been successfully used in environmental monitoring programs such as the U.S "Mussel Watch" (Goldberg et al., 1978) and the Mediterranean "Mussel Watch" (UNEP/FAO, 1986).

The purposes of the present study were to evaluate the levels of cadmium, lead and mercury in *M. edulis* soft tissues during a one year period and to determine the range of metal contamination of some sampling locations of the Moroccan Atlantic coast in order to correlate levels detected with the possible anthropogenic sources of these metals.

MATERIAL AND METHODS

A population of *M. edulis* along Atlantic coast of Morocco from Sale to Mohammadia (Figure 12) was sampled at regular intervals between March 1990 and January 1991. The sampling sites consisted of coastal areas where some urban and industrial sources were suspected of discharging heavy metals. The characteristics of the chosen sampling sites are:

- Plage des Nations: this site is far from the urban industrial area where there are no particular pollution sources; this area is considered as a reference site.
- Sale (Sidi Moussa): Characterized by the multiple urban industrial discharges.
- Rabat: four sampling points were chosen as following:

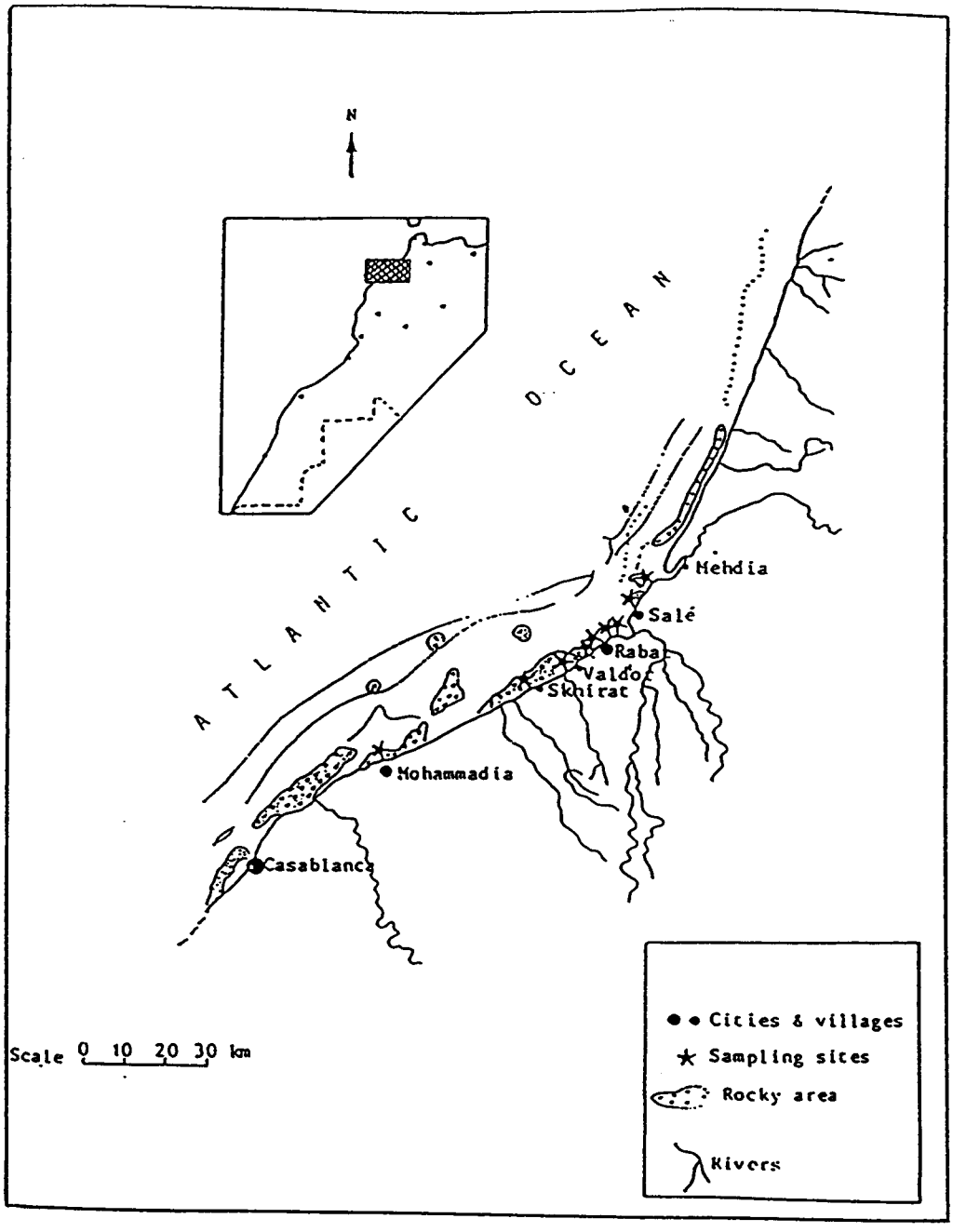

Figure 12. Map showing the sampling sites of mussels.

P1: The mouth of the Bou Regreg River which collects some agricultural and industrial effluents.

P2 and P3: Were chosen near sources of industrial discharges.

P4: Characterized by its proximity to the slaughterhouse of Rabat.

- Skhirat and Val d'or: these two sites are relatively far from any important metallic pollution source.

- Mohammadia: is a highly industrialized area (petrochemical and oil refinery industries, electrical power industry...etc). These activities are suspected to be an important source of metallic pollution.

The sampled mussel sizes were maintained between 5-6 cm throughout the study to reduce the variation due to size and age and to insure that the collected specimens were sexually mature.

After transfer to the laboratory the length of the organisms was measured, soft tissue was removed, drained for 10 min and placed in a teflon beaker. Tissues of 20 specimens were then combined and homogenized using a blender. A wet procedure was used for digesting tissue to minimize any possible contamination or losses. One g of wet tissue was placed into screw-top culture tubes (175 mm x 22 mm) and 5 ml of concentrated HNO_3 was added (reagent grade). The tubes were left at room temperature overnight for predigestion and the following day were placed on heat block at 140 °C for 4 hours for cadmium and lead, and in water bath at 90°C for 3 h in the case of mercury. The tissue digest was then made up to a volume of 10 ml with 4% of HNO_3 with redistilled water. Electrothermal atomic absorption was used to determine concentrations of cadmium and lead (UNEP/FAO/IAEA/IOC, 1984a). Mercury was measured by flameless atomic absorption spectrophotometry using a Colman mercury analyzer (MAS B 50) (UNEP/FAO/IAEA/IOC, 1984b). Metal recovery was assessed by analysis of IAEA reference material (MA-B-3/TM).

The sampling sites and seasonal variations are tested by one-way analysis of variance with pair differences tested with the Fisher's least significant difference test (LSD).

RESULTS AND DISCUSSION

1. Trends of contamination levels

Contamination levels of the mussels soft tissues determined in this study are listed in Table 16. Each value is a mean of 9 composite samples (20 mussels); analyses were done in triplicate.

Table 16. Concentrations of lead, cadmium, and mercury in soft tissue of *Mytilus edulis* from Atlantic Coast of Morocco.

Sampling Location		Metal Concentration (mg/kg f.w.)		
		Lead	Cadmium	Mercury
Plage des nations		0.473 (0.401-0.542)	0.222 (0.205-0.287)	0.504 (0.431-0.624)
Sale (Sidi Moussa)		0.560 (0.516-0.595)	0.313 (0.296-0.330)	0.600 (0.498-0.654)
Rabat	P1	0.609 (0.597-0.631)	0.297 (0.248-0.363)	0.559 (0.489-0.631)
	P2	0.615 (0.574-0.623)	0.251 (0.260-0.3)	0.541 (0.512-0.598)
	P3	0.623 (0.531-0.699)	0.358 (0.309-0.397)	0.562 (0.490-0.644)
	P4	0.570 (0.513-0.656)	0.332 (0.250-0.399)	0.577 (0.489-0.641
Val d'or		0.535 (0.477-0.587)	0.268 (0.2-0.339)	0.435 (0.323-0.532)
Skhirat		0.572 (0.518-0.595)	0.308 (0.245-0.363)	0.530 (0.495-0.556)
Mohammadia		0.682 (0.612-0.699)	0.375 (0.399-0.314)	0.725 (0.674-0.799)

1.1. Cadmium

Cadmium was detected in the 90 mussel samples collected. Concentrations were between 0.222 and 0.375 mg/kg fresh weight. These concentrations are in the range of those found in mussels collected from The North Atlantic Ocean, where the reported values are between 0.09 and 0.33 mg/kg f.w. (ICES,1980). High levels have been reported in the Oslo Commission area where the concentrations ranged between 0.043 and 12.6 mg/kg f.w. with a mean value of 1.040 mg/kg f.w. (UNEP, 1989). However the concentrations in mussel are difficult to compare for samples taken from different locations and seasons. This is due to the fact that mussels are sensitive to local pollution influences. Several countries have established limits for cadmium in seafood. In the majority of cases limits range between 0.1 and 1 mg/kg (Nauen, 1983). When comparing the results obtained in this study with the established limits all the concentrations are within acceptable levels for human consumption.

1.2. Lead

Concentrations of lead vary between 0.473 and 0.682 mg/kg f.w. In all cases, these concentrations were found to be slightly lower than those published for *Mytilus galloprovincialis* collected from various Mediterranean areas where the levels were reported to vary between 0.6 and 1.8 mg/kg f.w. with an average of 0.8 mg/kg f.w. (UNEP, 1989). On the Atlantic coasts of Portugal and Spain, Manga reported concentrations of lead ranging from 2 to 15 mg/kg dry weight (the equivalent of 0.67-5 mg/kg f.w.) (UNEP, 1989).

1.3. Mercury

Mercury was found at all sampling sites with concentration levels between 0.528 and 0.713 mg/kg f.w. These concentrations are rather high for human consumption since most countries have established limits of 0.5 mg/kg f.w. in their seafood products (Nauen, 1983). For the Mediterranean Sea, levels varying from 0.004 to 7 mg/kg f.w. with an average of 0.232 mg/kg f.w. have been reported (UNEP/FAO/WHO, 1983).

2. Sampling site variations

The analysis of variance of the sampling site differences for the trace metals analyzed showed a significant variation ($P<0.05$) between sites. In general, Mohammadia showed the highest contamination levels for the three metals, followed by Rabat, Sale, Skhirat, Val d'or and finally Plage des Nations being the least contaminated site. These results are in good agreement with the relative contamination sources of the sampling sites and confirm the ability of mussels to detect any small differences in trace metal contamination. Mohammadia is the

most industrialized site with an oil refinery where mercury may be still used as catalyst, and an electrical power plant which uses about 800,000 tons/year of coal containing at least 15% ash rejected directly to the sea. The ash contains high levels of trace metals which may explain the high contamination levels detected in this area.

Rabat is the second most contaminated area but there were some variations between sites. For example, the mussels collected in Bou Regreg estuary (P1) are moderately contaminated by metals in comparison with those sampled from P3 which is located near the largest sewage discharge from the city.

3. Seasonal variations

The Fisher's LSD test showed significant variation between seasons ($P<0.05$). The LSD test values in Table 17, indicate that maximum concentration found are during spring season followed by autumn and summer. There was insufficient data for the winter season as the collection from most of the sampling sites was almost impossible. These variations may be explained by changes in the importance of metallic sources as well as by metabolic and reproductive cycle of the test organisms. It has been often mentioned that gametogenesis may influence metal content of the mussel. The explanation of this phenomenon is related to the fact that during spawning up to 40% of soft tissue weight is lost (Cossa et al., 1989). Furthermore, since metals in mollusks are preferentially associated with proteins, the changes of the principal energy source from glycogen in autumn to proteins in spring are likely to influence the metallic flux in mussels (Widdows, 1978). Temperature has also been shown to induce slight increase in mercury uptake in *M. galloprovincialis* (Fowler et al., 1978) and to cause an acceleration in the building of cadmium to soft tissue (Fisher, 1986).

Table 17. Seasonal mean concentration of metals ranked by the LSD test from the nine sampling sites.

Season	Mean metal concentration (mg/kg f.w.)		
	Mercury	Lead	Cadmium
Spring	0.695 A	0.614 A	0.339 A
Summer	0.644 B	0.553 B	0.274 B
Autumn	0.685 AB	0.581 AB	0.305 AB
LSD (5%)	0.068	0.061	0.053

LSD = Least Significant Difference.
The means are ranked by alphabetical order. Every letter corresponds to a class of mean; two letters, the class is intermediate between the two respective classes.

CONCLUSION

The use of mussels (*Mytilus edulis*) to assess trace metal contamination in coastal waters provides a good quantitative indication of the extent of this contamination. The importance of contamination has been well correlated with industrialized and heavily populated sites of the study area. The variations due to size of the mussels and to seasonal effects can be minimized by using a composite sample of mussels of similar sizes collected at different times of the year from the same sampling sites.

The data obtained on the contamination of the three metals analyzed does not show alarmingly high levels for cadmium and lead, but relatively high levels for mercury. However, these concentrations remain lower than the maximum permissible levels set by Mediterranean countries for human consumption. This preliminary investigation provides basic information about metal contamination of this part of the Atlantic coast of Morocco that will be useful for future assessment and monitoring of these pollutants in the area.

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CHAPTER VI

PESTICIDE RESIDUES IN SEAFOOD PRODUCTS FROM THE MEDITERRANEAN COASTAL WATERS OF MOROCCO

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ABSTRACT

The residue levels of organochlorine pesticides are determined in marine organisms (fish, crustaceans and mollusks) from the Mediterranean coast of Morocco on a regional and a seasonal basis. The analyzed compounds are: hexachlorobenzene (HCB), lindane, heptachlor epoxide, aldrin, dieldrin, endrin, p,p'dichlorodiphenyltrichloroethane (p,p'DDT), and its derivatives (p,p'DDD and p,p'DDE).

Most of the samples analyzed showed negligible contamination by these compounds, however residue levels remain below the acceptable limits and no human toxicological problems from this source are likely to occur for fish consumers. In general, the results did not show any significant differences according to seasonal and sampling sites variations.

INTRODUCTION

The environmental contamination with chlorinated hydrocarbons is particularly dangerous because of their relative resistance to breakdown. This persistence is the major concern with organochlorine pesticides which tend to accumulate in different media reaching sometimes alarming levels. These chemicals are still increasingly used in agriculture and in public health programs to control vector borne disease in most developing countries (Forget, 1991).

In Morocco, large quantities (about 10,000 metric tons per year) of pesticides are used, about half of which are organochlorine compounds (ODI, 1984). Previous studies of contamination of multiple foodstuffs (poultry eggs, poultry liver, bovine liver and kidney) demonstrated the presence of a large contamination by chlorinated hydrocarbons pesticide residues that sometimes exceeded international tolerance levels (Kessabi et al., 1990). A study of chlorinated hydrocarbons in urban, industrial and continental waters along the Mediterranean coast of Morocco has shown negligible amounts of these contaminants are discharged into the marine environment (Kessabi et al., 1988). Marine organisms are subjected to such contamination especially in coastal fishing areas where large amounts of these chemicals are discharged daily.

Most of circum Mediterranean countries (UNEP, 1989; Cossa et al., 1990; UNEP/FAO/WHO/IAEA, 1990) have conducted surveys about their fishery products. In Morocco little data are available to respond to the international requirements for chemical contamination. This study was undertaken to determine contamination levels of chlorinated hydrocarbons pesticides in the major marine species collected from coastal fishing areas along the Mediterranean coast of Morocco and to evaluate the safety of these products for human consumption.

MATERIAL AND METHODS

Fish samples were collected at monthly intervals from January 1988 to July 1990 from the ports of Tanger, Tetouan (M'diq), Al Hoceima and Nador (Figure 13). The sampling of marine organisms were conducted according to the UNEP/FAO/IAEA recommendations (1984).

The samples were analyzed using the method described by Marchand (1983) and El Nabawi et al. (1987) with little modifications. Ten grams of fish muscle tissue were extracted with 250 ml hexane for 8 hours using a soxhlet apparatus. This less exhaustive method was used as recommended by the Marine Environment Laboratory (IAEA) to allow participation in their intercalibration program and also to allow a comparison of our results to other MED POL studies using similar methods. The extract was concentrated and purified by column chromatography containing 15 g of florisil (60/100 mesh). The compounds were eluted with 70 ml hexane for the first fraction which contained the compounds hexachlorobenzene (HCB) and p,p' dichlorodiphenyl-dichloroethylene (p,p'DDE). The second fraction eluted with 50 ml hexane and methylene chloride (70/30,v/v) contained lindane, p,p' dichlorodiphenyltrichloroethane (p,p'DDT) and its homologue p,p'dichlorodiphenyldichloroethane (p,p'DDD), the drins (aldrin, dieldrin and endrin) and heptachlor epoxide. The final extract was analyzed by a gas chromatograph (HP 5730 A) equipped with a capillary column (HP1, 25 m x 0.2 mm i.d.) and an electron capture detector.

RESULTS AND DISCUSSION

1. Trends in residue levels of contaminants.

Table 18 shows the analytical results for the various marines organisms collected from the different sampling sites. Lindane, p,p'DDT and its analogues (p,p'DDD and p,p'DDE) are present in relatively high concentrations in comparison with other compounds. The mean concentration of fish samples varied between 1.32 μg/kg fresh weight (f.w.) and 5.40 μg/kg f.w. for lindane and between 1.48 μg/kg f.w. and 5.67 μg/kg f.w. for DDT. The other compounds (HCB, heptachlor epoxide, aldrin, dieldrin and endrin) showed moderate contaminations and the concentrations are below 2 μg/kg f.w. for the most contaminated species.

The highest contamination levels in this investigation were detected in mussels. The mean concentration of DDT and lindane were 6.38 μg/kg f.w. and 6.84 μg/kg f.w. This confirmed the high capacity of this species to accumulate organic pollutants (UNEP, 1989; UNEP/FAO/WHO/IAEA, 1990).

Among fish species analyzed, *Mullus barbatus* showed the highest levels of contamination for most of the compounds which may be explained by the living habits of this species. *M. barbatus* lives in estuaries and ports that are usually subjected to heavy contamination by

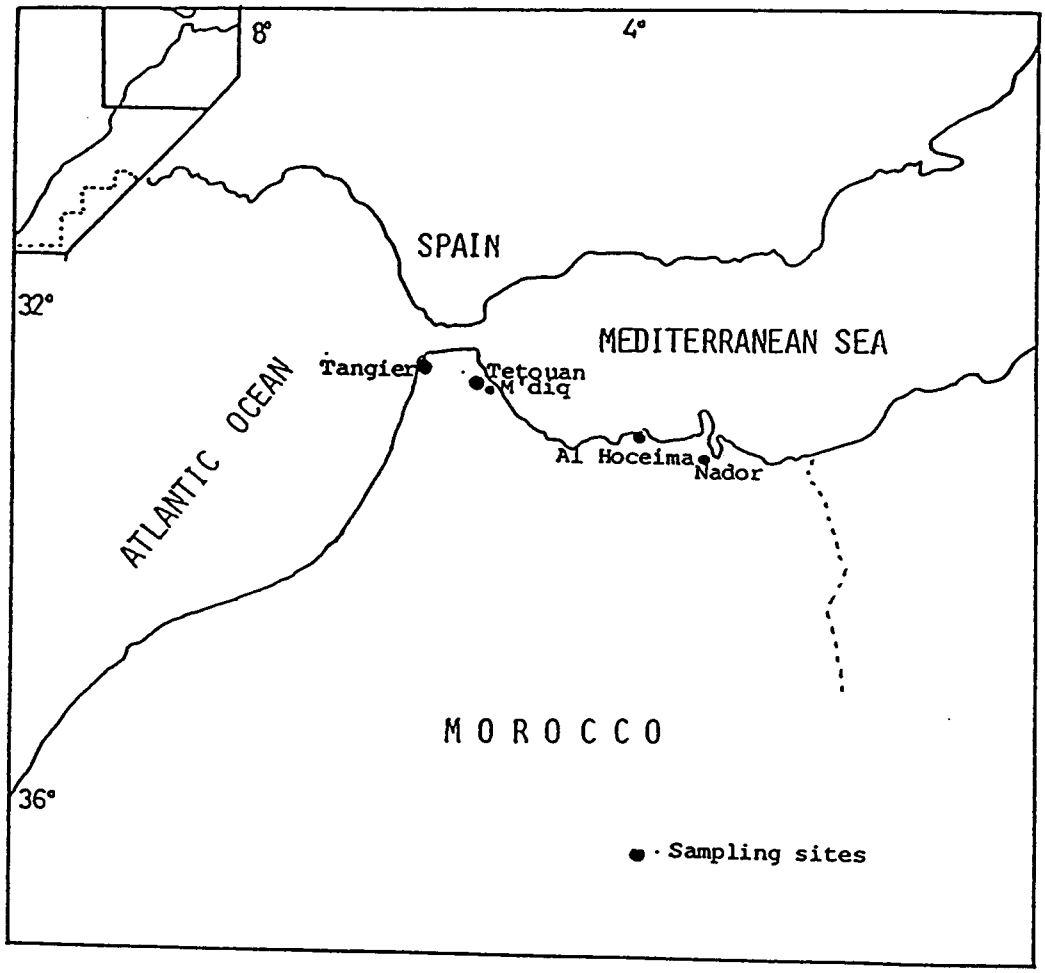

Figure 13. Map of northern Morocco showing the sampling sites of marine organisms.

Table 18. Chlorinated hydrocarbons in marine organisms from the Mediterranean Sea.

Species	Mean concentration (µg/kg f.w.) ± standard deviation										
	Sample	HCB	Lindane	Heptachlore	Aldrin	Dieldrin	Endrin	pp'DDE	pp'DDD	pp'DDT	DDTs
Boops boops	16	0.62±0.06	5.24±0.54	0.32±0.05	0.18±0.03	0.32±0.06	0.42±0.13	4.53±0.52	4.62±0.32	3.92±0.45	12.97
Chelidonichthys sp	8	0.44±0.09	4.59±0.84	0.27±0.19	0.28±0.03	0.20±0.15	0.48±0.06	4.53±1.06	4.29±0.27	3.61±0.46	12.43
Dentex sp.	14	0.53±0.10	5.43±0.26	0.41±0.16	0.32±0.15	0.72±0.06	0.65±0.05	3.64±0.92	5.47±0.14	3.40±0.45	12.51
Diplodus sp.	12	0.35±0.02	5.05±0.42	0.21±0.15	0.19±0.13	0.64±0.07	0.75±0.15	4.10±0.45	3.99±0.62	4.25±1.17	12.34
Merluccius sp.	14	0.20±0.04	3.84±0.18	0.14±0.03	0.18±0.03	1.07±0.15	1.02±0.22	1.96±0.05	3.54±0.21	3.96±0.94	9.46
Mullus barbatus	15	0.62±0.03	3.95±0.47	0.54±0.29	0.37±0.20	2.46±1.22	1.66±0.34	6.72±0.34	5.01±0.57	5.67±0.04	17.4
Pagellus acarne	13	0.49±0.09	1.32±0.53	0.56±0.05	0.86±0.07	1.05±0.06	0.95±0.03	1.43±0.27	1.46±0.52	1.46±0.36	4.39
Sardina pilchardus	14	0.29±0.04	3.26±0.36	0.63±0.09	0.47±0.07	0.83±0.09	0.57±0.05	2.85±0.03	3.23±0.53	3.85±0.67	9.93
Solea solea	10	0.45±0.03	3.58±0.62	0.68±0.05	0.63±0.07	0.24±0.03	0.63±0.09	4.58±0.83	4.92±0.42	3.38±0.92	12.88
Sparus pagrus	12	0.24±0.05	2.95±0.64	0.24±0.07	0.63±0.06	0.47±0.04	0.18±0.03	2.48±0.67	2.57±0.92	2.36±0.04	7.43
Trachurus trachurus	14	0.33±0.06	3.68±0.72	0.15±0.03	0.18±0.04	0.79±0.07	0.36±0.03	1.93±0.27	3.85±0.52	2.63±0.37	8.61
Sepia officinalis	8	0.23±0.04	2.65±0.07	0.36±0.07	0.42±0.06	0.43±0.05	0.36±0.04	2.58±0.67	3.36±0.73	3.48±1.07	9.44
M. galloprovincialis	15	0.74±0.16	6.38±1.32	1.27±0.52	0.92±0.09	3.34±0.42	2.92±0.63	7.92±1.34	4.48±0.96	6.64±1.54	19.24

various pollutants. Furthermore, this species was taken as an indicator of pollution in the Mediterranean Monitoring programs, and was shown to reflect local contamination conditions of the sampling site as well as a good tool to make comparisons of contamination between different regions (UNEP, 1989).

2. Ratio Between DDT and Its Analogue Products

The ratio of DDT to its metabolites is a way of measuring degradation efficiency by the organisms exposed and how recent is the exposure. Higher ratio could imply a more recent exposure (Olsson and Reutergardh, 1986). Table 19 summarizes the relative proportions of p,p'DDE, p,p'DDD and p,p'DDT to the total DDT. p,p'DDD was the predominant contaminant in all fish samples with 35.6% and p,p'DDE and p,p'DDT showed the same proportions with 32.2%. These data are not suggestive of any recent exposure to DDT of the marine organisms analyzed.

Table 19. Relative abundance of p,p'DDT, p,p'DDD and p,p'DDE to total DDTs in all fish samples analyzed.

Mean Concentrations (µg/kg f.w.) and range	**p,p'DDE**	**p,p'DDD**	**p,p'DDT**	**DDTs**
	3.87 (1.34-6.72)	4.28 (1.48-5.47)	3.87 (1.48-5.67)	12.02 (9.48-17.40)
% Mean	32.2	35.6	32.2	100

3. Comparison With Other Mediterranean Studies

Since both *Mullus barbatus* and *Mytilus galloprovincialis* were chosen as indicators in Mediterranean Pollution Programmes (MED POL), these two species were taken for comparison of the results of this program with those found in this study (Table 20; UNEP, 1989). This comparison revealed levels of chlorinated hydrocarbons 2 to 4 fold lower in the present study as compared to the mean levels of 1985 for the species *Mullus barbatus* (UNEP, 1989). Similar, but less pronounced, decreases were also noted for residual levels of these contaminants in *Mytilus galloprovincialis*. A possible explanation of this decrease in residue levels from 1985 to 1989 could be related to probable degradation associated with less use of these pesticides as results of their ban in most of Mediterranean countries. However, the contamination levels in both species for lindane and dieldrin did not show any substantial decrease and even an increase during this period. This finding suggests the continuing use of this compound in many formulations to control animal ectoparasites and vector borne disease in public health programs.

Table 20. Chlorinated hydrocarbon pesticides in Mediterranean pollution indicator organisms.

Mullus barbatus		
Chlorinated hydrocarbons	Mean concentrations (mg/kg f.w.) (Range)*	Mean concentrations (mg/kg f.w.) Mean±SD ¥
pp'DDT	22.8 8-38	5.67±0.04
pp'DDD	19.9 1.6-38	5.01±0.57
pp'DDE	28.5 8 - 53	6.72±0.34
HCB	3 2.6-5	0.62±0.03
Lindane	7 0.7-19	3.95±0.47
Aldrin	1 0.5-1.5	0.37±0.20
Dieldrin	3.57 0.4-6	2.46±1.22
Mytilus galloprovincialis		
Chlorinated hydrocarbons	Mean concentrations (mg/kg f.w.) (Range)*	Mean concentrations (mg/kg f.w.) Mean±SD ¥
pp'DDT	14.7 7 - 22	6.84±1.54
pp'DDD	23.7 7 - 49	4.84±1.54
pp'DDE	8.5 5 - 13	7.92±1.34
HCB	1.5 1.1-1.9	0.74±0.16
Lindane	2.3 0.7-19	6.38±1.32
Aldrin	2 0.5-5	0.92±0.09
Dieldrin	2.37 0.8-3.5	3.34±0.42

* UNEP, 1989
¥ This study.

4. Seasonal variations.

Two species *Boops boops* and *Trachurus trachurus* were taken for seasonal variation analysis as the sampling covered spring, summer and fall. The two species did not demonstrate any seasonal variations for most of the compounds except in case of DDT which shows an increase during the spring season (Figure 14). This increase may be related to the run-off effects that bring large amounts of this contaminant during this season (Burns and Villeneuve, 1982; Villeneuve, 1986). This finding was also noted in the study of Kessabi et al. (1988) that shows an increase of DDT in urban, industrial and continental waters of the Mediterranean region during spring season. This variation could, at least partly, be accounted for by local agricultural and pest control practices during this season in spite of the official use of chlorinated hydrocarbon pesticides for agricultural purposes (M.A.R.A., 1985).

5. Sampling site variations.

Four species; *Boops boops, Pagellus acarne, Sardina pilchardus and Trachurus trachurus* were subjected to comparison between the four sampling sites (Tanger, Tetouan, Al Hoceima and Nador). Figures 15a and b show the relative concentrations of the chlorinated hydrocarbons in the four sampling sites. There was no variation in contamination between the sampling areas even though there are some variations as far as urbanization, industrial and agricultural activities between these regions.

6. Comparison of these levels with regulatory limits

As there are no regulatory limits for chlorinated hydrocarbon in fish products in Morocco, the levels detected in this study were compared with the acceptable levels established by other countries (Table 21). This comparison shows that they are below the acceptable limits established by all these countries and human health risks are likely to occur with these contamination levels.

CONCLUSION

All the species analyzed showed low contamination levels by chlorinated hydrocarbon pesticides. DDT and its analogues (DDD and DDE) and lindane were detected at the highest concentrations for most of the species. HCB, heptachlor epoxide, aldrin, dieldrin and endrin were present at relatively lower concentrations.

There was no seasonal variation for the compounds analyzed except for DDT which shows an increase during spring season. Also no sampling site variations were observed in this study. No immediate health risks are likely to be generated through the consumption of seafood

Table 21. Compilation of legal limits for organochlorine pesticides in fish and fishery products (mg/kg f.w.).

Countries	HCB	Aldrin/Dieldrin	DDT	DDE	DDD	DDTs	Endrin	Lindane
Canada		0.1	5.0	5.0	5.0	5.0	0.1	0.1
Denmark			2.0-5.0					
Germany	0.5	0.5-1.0				2.0-5.0	0.01	
Iceland								0.5
Sweden	0.2	0.1				5.0		0.2
Thailand	0.1-0.3		5.0				0.3	0.5
USA		0.3	5.0	5.0	5.0	5.0	0.3	
Range Minimum Maximum	 0.2 0.5	 0.1 1.0	 2.0 5.0	 5.0 5.0	 5.0 5.0	 5.0 2.0	 0.01 0.3	 0.1 2.0

Reference: Nauen (1983); USFDA (1982, 1984).

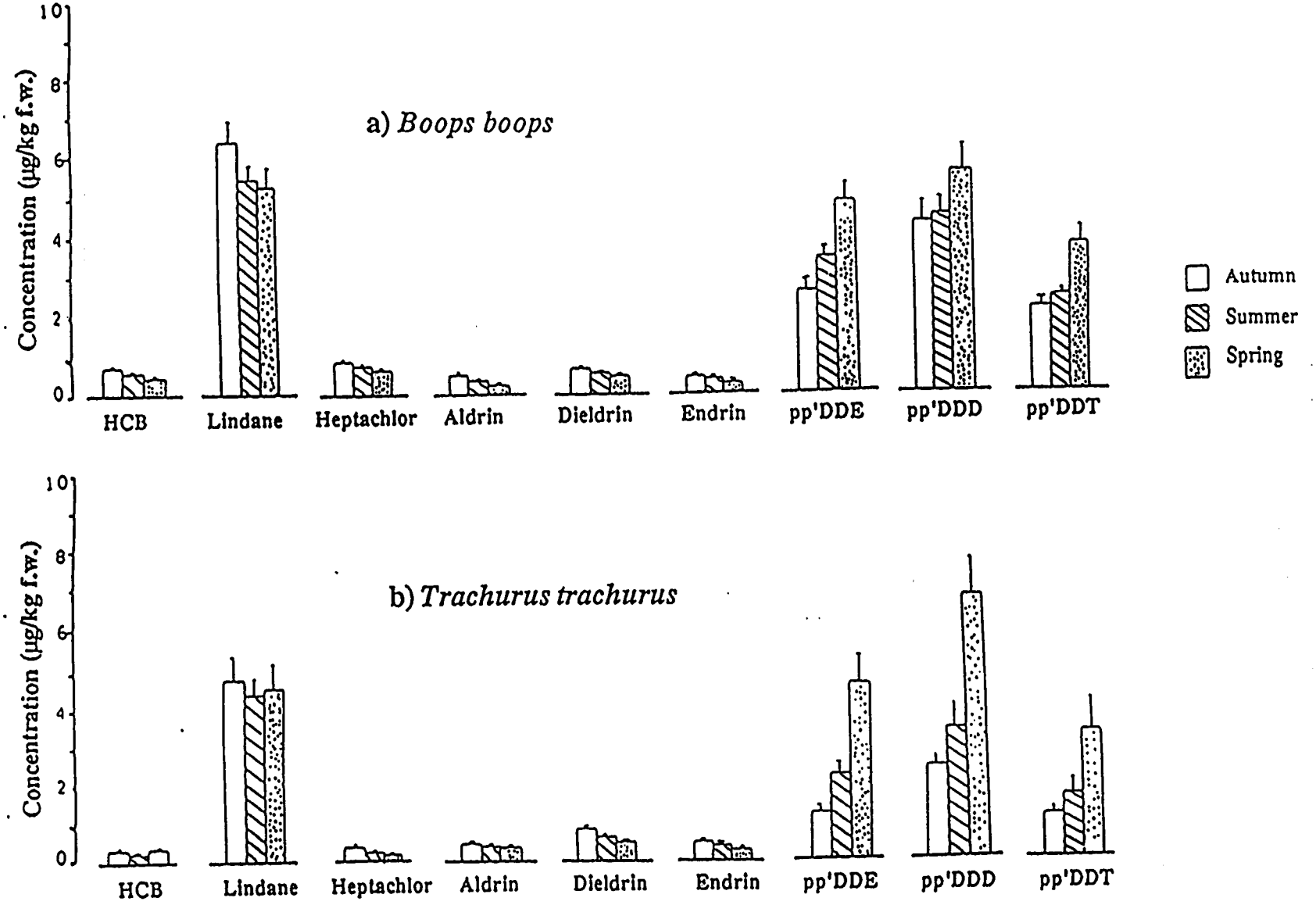

Figure 14. Concentration of chlorinated hydrocarbon pesticides during spring, summer, and autumn in (a) *Boops boops* and (b) *Trachurus trachurus*.

Figure 15a. Comparison of chlorinated hydrocarbon pesticides concentration in the four sampling sites for (a) *Boops boops* and (b) *Pagellus acarne*.

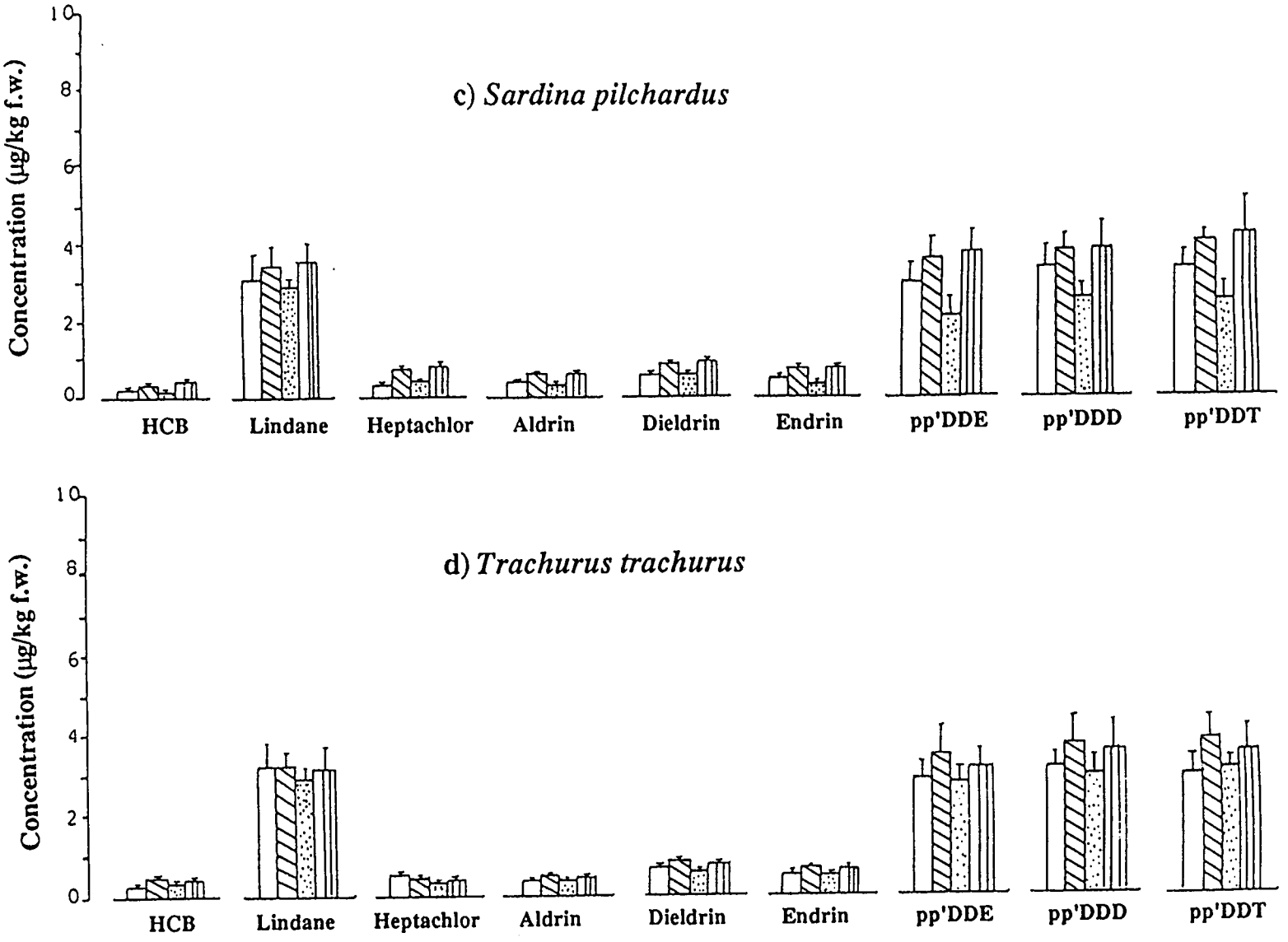

Figure 15b. Comparison of chlorinated hydrocarbon pesticides concentration in the four sampling sites for (c) *Sardina pilchardus* and (d) *Trachurus trachurus*.

products but a continuous monitoring is needed to insure both human and environmental health.

ACKNOWLEDGMENTS

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CHAPTER VII

RESIDUE LEVELS OF ORGANOCHLORINE PESTICIDES IN FISH FROM MOROCCAN COASTAL WATERS

ABSTRACT

Chlorinated hydrocarbon pesticide residues were determined in five fish species from two sampling sites: M'diq (Mediterranean Sea) and Mehdia (Atlantic Ocean).

The results showed that p,p'DDT and its analogues (p,p'DDE and p,p'DDD) and lindane were present in all samples at relatively high concentration in comparison with other organochlorine compounds. A positive correlation was noted between contaminant concentrations, body size, and lipid contents. No difference in contamination between Mediterranean and Atlantic species was noted. From public health point of view, the levels of organochlorine determined in this study were lower than the acceptable limits set for these contaminants by several countries.

INTRODUCTION

Contamination by chlorinated hydrocarbons has been extensively studied in most of Mediterranean countries (UNEP/FAO/WHO/IAEA, 1990) but very little is known about this contamination in Morocco. A previous study has shown that most industrial, agricultural and urban effluents, especially the ones discharged into marine environments, contain high concentration of these xenobiotics (Kessabi et al., 1988). With this concern and in view of human health and environmental hazards associated with these chemicals, the present investigation was undertaken to determine residue levels of chlorinated hydrocarbon pesticides in different fish species collected from two sampling sites: M'diq (Mediterranean Sea) and Mehdia (Atlantic Ocean).

MATERIAL AND METHODS

1. Sampling Strategy

Collections of five specimens from five species of fish were made at monthly intervals over a period of six months during 1990/91 at both M'diq (Mediterranean Sea) and Mehdia (Atlantic Ocean) (Figure 16). Samples of muscle were collected and immediately frozen for subsequent analysis. The edible muscle tissue was analyzed for chlorinated hydrocarbon pesticides.

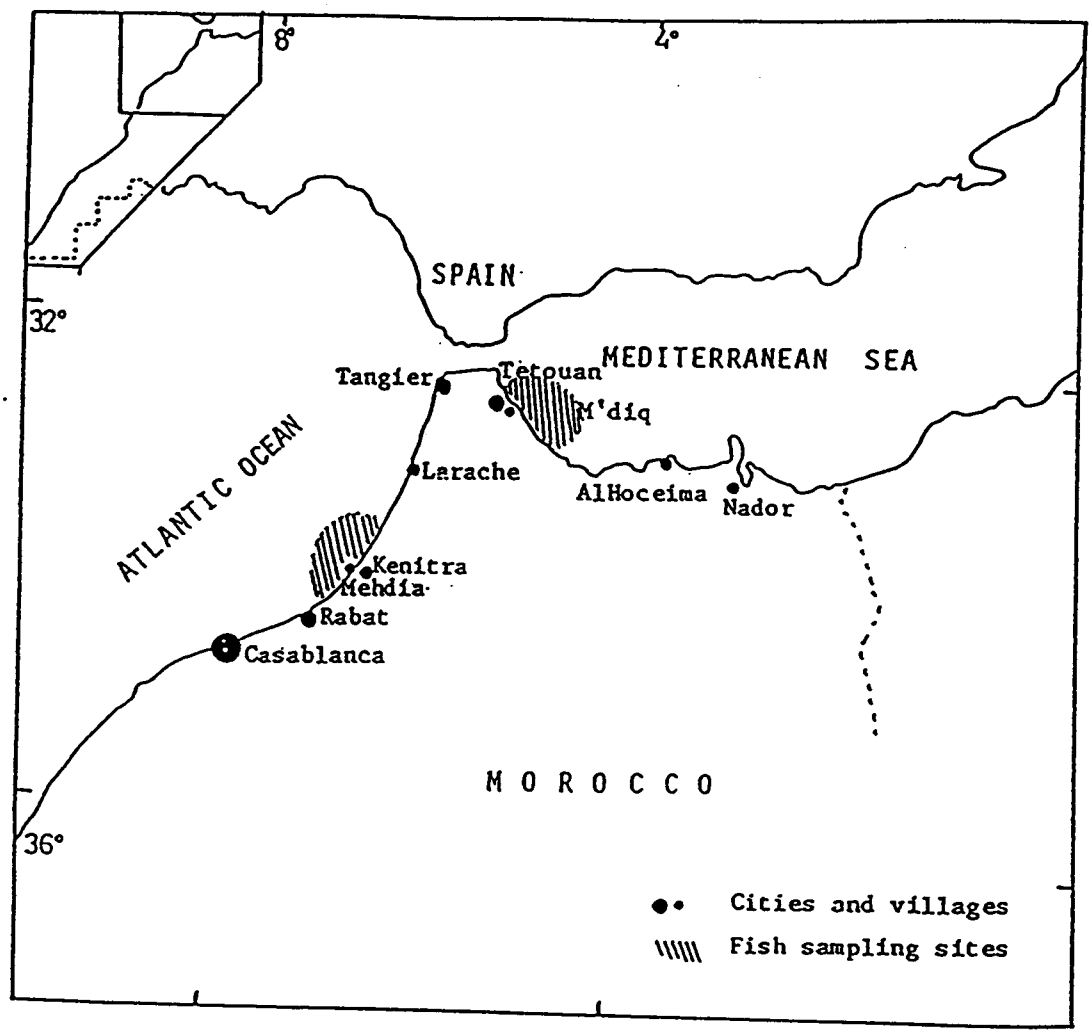

Figure 16. Map of northern Morocco showing the sampling locations of fish.

2. Collection and Sample Preparation

Five fish species were selected for this study: European hake (*Merluccius merluccius*), horse mackerel (*Trachurus trachurus*), axielary seabream (*Pagellus acarne*), bogue (*Boops boops*) and pilchard (*Sardina pilchardus*). Species selection was based on their presence both in Mediterranean Sea and Atlantic Ocean and their large consumption by Moroccans. Fish chilled in ice were collected from the port immediately after the return of the fishing boats. After being identified, the weight and the length recorded, the fish were dissected, an aliquot of muscle taken and immediately frozen and stored in the freezer at -20 °C until analysis.

Ten grams of the thawed dorsal fish muscle was mixed with 20 g of anhydrous sodium sulfate, then transferred to a soxhlet apparatus and extracted with 250 ml of hexane for 8 hr. The lipid content of the extract was determined gravimetrically. This less exhaustive method was used by the Marine Environmental Laboratory (IAEA) to allow participation in their intercalibration program and also to allow a comparison of our results with other MED POL studies using the similar methods.

3. Purification and Fractionation

Sample purification and fractionation were performed by florisil column chromatography. The column (20 mm i.d. x 300 mm) was filled with 14 g of 60/80 mesh florisil (Fisher Scientific) deactivated with 0.5 ml water and topped with 1 cm of anhydrous sodium sulphate. The column was washed with hexane and eluted with 70 ml hexane for the first fraction and with 50 ml hexane + methylene chloride (70/30, v/v) for the second fraction. The first eluant, contained the compounds hexachlorobenzene (HCB) and p,p' dichlorodiphenyldichloroethylene (p,p'DDE) in addition to polychlorinated biphenyls (PCBs). The second eluant (pesticide fraction) contained lindane, p,p' dichlorodiphenyltrichloroethane (p,p'DDT) and its homologue p,p' dichlorodiphenyldichloroethane (p,p'DDD), the drins (aldrin, dieldrin, and endrin) and other compounds.

4. Sample Analysis

Residues were analyzed by capillary gas chromatography (GC), using a Varian 3700 equipped with a ^{63}Ni electron capture detector (ECD), a split injection system, and an integrator model LCI 100 (Perkin-Elmer). The fused silica capillary column was 15 m x 0.25 mm i.d. SE-54 (Altech Associates, Inc). The carrier gas was helium with a flow rate of 1.5 ml/min.

The temperature for the injector and detector was respectively 280°C and 350°C, an initial temperature of 70°C was held for 2 min. raised to 260°C at 3°C/min, and held for the final temperature for 2 min. The detector make-up gas was nitrogen at a flow rate of 30 ml/min.

Quality assurance measurement include the analysis of reagent blanks with each set of analysis along with an IAEA certified reference material. 2,4,5-trichlorobiphenyl (2,4,5 TCB) was used as an internal standard. Average percent recovery in spiked samples ranged from 92 to 98%. Residues were not corrected for percent recovery.

Analyte identifications were confirmed using a Finnigan mass spectrometer, model 4023, operating in ion scan mode. Instrumental conditions included electron ionization (E.I) with a voltage of 2800 eV and under scan condition of m/z 50-400 at one scan per half second. Data for variation between different species and sampling site contamination is tested by analysis of variance (ANOVA).

RESULTS AND DISCUSSION

1. Trends in Residue Levels of Contaminants

The analytical results for chlorinated hydrocarbons residues in fish muscle tissue of the six species examined are presented in Tables 22 and 23 for Mehdia and Tetouan (M'diq), respectively.

DDT and its analogues were present in relatively high quantities in all fish species in comparison with other organochlorine compounds, except for lindane which is by far the most predominant compound in this study. The other compounds are present in relatively low concentrations. The levels of DDTs (p,p'DDT+p,p'DDD+p,p'DDE) varied between 5.61 and 17.96 μg/kg f.w. for all the species collected from Mehdia, and between 3.02 and 13.31 μg/kg f.w. for the species collected from Tetouan. These levels are slightly below the average concentration for Mediterranean species which is 25 μg/kg f.w. (UNEP/FAO/WHO/IAEA, 1990). The average residue levels of DDTs are 9.69 μg/kg in Tetouan and 11.36 μg/kg in Mehdia. These levels are comparable to the average levels of these contaminants in fish species collected from the Gulf of Mexico where the concentrations ranged between 10 and 19 μg/kg as summarized from several studies by Kennicutt II et al. (1988).

Residues of hexachlorobenzene (HCB) ranged between 0.10 and 0.26 μg/kg f.w in Mehdia and between 0.04 and 0.99 μg/kg f.w in Tetouan. These levels are in the range of the other Mediterranean species where the levels range between 0.10 and 1.00 μg/kg f.w. (UNEP/FAO/WHO/IAEA, 1990). Residues of lindane are present in all fish samples, with levels between 8.40 and 39.2 μg/kg f.w. for species collected from Tetouan and between 7.80 and 26.98 μg/kg f.w. in Mehdia. The presence of this compound in high concentrations in fish may reflect its continuing agricultural uses even though it has been recently banned for this purpose (MARA, 1985).

Table 22. Residue levels of organochlorines in musle tissue of different fish species from Tetouan.

Fish species	Samples	Mean concentration (µg/kg f.w.) (Range)								
		HCB	Lindane	Aldrin	Dieldrin	Endrin	p,p' DDE	p,p' DDD	p,p'DDT	DDTs
Boops boops	7	0.08 (ND-0.17)	8.31 (0.23-20.49)	0.47 (ND-3.26)	ND	0.39 (ND-1.85)	0.35 (ND-0.99)	0.35 (ND-1.00)	2.32 (0.05-4.8)	3.02
Merluccius merluccius	4	0.09 (0.08-0.11)	39.2 (4.63-79.77)	ND	ND	ND	0.65 (0.23-1.08)	2.07 (0.16-3.98)	2.15 (1.99-2.32)	4.87
Pagellus pagellus	7	0.04 (ND-1.03)	10.16 (ND-22.84)	0.08 (ND-0.31)	0.05 (ND-0.37)	0.31 (ND-1.18)	2.05 (ND-12.18)	3.62 (ND-12.43)	6.96 (0.14-33.95)	12.63
Sardina pilchardus	4	0.59 (ND-1.19)	14.84 (ND-49.32)	0.09 (ND-0.31)	0.29 (ND-1.18)	1.41 (0.05-4.3)	3.09 (ND-8.21)	1.72 (0.28-3.89)	8.49 (ND-25.26)	13.31
Trachurus trachurus	4	0.99 (ND-2.15)	16.00 (ND-26.35)	0.13 (ND-0.33)	ND	0.48 (0.08-1.25)	3.28 (ND-6.62)	0.94 (ND-1.75)	7.42 (0.51-19.4)	11.64
All species mean	26	0.36 (0.04-0.99)	17.7 (8.4-39.2)	0.15 (ND-0.47)	0.25 (ND-0.29)	0.52 (ND-1.41)	1.88 (0.35-3.28)	1.74 (0.35-3.62)	5.67 (2.15-8.49)	9.69

ND = Not detected.
DDTs = p,p' DDE+ p,p' DDD+ p,p' DDT.

Table 23. Residue levels of organochlorines in muscle tissue of different fish species from Mehdia.

Fish species	Samples	Mean concentration (µg/kg f.w.) (Range)								
		HCB	Lindane	Aldrin	Dieldrin	Endrin	p,p' DDE	p,p' DDD	p,p'DDT	DDTs
Boops boops	6	0.1 (ND-0.26)	11.74 (ND-33.62)	3.18 (ND-10.02)	0.08 (ND-0.45)	0.97 (ND-4.47)	0.44 (ND-1.57)	2.18 (ND-12.12)	4.71 (0.18-12.79)	6.79
Merluccius merluccius	4	0.24 (ND-0.59)	8.44 (ND-18.70)	6.01 (ND-23.09)	0.14 (ND-0.56)	0.53 (ND-1.29)	0.18 (ND-0.75)	0.45 (ND-0.68)	9.96 (ND-36.9)	10.59
Pagellus pagellus	4	0.26 (0.04-0.48)	26.98 (13.76-40.2)	ND	ND	0.61 (0.36-0.84)	2.71 (0.1-5.33)	1.92 (0.31-3.53)	11.22 (6.64-15.79)	15.85
Sardina pilchardus	4	0.24 (0.1-0.53)	7.8 (6.5-10.82)	1.55 (0.85-3.51)	0.25 (ND-0.48)	ND	1.07 (ND-4.31)	2.3 (1.60-4.12)	2.24 (0.41-4.31)	5.61
Trachurus trachurus	4	0.14 (0.06-0.19)	19.32 (1.73-36.99)	1.57 (ND-5.69)	0.05 (ND-0.19)	0.31 (0.10-0.58)	0.42 (0.06-1.46)	5.34 (ND-18.75)	12.2 (1.5-22.13)	17.96
All species mean	22	0.2 (0.10-0.26)	14.85 (7.8-26.98)	2.46 (ND-8.01)	0.1 (ND-0.97)	0.48 (ND-0.97)	1.61 (0.18-4.31)	2.44 (0.45-5.34)	8.06 (2.24-12.20)	11.36

ND = Not detected.
DDTs = p,p' DDE+ p,p' DDD+ p,p' DDT.

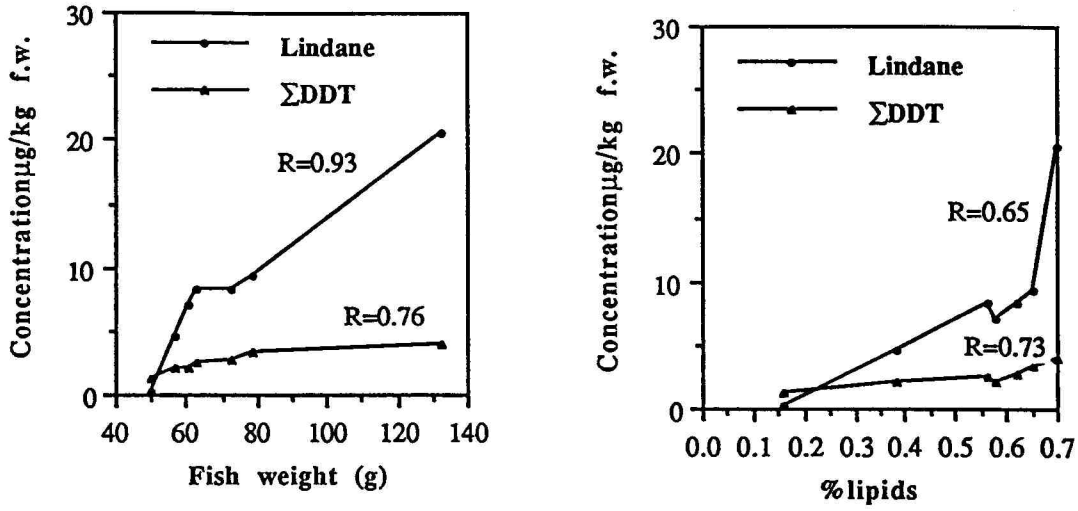

Figure 17. Correlation between concentrations of lindane and DDTs with fish body weight and lipid content of Bogue (*Boops boops*).

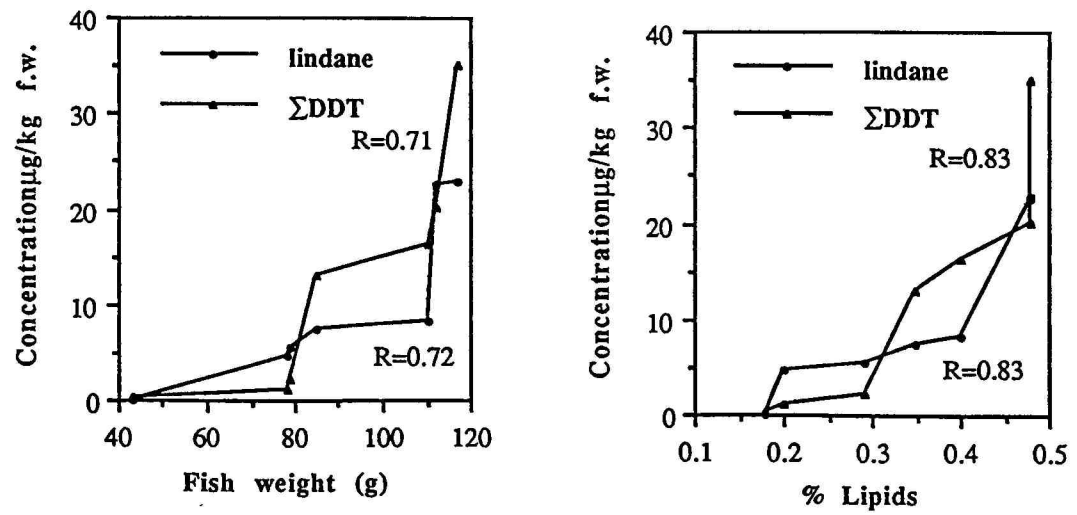

Figure 18. Correlation between concentrations of lindane and DDTs with fish body weight and lipid content of Axielary seabream (*Pagellus acarne*).

The drins (aldrin, dieldrin, and endrin) are present in relatively lower concentrations and frequencies in fish samples from both sampling sites, with an exception of the high concentrations of aldrin in Mehdia which may reflect its heavy use in this area. The average levels for all species are 0.15, 0.25 and 0.52 μg/kg f.w. for aldrin, dieldrin and endrin in Tetouan and 2.46, 0.10 and 0.48 μg/kg f.w. in Mehdia respectively.

A correlation between lindane and DDT concentrations with lipids content and body weight was carried out in both bogue and axielary seabream species. The results revealed an increase of chlorinated hydrocarbons with increase in body weight and lipid contents in all fish samples analyzed. The linear accumulation of p,p'DDT and other chlorinated hydrocarbons with body size was noted previously in several marine and fresh water fish species and was attributed to lipid concentration with larger fish (Satjmadjis and Gabrielides, 1979; El Nabawi et al., 1987). However, Fossato and Craboledda (1981) found that the sequence of species (*Nephrops*, *Carcinus*, *Mytilus*, *Mullus*, *Engraulis*, *Thunnus*) according to tissue level of chlorinated hydrocarbons only partly reflects their lipid content. In fact, levels in anchovies were comparable to those found in mullets, although the lipid contents of the two species were very different. It was concluded that food, habitat and the physiology of the various organisms strongly influence their accumulation capability.

Analysis of variance for fish species contamination byt he two sampling sites did not show significant differences at a 5% level, but multiple range analysis tests for fish species contamination by sampling location yielded low power (variability in the data and low sample size) as indicated by confidence interval ratios of Tetouan and Mehdia. The levels ranged from 0.21 to 2.77 for lindane; 0.57 to 5.10 for HCB; 0.12 to 2.22 for Drins and from 0.32 to 0.47 for DDTs.

The Mediterranean Sea is an almost completely land-locked body of water which is highly contaminated by the dumping from numerous pollution sources and generally contamination levels for persistent chemicals detected there are higher than those in other open seas. In the present study, for most of organochlorine contaminants analyzed, the concentration levels in the same fish species collected from both sampling sites are not significantly different. This similarity may be explained by the intense agricultural activity in the Mehdia (Gharb area) which certainly contribute to increase this contamination. Another factor that has to be considered is the proximity of Tetouan to the Straight of Gibraltar, which is a point of an extensive water exchange between the Mediterranean Sea and the Atlantic Ocean.

The ADI of DDT for humans is estimated by FAO/WHO to be 0 to 0.02 mg per kg body weight (FAO/WHO, 1984). Morocco has not established maximum tolerable levels for human

consumption, but by considering the levels in many other countries, the maximum DDT level allowed in fish is 5 mg per kg fish. The corresponding values for aldrin and HCB are 0.2-0.5 mg/kg,and 0.1-2 mg/kg for lindane (Nauen, 1983; USFDA, 1982, 1984). These values, based on the fresh weight of the edible portion of the food, were not exceeded in any of the samples analyzed and the concentrations found were well below the risk levels. However, even if the pesticide levels are not likely to cause health hazards to humans, they may still present a future danger, thus requiring continuous monitoring of these contaminants to insure the protection of both the environment and human health.

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CHAPTER VIII

POLYCHLORINATED BIPHENYL RESIDUES IN MOROCCAN SEAFOOD PRODUCTS

ABSTRACT

Polychlorinated biphenyls (PCBs) were analyzed in five fish species collected from M'diq (Mediterranean Sea) and from Mehdia (Atlantic Ocean). Residue analysis were performed by capillary gas chromatography and mass spectrometry techniques.

Total PCB concentrations detected in the samples from Tetouan varied from 5.8-21.92 μg/kg fresh weight with an average of 10.8 μg/kg fresh weight. Mean concentrations for the species collected from Mehdia range from 8.75-33.51 μg/kg with an average of 17.92 μg/kg. The most prevalent congeners are present in concentration between 1-2 μg/kg f.w. in all fish species, and have 4-7 chlorine atoms in their molecules.

PCB contamination of seafood products was relatively light compared to other locations in the world. No problems are likely to occur with respect to human consumption of fishery products since the levels are below acceptable limits in fish and fishery products as established by several countries. These threshold values vary between 1-5 mg/kg fresh weight. There was no significant difference in contamination between sample collected from Mediterranean Sea and Atlantic Ocean.

INTRODUCTION

Polychlorinated biphenyls (PCBs) are one of the most ubiquitous and persistent pollutants occurring in the environment because of their extensive utilization and their chemical properties. They enter the marine environment from both aerial sources and run-off and where fish and shellfish accumulate high concentrations of these compounds in their tissue. It has been shown that fish products generally contain higher levels of PCBs than any other food category (UNEP/FAO/WHO, 1988) which can result in potential health risks for humans who consume large amounts of seafood products.

The aim of this study was to analyze commercially exploited fish species taken along the coast of Morocco from the Mediterranean Sea and the Atlantic Ocean and to evaluate the respective state of contamination by PCBs of these two sampling locations.

MATERIAL AND METHODS

1. Sampling Strategy

Collections of five fish from five species were made at monthly intervals over a period of six months during 1990/91 at both Tetouan (M'diq) (Mediterranean Sea) and Mehdia (Atlantic Ocean) (Figure 19). Samples of both muscle were collected and immediately frozen for subsequent analysis. The edible muscle tissues were analyzed for polychlorinated biphenyl (PCBs).

2. Collection and Dissection

Five fish species were selected for this study: European hake (*Merluccius merluccius)*, horse mackerel *(Trachurus trachurus)*, axielary seabream *(Pagellus acarne)*, bogue *(Boops boops) and* pilchard *(Sardina pilchardus*). Species selection was based on their presence both in Mediterranean Sea and Atlantic Ocean and a large consumption by Moroccans. Fish chilled in ice were collected from the port immediately after the return of the fishing boats. After being identified, the weight and the length recorded, the fish were dissected and aliquot of muscle taken immediately frozen and stored in freezer at -20 °C until analysis.

3. Sample preparation and extraction

Ten grams of the thawed dorsal fish muscle were mixed with 20 g of anhydrous sodium sulfate, then transferred to a soxhlet apparatus and extracted with 250 ml hexane for 8 hr. The lipid content of the extract was determined gravimetrically.

4. Purification

The samples were purified by florisil column chromatography. The column (20 mm i.d. x 300 mm) was filled with 14 g of 60/80 mesh florisil (Fisher Scientific) deactivated with 0.5 ml water and topped with 1 cm of anhydrous sodium sulphate. The column was washed with hexane and eluted with 70 ml hexane. The elutant, in addition to PCBs contained the compounds hexachlorobenzene and p,p'DDE.

5. Sample analysis

Residues were analyzed by capillary gas chromatography (GC) using a Varian 3700 equipped with a ^{63}Ni electron capture detector (ECD), a split injection system, and an integrator model LCI 100 (Perkin-Elmer). The fused silica capillary column was 15 m x 0.25 mm i.d. SE-54 (Altech Associates, Inc). The carrier gas was helium with a flow rate 1.5 ml/min. The temperature for the injector and detector was 280°C and 350°C, respectively; an initial

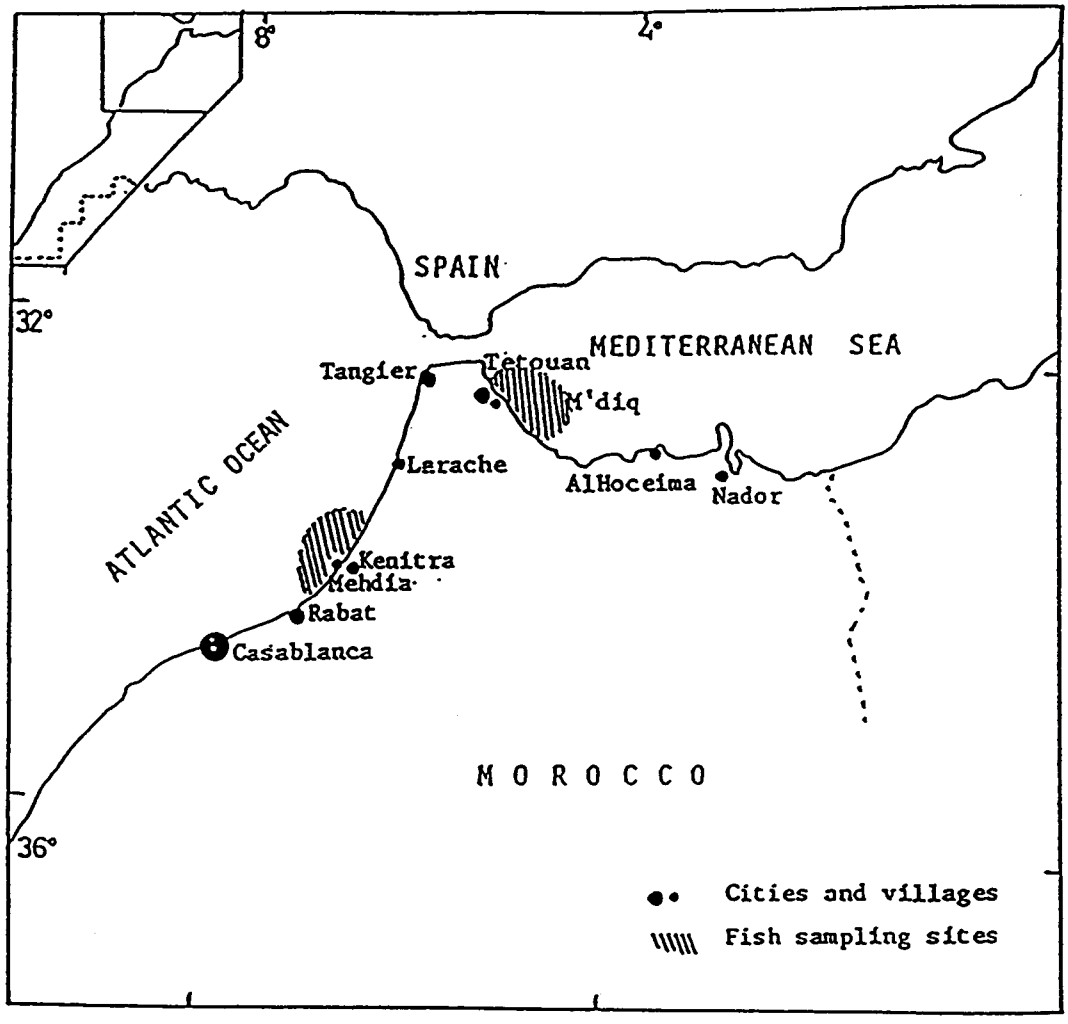


Figure 19. Map of northern Morocco showing the sampling location of fish.

temperature of 70°C was held for 2 min. raised to 260°C at 3°C/min, and held at final temperature for 2 min. The detector make-up gas was nitrogen at a flow rate of 30ml/min. Quality assurance measures include the analysis of reagent blanks with each set of analyses. 2,4,5-Trichlorobiphenyl (2,4,5 TCB) was used as an internal standard. Average percent recovery in spiked samples was 94±5%. Residues were not corrected for percent recovery.

For accurate analysis of PCBs all congeners which elute as identified peaks from the column (SE-54) GC-ECD were considered (Figures 20a,b,c). The congeners were identified through direct comparison of the chromatograms of commercial Aroclor 1242, 1254, and 1260 mixtures and relative retention time of all the congeners on the same column type (Mullin et al., 1984; Capel et al., 1985). The PCBs were identified on the basis of International Union of Pure and Applied Chemists (IUPAC) congeners numbering system (Ballschmiter and Zell, 1980). Quantification was done using Aroclor 1254, and 1260 standard mixture (IAEA standards) and the relative congeners percentages of the same mixtures for specific congeners determination (Capel et al., 1985).

Analyte identifications were confirmed using a Finnigan mass spectrometer, model 4023, operating in ion scan mode (Figures 21a,b). Instrumental conditions included electron ionization (E.I) voltage of 2800 eV and scan condition of m/z 50-400 at one scan per 1/2 second (Pellizzari et al, 1985). Data for variation between different fish species and sampling sites were tested by analysis of variance.

RESULTS AND DISCUSSION

The congeners analyzed represent different degrees of chlorination encountered in Aroclor mixtures, and are considered most likely to have toxicological importance and to varying in degrees and types of induction of the mixed function oxidase activities in animals (McFarland and Clarke, 1989). Only congeners that could be identified and quantified were considered. The mean concentration of 20 PCB congeners and total PCB by site and species are reported in Tables 24 and 25. Total PCB concentrations were calculated by summing the concentrations of all individual congeners including those that are not listed in Tables 24 and 25.

1. Trends in residue levels of contaminants

The analytical results of polychlorinated biphenyls residues in fish muscle tissue of the five fish species examined are presented in Tables 24 and 25 for Mehdia and Tetouan, respectively. Total PCB concentrations detected in the samples from Tetouan varied between 5.8-21.92 μg/kg fresh weight with an average of 10.8 μg/kg fresh weight. The comparison of these levels with other Mediterranean locations showed moderate contamination of this area

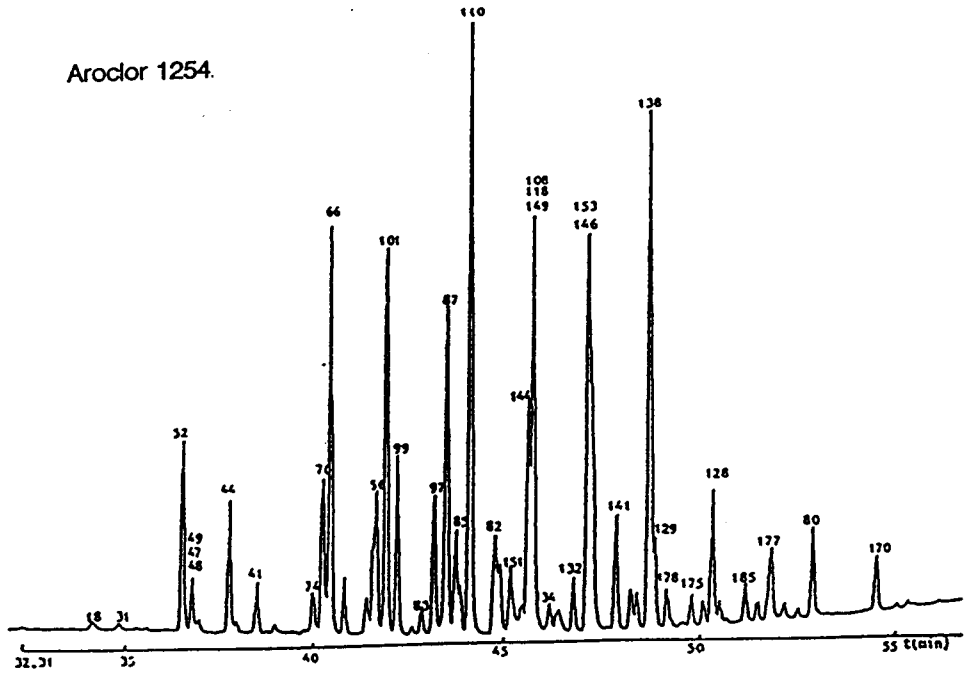


Figure 20a. Capillary column gas chromatogram of Aroclor 1254.

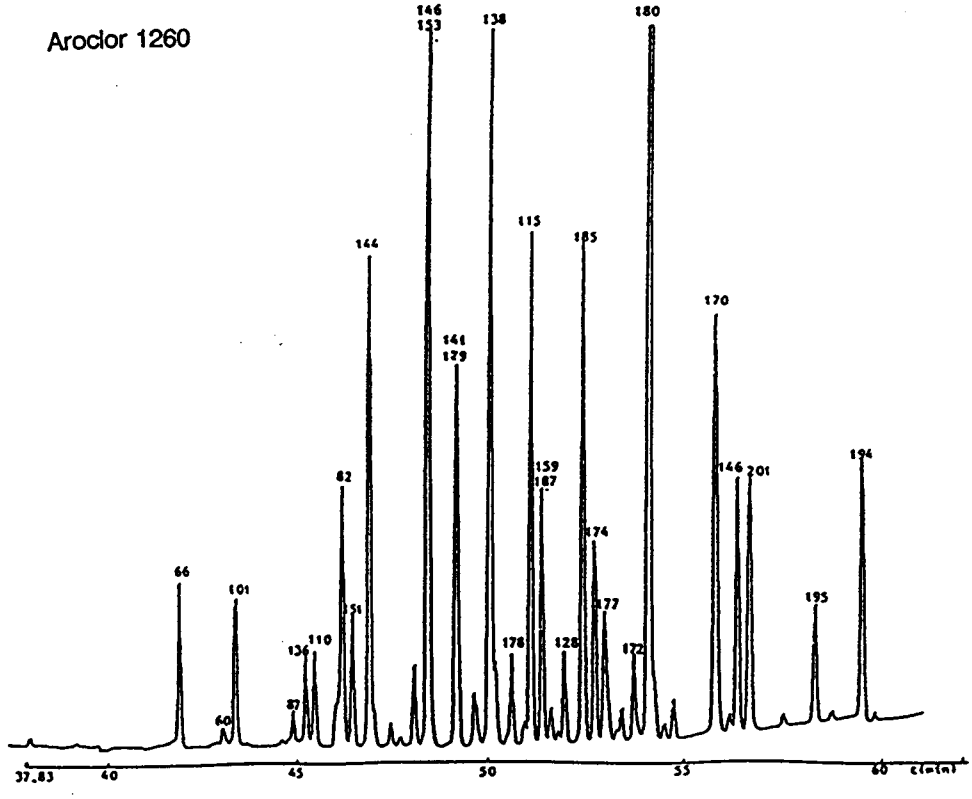


Figure 20b. Capillary column gas chromatogram of Aroclor 1260.

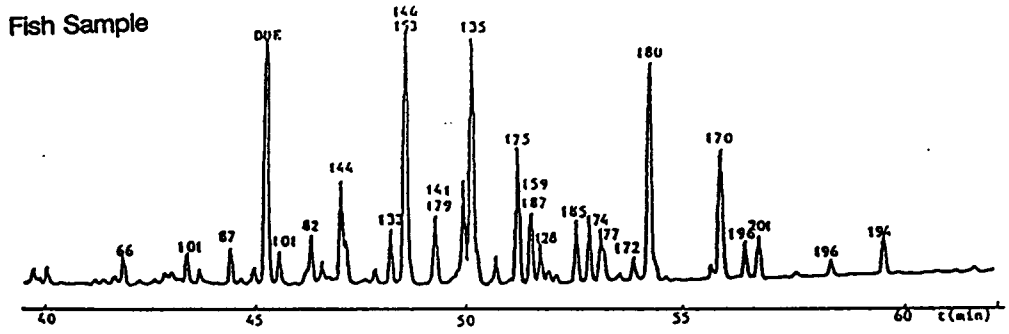


Figure 20c. Capillary column gas chromatogram of fish sample.

(Table 26). Mean concentrations for the species collected from Mehdia ranged from 8.75-33.51 μg/kg with an average of 17.92 μg/kg.

Even though the average concentration in Mehdia is higher than Tetouan, the PCBs contamination differences of the two sampling sites tested by analysis of variance for different species in both areas did not show any significant variation at level 5% between the two sampling sites. But multiple range analysis test for fish species contamination by sampling site showed low power variability in the data and low sample size as indicated by a wide confidence interval ratio of Tetouan site to Mehdia ranging from 0.23 to 2.96.

PCB contamination of seafood products of the two sampling sites showed a lower level of contamination as compared to other polluted locations of the world. For example, the PCB levels varied between 11-203 μg/kg in fish from the Gulf of Mexico (Kennicutt et al., 1988). A very high accumulation of PCBs (0.2-0.5 mg/kg fresh weight) was reported in a polluted area of Puget Sound in Washington, U.S.A. (Malins et al., 1984). A Swedish study showed a mean PCB values between 0.3-0.7 mg/kg fresh weight in herring from the Baltic (Andersson et al.,1984).

2. PCB Congeners Pattern in Fish Species

The prevalent congeners are present in concentration between 1-2 μg/kg f.w. in all fish species, and have 4-7 chlorine atoms in their molecules, i.e., numbers 118/108/149,138, 170, 101, 153/146, 180, 18, 47/48/49, 70, 74, and 159/187. These moderately chlorinated groups (penta, hexa, and heptachlorobiphenyls) contain 112 of the 209 possible PCB configurations. They were synthesized in high proportions in many PCB formulations and are the most bioaccumulating PCB congeners which make them likely to be prevalent in environmental samples (Alford-Stevens, 1986; Hutzinger et al., 1974). The more highly chlorinated (7-8 chlorines) congeners such as 194, 177, 159/187 and 201 were present at lower concentrations less than 1 μg/kg f.w. It has been shown that the most highly chlorinated congeners are generally less available to organisms because they are usually present at lower quantities in the environment and are more tightly bound to soil and sediments (McFarland and Clarke, 1989). Congeners with less chlorination i.e., 87, 99, 44, 52 were present in lower concentrations less than 0.5 μg/kg f.w.,with exception of congener number 18, because they are readily metabolized and eliminated and do not tend to bioaccumulate. None of samples in this study matched with the commercial mixture Aroclor 1242 but showed a pattern similar to either Aroclor 1254 or 1260. The commercial mixture 1242 contains congeners of low chlorine substitution that are generally more readily metabolized by the organisms and consequently would not be present in the tissue analyzed.

Table 24. Concentration of PCB congeners in fish muscle tissue of different fish species from Tetouan.

PCBs	Mean concentration (µg/kg f.w.) (range)				
	Trachurus n=4	Sardina n=7	Merluccius n=3	Boops n=6	Pagellus n=7
118/108/149	1.45 (0.52-2.38)	0.08 (0.043-0.122)			
128	0.17 (0.03-0.32)	0.04 (0.025-0.06)	0.154	0.06 (0.01-0.13)	0.03
138	1.66 (0.45-4.74)	1.12 (0.12-3.62)	0.16 (0.13-0.2)	0.55 (0.03-1.63)	0.8 (0.01-1.56)
170	0.96 (0.17-1.29)	1.42 (0.033-3.39)	1.97 (0.01-5.81)	0.34 (ND-0.81)	0.4 (0.05-1.09)
87	0.77 (0.0044-1.8)	0.22 (0.014-0.56)	0.27	0.08 (ND-0.23)	0.29 (ND-1.18)
99	0.57 (0.273-0.873)	0.31 (0.19-0.52)	ND	0.44 (0.21-0.68)	0.11 (0.06-0.15)
101	1.16 (0.43-1.83)	0.42 (0.144-0.75)	1.54 (0.06-4.37)	0.31 (0.14-0.44)	0.19 (0.003-0.38)
146/153	1.19 0.61-2.19)	1.28 (0.09-3.18)	0.61 (0.125-0.94)	0.7 (0.11-1.53)	0.59 (0.1-1.59)
180	0.79 0.112-1.97)	0.39 (0.007-1.3)	1.25 (0.04-3.44)	0.45 (0-1.04)	0.31 (0.012-0.69)
194	0.145 (0.1-0.19)	0.045 02-0.07)	0.011	0.03	0.007
18	ND	2.23 (2.23-14.28)	0.26	2.84 (2.84-7.03)	0.7 (0.27-1.57)
44	0.26 (0.02-0.7)	0.81 (0.51-1.36)	0.26	0.129	0.1 (0.05-0.14)
47/48/49	1.49 (0.62-2.37)	1.96 (0.18-5.46)	0.02	ND	0.56 (0.07-1.05)
52	0.81 (0.098-1.417)	0.52 (0.32-0.76)	0.36 (0.15-0.56)	0.24 (0.24-0.25)	0.27 (0.08-0.42)
70	0.74	2.88 (0.65-5.12)	1.08	0.41	0.90 (0.47-1.31)
74	2.59 (2.48-2.7)	1.50 (1.37-1.57)	0.51	0.15	0.83 (0.40-1.25)
151	0.18 (0.1-0.27)	0.13 (0.07-0.21)	0.19	0.07 (0.05-0.09)	ND
177	0.38 0.19-0.57)	0.07 (ND-0.13)	0.06 (0.054-0.06)	0.06 (ND-0.13)	0.24 (0.03-0.45)
159/187	1.37 (0.12-3.55)	0.53 (0.05-1.50)	0.36 (0.063-0.066)	0.94	0.20 (0.07-0.32)
201	0.603	0.18 (0.11-0.25)	0.039	(0.26-1.63) 0.14	0.03
Total PCBs	21.92 (5.55-33.11)	11.1 (0.46-19.34)	8.87 (1.63-20.63)	5.8 (3.65-55.29)	6.26 (6.79-9.11)

ND= Not detected

Table 25. Concentration of PCB congeners in fish muscle tissue of different fish species from Mehdia.

PCBs	Mean concentration (µg/kg f.w.) (range)				
	Trachurus n=4	Sardina n=7	Merluccius n=3	Boops n=6	Pagellus n=7
118/108/149	1.12 (0.78-1.56)	3.39	5.29	0.80 (0.24-1.34)	ND
128	0.07 (0.006-0.195)	ND	0.09 (0.07-0.12)	0.14	0.98
138	2.02 (0.106-9.15)	1.70	0.05 (0.02-0.10)	1.31 .(0.46-2.87)	2.21 (0.194-4.22)
170	1.76 (0.012-5.86)	1.41	2.30	0.69 (0.25-1.41)	0.82 (0.044-1.6)
87	0.04 (0.01-0.102)	0.44	0.99	0.1 (0.07-0.143)	ND
99	0.16 (0.045-0.275)	0.49	0.10	0.49 (0.16-0.81	ND
101	0.49 (0.16-0.95)	0.28	1.39 (0.64-2.78)	0.42 (0.32-0.52)	0.74
146/153	1.22 (0.36-2.40)	2.49	2.74 (0.20-5.29)	1.66 (0.32-2.57)	5.05
180	0.80 (0.12-2.42)	1.01	0.40 (0.14-0.65)	0.85 (0.65-1.15)	2.34
194	0.023 (0.005-0.046)	ND	0.21	0.06 (0.05-0.06)	ND
18	2.07 (1.54-2.61)	ND	1.6	1.24 (0.54-1.94)	ND
44	0.47 (0.07-0.87)	0.53		0.23	ND
47/48/49	0.165	0.28	0.72	0.20	0.12
52	0.30 (0.0212-0.397)	0.84	2.08	0.07 (0.04-0.114)	ND
70	1.26 (0.46-2.05)	7.53	9.51	0.63 (0.20-1.06)	1.131
74	0.91 (0.74-1.08)	2.2	1.86 (ND-1.86)	0.19 (0.102-0.27)	ND
151	0.22 (0.03-0.42)	0.33	0.59	0.23	ND
177	0.02 0.002-0.043)	0.26	0.55 (0.39-0.713)	0.16 (0.05-0.24)	0.59
159/187	0.54 (0.043-2.32)	0.49	0.45 (0.45-0.46)	0.24 0.22-0.27)	0.77 (0.713-0.83)
201	0.11 (0.02-0.19)	ND	ND	0.17 (0.17-0.174)	0.51
TOTAL PCBs	16.43 (1.78-45.65)	33.51	19.41 (12.95-48.32)	8.75 (3.15-14.74)	11.5 (9.18-20.82)

ND = not detected

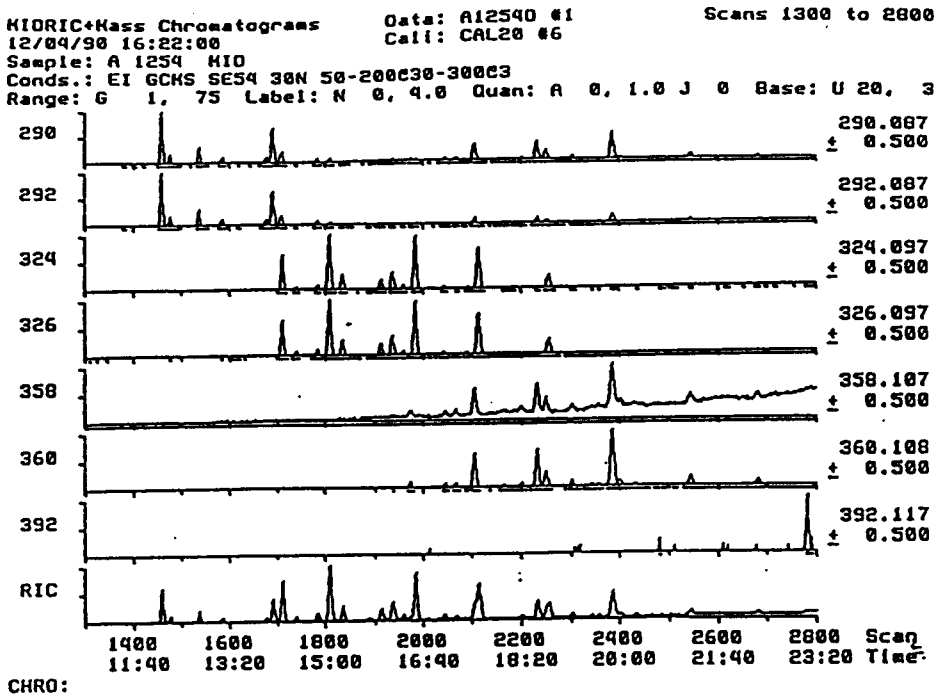
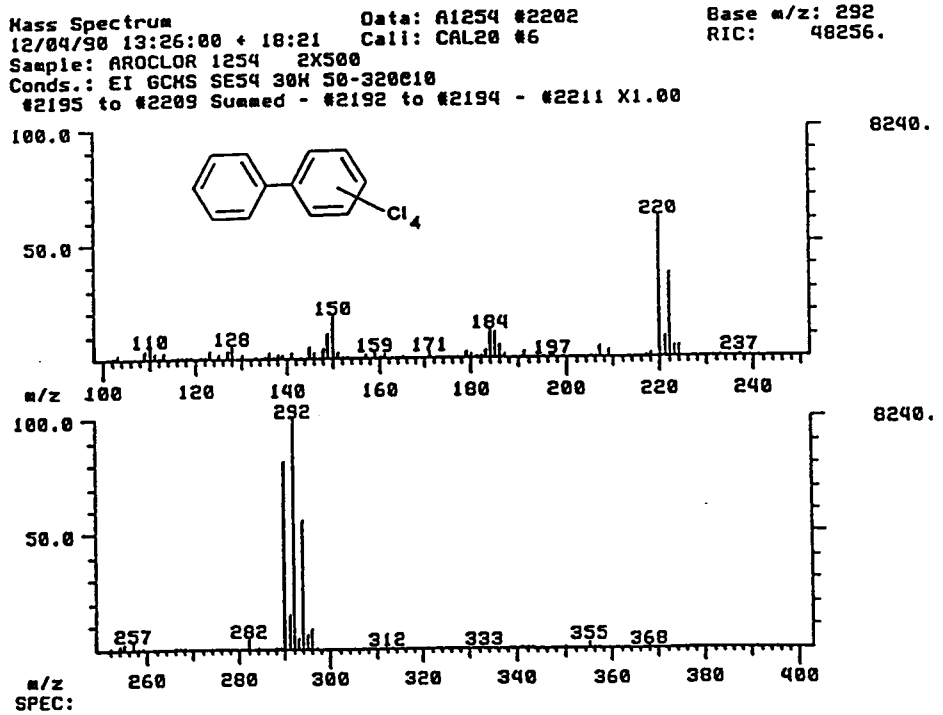


Figure 21a. Negative ionization mass chromatogram of Aroclor 1254.



MW = 292
Intense Peaks: 292 (100); 290 (841); 220 (70).

Figure 21b. Negative ionization mass spectrum of tetrachlorobiphenyl.

Table 26. Comparison of PCBs in fish from Mediterranean regions, Persian Gulf and Moroccan Mediterranean coast.

Regions	Concentration (µg/kg f.w.)		References
	Range	Average	
Adriatic Sea Yugoslavia	ND-15000	960	Smodlaka et al.,1981
Sicilian coast Italy	ND-6100	990	Castelli et al., 1983
Sicilian coast Italy	10-370	70	Amico et al., 1979
North Adriatic	100-870	50	Viviani et al., 1974
Israeli coast	ND-1200	160	Ravid et al., 1985
Gulf (Coast of Oman)	0.24-0.54	0.4	Burns et al., 1984
Gulf (Coast of Kuwait)	1.3-11.4	4.2	Villeneuve et al.,1987
Mediterranean	5.8-21.92	10.8	This study

ND=Not detected.
From Villeneuve et al. (1987).

CONCLUSION

In this study PCB contamination of seafood products showed very limited contamination as compared to other locations in the world. No problems are likely to occur withrespect to human consumption of fishery products since the levels are below acceptable limits in fish and fishery products as established by several countries. These threshold values vary between 1-5 mg/kg fresh weight (Nauen, 1983).

There were no significant differences in contamination between samples collected from the Mediterranean sea and Atlantic Ocean.

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CHAPTER IX

APPROACH TO ASSESSMENT OF HUMAN HEALTH RISKS FROM THE CONSUMPTION OF CONTAMINATED SEAFOOD

ABSTRACT

Human health risks for some chlorinated hydrocarbons and three heavy metals in fish are evaluated in terms of comparison with acceptable levels set by FAO/WHO regulatory policy. The risk is evaluated for fishermen and their families in coastal village area in the Mediterranean coast of Morocco where people consume daily large amounts of fish.

Results revealed that chlorinated hydrocarbons pesticides and polychlorinated biphenyls (PCBs) detected in seafood products are not likely to cause any immediate toxicological problem for this high fish consumer group.

For trace metals the daily intake is respectively about 10%, 11.9 to 14.5% and 143% of the acceptable daily intake for lead, cadmium and mercury. The mercury is likely to cause some health problems. Further toxicological evaluation (blood and hair mercury levels) are necessary to decide on the risk for this group of population.

Presented at the Third International Symposium on Foodborne Diseases and Intoxicants. June 16-19, 1992; Berlin, Germany.

INTRODUCTION

Seafoods are harvested and extensively consumed in Morocco especially in coastal areas. Relatively high concentrations of contaminants such as mercury, lead, cadmium, chlorinated hydrocarbons pesticides and polychlorinated biphenyls (PCBs) have been found in some edible marine organisms previously analyzed for these contaminants (El Hraiki et al., 1992a). High intake of seafood from these locations thus could adversely affect the health of exposed individuals.

Risk analysis for chemical contaminants may be conducted by one or more of the methods that are classified as qualitative, semi-quantitative and quantitative (Scheuplein, 1987; Barnes and Dourson, 1988; Portier, 1989). However, the distinction between these approaches are not clear-cut because of limitations and uncertainties associated with the kind of data that are typically available for risk characterization. In qualitative approaches, the characterization of risk is based only on relative ranking (e.g. high, medium and low). In the semi-quantitative approaches, a numerical risk index is calculated, but absolute risks are not estimated. In quantitative approaches, dose-response models are completely specified and are used to calculate absolute estimate of risk or to derive "action level" guidelines for tissue contamination from an "allowable risk" set by regulatory policy.

The aim of the present study is to evaluate the health risks associated with specified patterns of seafood consumption. Fishermen and their families were selected as a high potential risk group because of their heavy seafood intake.

MATERIAL AND METHODS

To assess the human health risks from the contaminated seafood a study was carried out to determine the contamination of different fish species by some major contaminants such as chlorinated hydrocarbons pesticides, polychlorinated biphenyls (PCBs) and trace metals (cadmium, mercury and lead). Fish samples were collected at monthly intervals from January 1988 to July 1990 from the ports of Tangier, Tetouan (M'diq), Al Hoceima and Nador. The sampling of marine organisms were conducted according to the UNEP/FAO/IAEA/IOC recommendations (1984 a, b). Chlorinated hydrocarbons were analyzed according to the method described by Villeneuve (1986) and El Nabawi et al. (1987). PCBs were analyzed by gas chromatograghy with an electron capture detector. Identification and quantification were carried out using Aroclor 1254 and 1260 standard mixtures (Ballschmitter and Zell, 1980; Capel et al., 1985). Cadmium and lead concentrations were determined by electrothermal atomic absorption spectrophotometry (AAS). Cold vapor AAS was used for mercury analysis (Lazzlo, 1972); UNEP/FAO/IAEA/IOC, 1984 c, d).

RESULTS

Concentration of Major Pollutants in Fish

The results are summarized in Table 27. The data selected for risk evaluation were obtained from analysis of some major contaminants in various fish species collected from the Mediterranean coasts of Morocco (El Hraiki et al, 1992a, b, c, d). These results indicate that lindane was the most abundant pesticide in all fish species probably due to the continuing heavy use of this pesticide in animal and human health programs to control vector borne diseases. PCB mean concentration of 10 μg/kg fresh weight are low in comparison with other areas of the Mediterranean where the average concentrations have been reported around 100 μg/kg fresh weight (UNEP/FAO/WHO/IAEA, 1990). DDTs with mean concentration of 7 μg/kg fresh weight is still considered high value since these compounds have been banned in most Mediterranean countries several years ago. Mercury was the most predominant metal found in this study with the levels for some fish species exceeding the acceptable limits of 0.5 mg/kg adopted by several countries (Nauen, 1983; UNEP/FAO/WHO, 1988).

The Use of Data for Intake Estimates

For appropriate estimation of risk, a survey was conducted in the study area on 60 families of fishermen, considered as population at risk because of their heavy fish consumption. The results show that the fish was eaten from 3 to 7 days a week with intakes ranging between 50 to 190 g/day. The species consumed most frequently were those regarded as less desirable and hence of low value. These were *Trachurus trachurus, Boops boops* and *Sardina pilchardus* with 40 %, 32 % and 22 %, respectively and 6 % for all other species. These percentages with the highest fish consumption were taken into consideration for intake estimate.

Risk Related to Acceptable Daily Intake

The tissue concentrations of contaminants in seafood species collected from the study area (Table 27) were used to calculate the daily intake levels. The intakes are compared with the acceptable daily intake (ADI) for the various contaminants established by the FAO/WHO recommendations or by United States Food and Drug Administration (USFDA) in cases where no ADI has been proposed for certain chemicals by the FAO/WHO committee (Table 28).

Acceptable daily intake denotes the dose, expressed on a body weight basis, of a substance which can be acceptable over a lifetime without appreciable health risk. The starting point is usually the No Observed Adverse Effect Level (NOAEL) estimated in 90-day animal studies and extrapolated to man with some margin of safety (WHO, 1987a).

Table 27. Mean concentration of major pollutants in fish harvested on the Mediterranean coast of Morocco.

Fish species	Mean concentration (µg/kg f.w.)							
	Total PCBs	HCB	Lindane	Aldrin+ Dieldrin	DDT	Total Hg	Pb	Cd
Boops boops	5.8	0.08	8.4	1.66	2.32	376	247	64.5
Merluccius merluccius	8.87	0.09	39.2	ND	2.15	262	27	81
Pagellus acarne	6.26	0.05	11.86	0.51	6.96	193	257	44.5
Sardina pilchardus	11.1	0.79	19.79	1.3	11.32	593	249	24
Solea solea	7.96	0.31	15.32	ND	11.89	230	289	31.5
Trachurus trachurus	21.92	1.48	24	0.2	7.42	301	261	23
Average	10.5	0.53	19.76	0.61	7.01	325	262	44.7

Total PCBs = Total of resolved peaks. HCB = Hexachlorobenzene.

Polychlorinated Biphenyls (PCBs)

Fish generally contains higher levels of PCBs than any other food category (WHO, 1976; UNEP/FAO/WHO, 1988). National regulatory limits for PCBs in fish and shellfish range from 500 to 5000 μg/kg, certain countries setting different limits depending on fishing grounds and fish species (WHO, 1987b). Generally, the median levels reported were less or slightly above 100 μg/kg (UNEP/FAO/WHO, 1988). The average levels for the most consumed species found in this investigaiton is 10.3 μg/kg. No ADI has been established by FAO/WHO Codex Alimentarus Commission. The daily intake for the high fish consumers is 1.96 μg/kg for PCBs which contributes to the ADI about 2.8%.

Aldrin and Dieldrin

The ADI for aldrin and dieldrin is 0.1 μg/kg body weight/day. Dietary intake, expressed as a percentage of the ADI for aldrin and dieldrin shows that current intake is about 1.7%.

Dichlorodiphenyltrichloroethane (DDT)

The ADI is 5 μg/kg body weight/day FAO/WHO, 1979). Dietary intake expressed as a percentage of the ADI shows that intake is about 0.4% of the ADI.

Lindane

The ADI for lindane is 10 μg/kg body weight/day FAO/WHO, 1979). Dietary intake of lindane throug seafood consumption indicates very low contribution (about 0.4% of this product to the ADI).

Hexachlorobenzene (HCB)

The ADI of 420 μg for HCB has been canceled since it was considered too high (FAO/WHO, 1979). The value in Table 28 is one tenth of the canceled ADI. The calculated dially intake of l0.10 μg/kg contributes to about 0.24% of the established value for HCB.

Lead

In 1972, the Joint Expert Committee on Food Additives (JECFA) established a provisional tolerable weekly intake (PTWI) for lead of 50 μg/kg body weight (500 μg/person/day), applicable to adults only (WHO, 1972). In 1986, the JECFA evaluated the health risks of lead for infants and children and established a PTWI of 25 μg/kg of body weight. The latter population group is more vulnerable to lead than adults because of its metabolic and behavior differences (WHO, 1987c, d).

Table 28. The comparison of ADI with the daily intake of high fish consumers (190 g seafood/day).

Contaminant	Acceptable daily* intake µg/70 kg	Concentration in fish µg/Kg	Daily intake µg/person	Daily intake In % ADI
Aldrin + Dieldrin	7	0.61	0.12	1.71
DDT	350	7.01	0.12	0.4
HCB	42	0.53	0.1	0.24
Lindane	700	19.76	3.75	0.54
PCB	70	10.32	91.96	2.8
Total Hg	43	325	61.75	143.6
Cd	57.1-71.4*	44.75	8.5	11.9-14.9
Pb	500*	262	49.78	9.96

* ADIs for HCB and PCB are not FAO/WHO recommendations.
*Calculated ADIs from the Provisionald Tolerable Weekly Intake (PTWI). (WHO, 1972).

The highest concentrations of lead in food products are found in molluscs, crustaceans, and fish and animal offals (UNEP/FAO/WHO, 1988). In fish examined in this study the average lead contamination level was 262 μg/kg. The calculated daily intake for the selected population is, therefore, about 49.78 μg/person/day which contributes to about 10% of the ADI (500 μg/person/day).

Cadmium

Provisional Tolerable Weekly Intake (PTWI) established by the JECFA for cadmium is 400 to 500 μg Cd/week/person (57.1-71.4 μg/day) (WHO, 1972). In 1988, JECFA reassessed cadmium exposure and established a PTWI of 7 μg/kg body weight applicable to adults as well as children. Unlike mercury where the main source of intake by man is through the consumption of contaminated seafood, for nonoccupationally exposed humans, terrestrial food and cigarettes constitute the most important source of cadmium intake. For example, smoking 20 cigarettes/day yields an intake of about 20 μg Cd per week (Stoeppler, 1984). The average concentration in fish products in this study is about 44.75 μg/kg f.w. which gives a daily intake of about 8.5 μg per person for the high fish consumers and contributes to about 11.9 to 14.9% of the ADI.

Mercury

Mercury as methylmercury (MeHg) is still considered as a prime pollutant in fish, including marine fish all over the world. It was estimated that the earliest symptoms may appear following long-term daily ingestion of 200-500 μg Hg as methylmercury for 70 kg person (WHO, 1976). The JECFA has estalbished a PTWI of 300 μg of total Hg/week/person (42.8 μg/day), of which no more than 200 μg should be present as methylmercury (WHO, 1972). The PTWI has been confirmed (UNEP, 1980) but it was pointed out that more attention should be paid to the prenatal stage as there are some indications of a higher fetal sensitivty to MeHg.

As fish is the major source of mercury (Clarkson, 1989), several countries have established limits for mercury in their seafood products. The limit of 0.56 mg/kg has been adopted by the majority of countries (Nauen, 1983; USFDA, 1982). The mean mercury concentrations in fish presented in Table 27 is about 325 μg/kg as total mercury and this is estimated to contribute about 143% of the ADI for this particular population group of fishermen and their families.

DISCUSSION

Levels of chlorinated hydrocarbon pesticides and PCBs, detected in this study are not likely to cause any toxicological problem. The daily intake of PCBs contributes to only a small percentage (0.24 to 2.8%) of the FDA's recommended consumption guideline for adults (1 µg/kg/day; Swain, 1988). However, most of chlorinated hydrocarbon pesticides and PCBs showed limited evidence of carcinogenicity in humans but sufficient evidence of carcinogenicity for experimental animals and as such it is prudent to regard these organic contaminants as presenting a carcinogenic risk to humans (IARC, 1987). They may increase cancer risk not only for high fish consumers, but also for low fish consumers. Furthermore, it is assumed that cancer initiating agents have no threshold dose and their effect is irreversible and additive (Barnes and Dourson, 1988; UNEP/FAO/WHO/IAEA, 1990).

Cadmium intake, could present an important toxicological problem in high fish consumers. Even though the daily cadmium consumption is only a couple of hundred micrograms, over a period of many years of exposure, the level may accumulate in the critical organ in humans (the kidney) to toxic levels (Friberg, 1988). Cadmium has been shown to be carcinogenic in animals, and although there is no strong evidence for the carcinogenicity of cadmium to humans following oral exposure, a high incidence of cancers of the prostate and lung have been noted in cadmium smelters (USEPA, 1989). In addition, smoking constitutes an important source of human exposure (Stoeppler, 1984). In the population group surveyed in this study 70% of the adults were smokers which may elevate the PTWI above the acceptable level.

In this particular group of fishermen and their families, in a coastal fishing village, the amounts of fish and other seafood consumed could result in an intake exceeding acceptable levels. An assessment of the state of pollution of the Mediterranean Sea by mercury (UNEP/FAO/WHO, 1983) reached the conclusion that the general population did not appear at risk but there was evidence that specific population groups, particularly fishermen and their families in coastal fishing areas, could have an intake of mercury exceeding acceptable levels. A pilot study, which started in 1984, of selected coastal areas in three Mediterranean countries, did not confirm the previous assumption considering that the bulk of the total mercury was in the form of methylmercury. In contrast, there was considerable variation in the proportion of the methylmercury content ot the total mercury among the species analyzed (UNEP, 1989). In this specific case, the methylmercury in percent of the total mercury in the species most consumed (*T. trachurus, B. boops, and S. pilchardus*) was respectively 48%, 33%, and 28% (UNEP, 1989) which reduced the toxic risk for this group of population. However, special

attention should be given to pregnant women who are at greater toxic risk of mercury, because of their lesser body weight and the greatest sensitivity of the fœtus to mercury.

Assuming there is no dietary intake of lead from other sources than fish, the PTWI of 50 µg/kg body weight for adult populations is not exceeded for the inhabitants of this coastal village who consume large amounts of fish. For children, if we consider the same daily fish consumption established for adults of 190 g/day, the consequent daily intake of 49 µg/person will give a weekly intake of 343 µg/person. Taking an infant weight of 30 kg, the calculated weekly intake is about 11.4 µg/kg body weight which is still below 50% of the PTWI of 25 µg/kg body weight established for children.

In assessing risk to human health one must consider that, the levels of chlorinated hydrocarbons and heavy metals in seafood are only one part of the total exposure to these contaminants. If the intake from other sources (atmospheric, occupational exposure, drinking water, other food items) is near the hazardous level, contaminant amounts in seafood products may elevate the daily intake above the acceptable level.

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CHAPTER X

THE USE OF MIXED FUNCTION OXIDASE SYSTEMS AS INDICATOR OF ENVIRONMENTAL CONTAMINATION IN FISH

ABSTRACT

Analysis of residue levels of polychlorinated biphenyls, DDTs (p,p'DDT, p,p'DDD and p,p'DDE) and some trace metals such as cadmium, lead and mercury in five fish species were carried out along with specific P4501A1 determinations to investigate the feasibility of using the hepatic cytochrome P450 induction as an indicator of contamination of fish. Chlorinated hydrocarbons were determined by capillary gas chromatography. Trace metals analysis was performed by atomic absorption spectrophotometry (AAS) using cold vapor AAS for mercury and graphite furnace AAS for lead and cadmium. Hepatic microsomal cytochrome P450 determinations were analyzed by immunoblotting (western blotting) technics.

There was moderate contamination of fish samples by all pollutants analyzed, but there is no direct correlation between pollutants levels and cytochrome P4501A1 induction.

INTRODUCTION

Most industrial, agricultural, and urban effluents, especially the ones discharged into marine environments, contain high concentration of xenobiotics (Kessabi et al., 1988) as chlorinated hydrocarbons and polychlorinated biphenyls (PCBs).

Organic contaminants are metabolized by mixed function oxidase (MFO) systems which detoxify organic compounds (Buhler and Rasmusson, 1968). The MFOs are a multienzyme system which convert a foreign compounds to less toxic substances and in some cases to highly reactive intermediates. The MFO fish systems appears to be induced by compound such as polyaromatic hydrocarbons and PCBs (Buhler and Williams, 1989; George and Young, 1986). Activities of these enzymes have been suggested as a bioindicator system to monitor the organic environmental contamination (Lee et al., 1980; Payne,1984) and has recently been evaluated in depth by Payne and coworkers (1987) and Rattner et al. (1989).

The aim of the present work is to analyze the residue levels of some organic environmental contaminants such as organochlorine pesticides and PCBs in some fish species and to investigate the feasibility of using the hepatic cytochrome P450 induction as an indicator of this contamination.

MATERIAL AND METHODS

1. Sample collection

Five fish species were selected for this study: *Merluccius merluccius, Trachurus trachurus, Pagellus acarne, Boops boops* and *Sardina pilchardus*. Fish were collected from the port immediately after the return of the fishing boats. After being identified, the weight and the length were recorded, then fish were dissected and aliquot of muscle taken along with the whole liver for analysis. Muscle and liver were stored in freezer at -20°C until analysis.

2. Hepatic microsomal cytochrome P450 determinations

The frozen livers were weighed, placed in ice-cold buffer A [10 mM potassium phosphate, pH 7.7, 20% glycerol, 1 mM EDTA, 0.2% chlorate, 0.1% Lubrol PX and 0.1M phenylmethylsulfonyl floride (PMSF) and homogenized in 4 vol. of buffer A. The microsomal pellet was obtained by centrifugation at 10,000 g for 30 min followed by 105,000 g for 90 min. All procedures involved in the solubilization and the purification were performed on ice or in 4°C room using buffers containing butylated hydroxytoluene and PMSF. The microsomes were resuspended to a protein concentration of about 20 mg/ml in buffer B (0.1M potassium phosphate pH 7.25, 20% glycerol and 1mM EDTA) (Williams and Buhler, 1982). Microsomal suspensions were frozen at -70°C until assayed.

Microsomal protein concentrations were determined according to Lowry et al., (1951) with bovine serum as standard.

Immunoblotting (Western blotting) was carried out by the method of Burnette et al. (1980). Microsomes and purified cytochrome P450 preparations were separated on 9% polyacrylamide gels containing 10% sodium dodecyl sulfate (Laemmli, 1970) and transferred to nitrocellulose sheets electrophoretically at 4°C. After transfer the nitrocellulose sheets were incubated in PBS-Tween containing 5% milk powder and incubated with rabbit IgG raised against purified rainbow trout cytochrome P4501A1 (Williams and Buhler, 1982) for 1h at room temperature, as primary antibody. Horseradish peroxidase goat anti-rabbit IgG (Amersham) was used as secondary antibody and visualized by the Enhanced Chemiluminescence reagents (ECL) (Amersham). P450 was estimated by direct comparison between the samples and purified trout P450 as a standard on the basis of the response intensity (0 = no response, 4 = maximum response).

3. Contaminant determinations

The sampling of marine organisms were conducted according to the UNEP/FAO/IAEA recommendations (1984a, b). Chlorinated hydrocarbons were analyzed according to the method described by Villeneuve (1986) and El Nabawi et al. (1987). PCBs were analyzed by gas chromatograghy with an electron capture detector. Identification and quantification were done using 1254, and 1260 standard mixture (Ballschmitter and Zell, 1980; Capel et al. 1985). Trace metals determinations were performed by atomic absorption spectrophotometry (AAS) using cold vapor AAS for mercury and graphite furnace AAS for the remaining elements (Lazzlo, 1972; UNEP/FAO/ IAEA/IOC, 1984 c, d).

RESULTS AND DISCUSSION

To determine if relationships might exist between contaminants in the fish and hepatic MFOs, P450 and contaminants determinations were carried out from all individual fish. Contaminants we have dealt with are mainly PCBs and DDTs, p,p'DDT, p,p'DDD, p,p'DDE, and some trace metals such as cadmium, lead, and mercury.

The contaminant concentrations in muscle fish tissue of the five fish species examined are presented in Table 29. The DDTs were present in relatively high quantities in all fish species. Their concentrations varied between 0.22 and 26.19 μg/kg f.w. for all the species. These concentrations are considered as a moderate contamination in comparison with other Mediterranean locations were the average concentrations are about 25 μg/kg f.w. (UNEP/FAO/WHO/IAEA, 1990). The total PCB concentrations are not very high as compared

Table 29. Chlorinated hydrocarbons, PCBs and three trace metals in tissue of some fish species and their cytochrome P450 content*.

Fish species	Liver weight/ Body weight	Total PCB (µg/kg)	DDTs (µg/kg)	Cd (mg/kg)	Pb (mg/kg)	Total Hg (mg/kg)	P450 ¥
Trachurus trachurus	0.025	29.04	NA	0.09	0.324	0.34	4
	0.009	11.13	NA	0.162	0.264	NA	1
	0.008	15.49	26.19	0.08	0.273	0.083	1
	0.016	18.28	NA	0.116	0.359	0.185	3
	0.01	8.55	23.73	0.152	0.193	0.126	1
	0.018	6.28	18.81	0.057	0.321	NA	1
	0.014	1.78	1.59	0.185	0.342	0.084	1
Pagellus pagellus	0.016	3.6	0.22	0.135	0.256	0.345	2
	0.008	21.78	1.12	NA	0.342	0.221	3
	0.007	2.21	2.27	0.123	0.377	0.12	1
	0.009	2.18	19.42	0.252	0.24	NA	1
	0.008	5.09	20.18	0.067	0.183	0.117	0
	0.015	20.82	12.27	0.172	0.153	0.308	1
Boops boops	0.008	7.79	1.97	NA	0.234	0.308	2
	0.014	14.74	NA	0.122	0.222	NA	3
	0.005	6.33	2.31	0.06	0.212	0.149	2
	0.006	3.64	3.83	0.145	0.357	0.079	NA
	0.007	3.86	1.26	0.138	0.254	0.206	1
	0.148	6.14	1.99	0.157	0.197	0.125	0
	0.006	3.29	1.88	0.134	0.238	0.264	0
	0.008	9.34	0.99	0.08	0.312	0.18	1
Merlucclus merlucclus	0.025	4.35	3.22	0.151	0.154	NA	1
	0.003	20.63	NA	0.144	0.306	0.158	3
	0.016	2.59	4.16	0.171	0.236	0.352	4
	0.03	1.62	6.52	0.28	0.21	0.125	4
Sardina pilehardus	0.01	19.34	NA	0.138	0.216	0.137	2
	0.008	16.39	NA	0.06	0.195	NA	1
	0.014	11.34	2.59	0.26	0.301	0.232	2
	0.011	6.46	NA	0.132	0.212	0.196	2
	0.01	7.39	2.71	NA	0.379	0.043	1

NA: not analyzed
*: Concentration presented on f.w. basis.
¥: numbers represent the intensity of the response as compaired to the standard (Pure P450 1A1)

to the other Mediterranean locations where the average concentration levels are in the range of 100 μg/kg f.w. (UNEP/FAO/WHO/IAEA, 1990). Liver sensitivity to certain PCB congeners involving an increase of cytochrome P450 and consequently of MFO activity in several fish species has frequently been reported during last decades (Hill et al., 1976; Addison et al., 1978; Melancon et al., 1981). Congeners that demonstrate 3-methylcholanthrene-type (3MC-type) MFO induction and mixed type (3MC and phenobarbital-type) MFO induction have the greatest toxic potential. The congeners numbers 77, 126 and 169 were shown to be the most potent pure-3MC-type inducers (McFarland et al., 1989). These congeners were not detected in all the samples analyzed. Among the mixed-type inducers congeners number 118, 128, 138, 156 and 170 were present in most samples in relatively high concentration as compared to the other congeners but they never exceeded 29 μg/kg f.w. (Table 30).

Analysis of hepatic ethoxyresorufin O-deethylase (EROD) and pentoxyresorufin O-deethylase (PROD) activities in liver microsomes of fish were negative in all fish samples probably because the denaturation of the enzyme with corresponding loss in activity due to the storage conditions during transportation to the laboratory.

The P4501A1 measured in this study indicates poor response in comparison with standard as β-naphthoflavone (BNF) inducible trout microsomes and P4501A1 purified trout microsomes. One important finding is the variable electrophoretic configuration of the proteins of cytochrome P450 area of different fish species with similar pattern within the same species (Figure 22).

There is a poor correlation between pollutant levels and those of P4501A1 (correlation coefficient $R < 0.16$). This could be the result of the low contaminant concentrations detected in fish that may be below the threshold levels required for induction. The pesticide DDT or its metabolites do not appear to affect MFO in fish (Buhler and Rasmusson, 1968), but some PCB congeners are high inducers of MFO (McFarland et al., 1989). However, It has been reported that concentrations of PCB (or other inducers) found in water, food or sediment may often be so low that they can be sequestered into cellular lipids and not be available in sufficient threshold concentrations for binding to the specific cytosol receptors to initiate the induction process (Tukey et al., 1982). Another explanation could be the poor cross-reactivity of the antitrout P4501A1 antibodies against P4501A1 from other fish species. However, Goksoyr et al. (1991) have recently shown that the antibodies against trout P4501A1 readily cross-react with similar P450s from many marine species. Pollutants mixture such as oil and PCBs may also influence the P450 induction response to pollutants. Both polyaromatic hydrocarbons (PAHs) and PCBs induce P4501A1. Fish metabolize PAHs very rapidly and one of the only ways of showing environmental exposure is to look for its "fingerprint" i.e. P450 induction. By contrast

Table 30. Concentration of PCB congeners in fish muscle tissue of different fish species.

PCBs	Mean concentration (µg/kg f.w.) (range)				
	Trachurus 4	Sardina 7	Merluccius 3	Boops 6	Pagellus 7
118/106/ 149	1.45 (0.52-2.36)	0.06 (0.043-0.122)	0.85	0.42 (0.09-0.74)	0.48 (0.14-0.82)
128	0.17 (0.03-0.32)	0.04 (0.025-0.06)	0.154	0.06 (0.01-0.13)	0.03
138	1.66 (0.45-4.74)	1.12 (0.12-3.62)	0.16 (0.13-0.2)	0.55 (0.03-1.63)	0.8 (0.01-1.56)
170	0.96 (0.17-1.29)	1.42 (0.033-3.39)	1.97 (0.01-5.81)	0.34 (ND-0.81)	0.4 (0.05-1.09)
87	0.77 (0.0044-1.8)	0.22 (0.014-0.56)	0.27	0.06 (ND-0.23)	0.29 (ND-1.16)
99	0.57 (0.273-0.873)	0.31 (0.19-0.52)	ND	0.44 (0.21-0.66)	0.11 (0.06-0.15)
101	1.16 (0.43-1.83)	0.42 (0.144-0.75)	1.54 (0.06-4.37)	0.31 (0.14-0.44)	0.19 (0.003-0.38)
146/153	1.19 0.61-2.19)	1.28 (0.09-3.18)	0.61 (0.125-0.94)	0.7 (0.11-1.53)	0.59 (0.1-1.59)
180	0.79 0.112-1.97)	0.39 (0.007-1.3)	1.25 (0.04-3.44)	0.45 (0-1.04)	0.31 (0.012-0.69)
194	0.145 (0.1-0.19)	0.045 02-0.07)	0.011	0.03	0.007
18	ND	2.23 (2.23-14.28)	0.26	2.84 (2.84-7.03)	0.7 (0.27-1.57)
44	0.26 (0.02-0.7)	0.81 (0.51-1.36)	0.26	0.129	0.1 (0.05-0.14)
47/48/49	1.49 (0.62-2.37)	1.96 (0.18-5.46)	0.02	ND	0.56 (0.07-1.05)
52	0.81 (0.098-1.417)	0.52 (0.32-0.76)	0.36 (0.15-0.56)	0.24 (0.24-0.25)	0.27 (0.08-0.42)
70	0.74	2.88 (0.65-5.12)	1.08	0.41	0.90 (0.47-1.31)
74	2.59 (2.48-2.7)	1.50 (1.37-1.57)	0.51	0.15	0.83 (0.40-1.25)
151	0.18 (0.1-0.27)	0.13 (0.07-0.21)	0.19	0.07 (0.05-0.09)	ND
177	0.38 0.19-0.57)	0.07 (ND-0.13)	0.06 (0.054-0.06)	0.06 (ND-0.13)	0.24 (0.03-0.45)
159/187	1.37 (0.12-3.55)	0.53 (0.05-1.50)	0.36 (0.063-0.066)	0.94 (0.26-1.63)	0.20 (0.07-0.32)
201	0.603	0.18 (0.11-0.25)	0.039	0.14	0.03
TOTAL PCBs	21.92 (5.55-33.11)	11.1 (0.46-19.34)	8.87 (1.63-20.63)	5.8 (3.64-55.29)	6.26 (6.79-9.11)

ND= Not detected

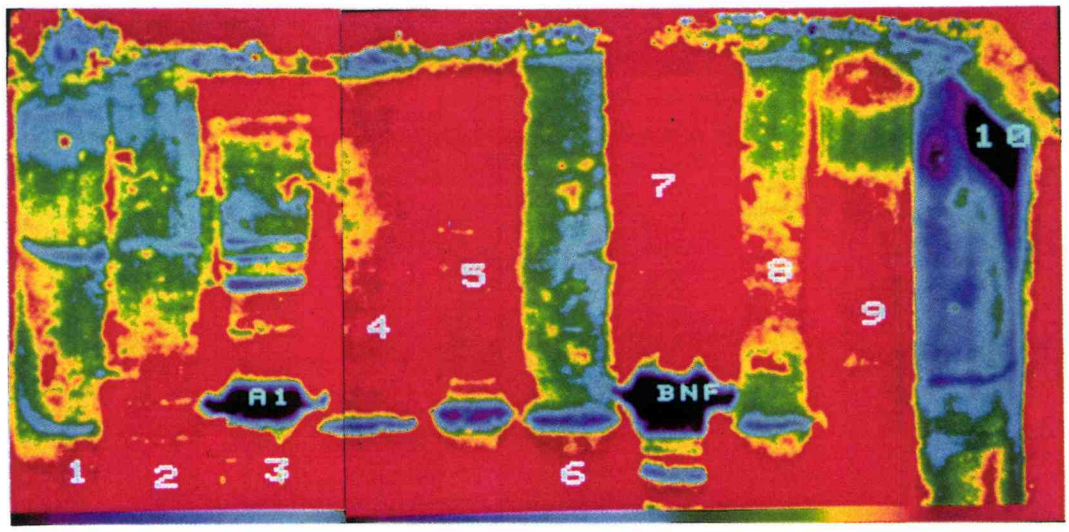

Figure 22. Western blot of fish liver microsomes, purified cytochrome P4501A1 and induced β-naphthoflavone (BNF) trout microsomes probed with anti-1A1 antibody and detected by Enhanced Chemiluminescence (ECL) technique.

1. *Boops boops*: 27.6 µg
2. *Sardina pilchardus*: 40 µg
3. Purified 1A1 trout microsomes
4. *Pagellus acarne*: 40 µg
5. *Boops boops*: 26.4 µg
6. *Pagellus acarne*: 40 µg
7. BNF-treated trout microsomes: 0.25 µg
8. *Pagellus acarne*: 40 µg
9. *Sardina pilchardus*: 40 µg
10. *Merluccius merluccius*: 40 µg.

PCBs are not readily metabolized and remain as residues in fish. Thus, induction of P450 varies according to location or species. The absence of correlation could also be attributed to pollutants inhibition or interference with P450 (MFO) systems. It has been speculated that high concentrations of strong inducing agents such as sulphite, which is used in some pulp bleaching processes, may inhibit MFO enzyme levels through cytochrome reduction and inactivation (Payne et al., 1987). The effects of trace metals on fish metabolizing enzymes remain relatively unknown. Yoshiba and coworkers (1976) observed a decreased synthesis in cytochrome P450 in mice exposed to cadmium. Alvares et al. (1972) reported a 40% to 50% decrease in cytochrome P450 activity in hepatic microsomes of rats after an iv injection of 5 mg/kg $PbCl_2$ and an ip injection of 5 mg/kg CH_3HgCl. Similarly, cadmium has been shown to inhibit ethoxyresorufin O-deethylase (EROD) and glutathione S-transferase (GST) activities in fish (George and Young, 1986).

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CHAPTER XI

DISCUSSION AND CONCLUSIONS

The objectives of this study were to assess the degree of pollutant contamination of edible marine fish and shellfish and to determine any related health risk. Another goal was to examine the utility of using mixed-function oxidase activities as an indicator for environmental contamination of fish. Specific studies include:

1) Examining the relative degrees of heavy metals and chlorinated hydrocarbons contamination of Moroccan fish products collected from several different locations and at several different times from the Mediterranean Sea and Atlantic Ocean;

2) Conducting risk assessment analysis of fishermen and their families as a segment of the population the most exposed to any health effects originating from the consumption of contaminated seafood products;

3) Assessment of the extent of heavy metals contamination of mussels along the western coast of Morocco; and

4) Studying the feasibility of using hepatic mixed function oxidase as bioindicator of exposure of fish to pollutants.

1. Chlorinated Hydrocarbons

1.1. Organochlorine Pesticides

To assess chlorinated hydrocarbons contamination of seafood products a study was conducted on the most consumed and represented species in both the Atlantic and Mediterranean Sea. Sampling and samples preparation were performed according to the UNEP/FAO/WHO/IAEA reference methods.

Among chlorinated hydrocarbon pesticides analyzed p,p'DDT and its derivatives (p,p'DDE and p,p'DDD) and lindane were the most predominant contaminants both in Mediterranean and Atlantic fish samples. The levels of DDTs (p,p'DDT+ p,p'DDD+ p,p'DDE) varied between 5.61 and 17.96 μg/kg f.w. for all the species collected from the Atlantic Ocean, and between 3.02 and 13.31 μg/kg f.w. for the species collected from Mediterranean Sea. These levels are slightly below the mean concentration for Mediterranean species which is 25 μg/kg f.w. (UNEP/FAO/WHO/IAEA, 1990) and are comparable to the contamination of fish species collected from the Gulf of Mexico where these concentrations are reported to range between 10 and 19 μg/kg (Kennicutt et al., 1988).

Residues of lindane were detected in all fish samples, with levels ranging between 8.40 and 39.2 μg/kg f.w. for species collected from Mediterranean Sea and between 7.80 and 26.98 μg/kg f.w. from Atlantic Ocean. The presence of this compound in high concentrations in fish may reflect its continuing uses even though it has been recently banned for agricultural purposes (MARA, 1985).

The other compounds: HCB, heptachlor epoxide, aldrin, dieldrin and endrin showed moderate contamination and the concentrations were below 2 μg/kg f.w. for the most contaminated species. Residues of hexachlorobenzene (HCB) ranged between 0.10 and 0.26 μg/kg f.w. in Atlantic samples and between 0.04 and 0.99 μg/kg f.w. in Mediterranean samples. These levels are in the range of the other Mediterranean species where mean concentrations range between 0.10 and 1.00 μg/kg f.w. (UNEP/FAO/WHO/IAEA, 1990). The drins (aldrin, dieldrin and endrin) are present in relatively lower concentrations and frequencies in fish samples from both sampling sites. Mean concentrations for all species are 0.15, 0.25 and 0.52 μg/kg f.w. for aldrin, dieldrin, and endrin for Mediterranean samples and 2.46, 0.10 and 0.48 μg/kg f.w. for Atlantic samples respectively.

1.1.1. Marine Species Variations

The highest contamination levels in this investigation were detected in mussels. The mean concentration of p,p'DDT and lindane were 6.38 μg/kg f.w. and 6.84 μg/kg f.w. This confirmed the high capacity of this species to accumulate organic pollutants (UNEP, 1989; UNEP/FAO/WHO/IAEA, 1990). Among fish species analyzed, red mullet (*Mullus barbatus)* showed the highest levels of contamination for most of the compounds which may be explained by the living habits of this species. Red mullet lives near estuaries and ports that are usually subjected to heavy contamination by various pollutants. This species, with *Mytilus galloprovincialis* was considered as pollution indicator in the Mediterranean Monitoring Programs (MED. POL), and was shown to reflect local contamination conditions of the sampling site as well as a good tool to compare contamination between different regions (UNEP, 1989). These two species were also taken for comparison between the results of this study and those of other Mediterranean areas. This comparison revealed that levels of chlorinated hydrocarbons are 2-to 4-fold lower in the present study as compared to the mean levels of 1985 for the species *Mullus barbatus* (UNEP, 1989). A similar but less pronounced decrease was also noted for residual levels of these contaminants in *Mytilus galloprovincialis*. The possible explanation of this decrease in residue levels from 1985 to 1989 could be related to probable degradation associated with less use of these pesticides as result of their ban in most Mediterranean countries. However, the contamination levels in both species for lindane and dieldrin did not

show any substantial decrease but increased during this period. This finding suggests the continuing use of these compounds in many forms to control animal ectoparisites and vector borne disease in public health programs.

1.1.2. Influence of Body Weight and Fat Content on DDT and Lindane Residue Levels in Fish

A correlation between lindane and DDT concentrations with lipids content and body weight was carried out in both bogue (*Boops boops*) and axielary seabream (*Trachurus trachurus)* species. The results revealed an increase of chlorinated hydrocarbons with increase in body weight and lipid contents in all fish samples analyzed. The linear accumulation of DDT and other chlorinated hydrocarbons with body size was noted previously in several marine and fresh water fish species and was attributed to lipid concentration with larger fish (Satjmadjis and Gabrielides, 1979; El Nabawi et al., 1987). However, Fossato and Craboledda (1981) found that the sequence of species (*Nephrops, Carcinus, Mytilus, Mullus, Engraulis, Thunnus*) according to tissue level of chlorinated hydrocarbons only partly reflects their lipid content. In fact, levels in anchovies were comparable to those found in mullet, although the lipid contents of the two species were very different. It was concluded that food, habitat and the physiology of the various organisms strongly influence their accumulation capability.

1.1.3. Seasonal and Sampling Site Variations

There were no seasonal variations for the compounds analyzed except for p,p'DDT which showed an increase during spring season. Also no significant sampling site variations were observed in this study.

The Mediterranean Sea is an almost completely land-locked body of water which is highly contaminated by the dumping from numerous pollution sources and generally contamination levels for persistent chemicals detected there are higher than those in other open seas. For most of organochlorine contaminants analyzed, the concentration levels in the same fish species collected from Mediterranean Sea and Atlantic Ocean sampling sites are not significantly different at level 5 percent. This similarity in contamination may be explained by the proximity of Moroccan Mediterranean coastal waters from the Straights of Gibraltar. It is a point of an extensive water exchange between the Mediterranean Sea and the Atlantic Ocean. Another explanation is the intense agricultural, industrial, and urban activities along the Atlantic coast which certainly contribute to equalizing the concentrations of contaminants between the two coastlines of Morocco.

1.2. Polychlorinated biphenyls

1.2.1. Residues Levels in Marine Organisms

Polychlorinated biphenyls were present in all samples. Mean concentrations of total PCBs ranged from 5.8 μg/kg f.w. to 33.51 μg/kg f.w. These levels are not high in comparison with other Mediterranean regions where the levels in fish are in the range of 100 μg/kg f.w. Total PCB concentrations detected in the samples from Tetouan varied between 5.8 and 21.92 μg/kg f.w. with an average of 10.8 μg/kg f.w. The mean concentration for the species collected from Mehdia range from 8.75 to 33.51 μg/kg f.w. with an average of 17.92 μg/kg f.w.

PCB contamination of seafood products of the two sampling sites showed a lower level of contamination as compared to other polluted locations of the world. For example, the PCB levels varied between 11 and 203 μg/kg f.w. in fish from the Gulf of Mexico (Kennicutt et al., 1988). A very high accumulation of PCBs (0.2-0.5 mg/kg f.w.) was reported in a polluted area of Puget Sound in Washington, U.S.A. (Malins et al. 1984). A Swedish study showed a mean PCB value between 0.3-0.7 mg/kg f.w. in herring from the Baltic (Andersson et al., 1984).

1.2.2. PCB Congeners Pattern in Fish Species

The highly toxic PCB congeners were not detected in any of our samples. However PCB congeners that are mixed type inducers were present in relatively high proportions in most of the samples. The prevalent congeners are present in concentration between 1 and 2 μg/kg f.w. in all fish species, and have 4 to 7 chlorine atoms in their molecules, i.e., numbers 118/108/149,138, 170, 101, 153/146,180, 18, 47/48/49,70, 74, and 159/187. These moderately chlorinated groups (penta, hexa, and heptachlorobiphenyls) contain 112 of the 209 possible PCB configurations. They were synthesized in high proportions in many PCB formulations and are the most bioaccumulating PCB congeners which make them likely to be prevalent in environmental samples (Alford-Stevens, 1986; Hutzinger et al., 1974). The more highly chlorinated (7-8 chlorines) congeners such as 194, 177, 159/187 and 201 were present at lower concentrations less than 1 μg/kg f.w. It has been shown that the most highly chlorinated congeners are generally less available to organisms because they are usually present at lower quantities in the environment and are more tightly bound to soil and sediments (McFarland and Clarke, 1989). Congeners with less chlorination i.e., 87, 99, 44, 52 were present in lower concentrations less than 0.5 μg/kg f.w., with exception of congener number 18 (an XYZ chlorobiphenyl), because they are readily metabolized and eliminated and so do not tend to bioaccumulate. None of samples in this study matched with commercial mixture Aroclor 1242 but showed similar pattern either with Aroclor 1254 or 1260. The commercial mixture 1242

contains congeners of low chlorine substitution that are generally more readily metabolized by the organisms and consequently would not be present in the tissue analyzed.

2. Trace Metals

2.1. Residue Levels in Seafood Products

A large number of trace metals are potentially harmful to the marine environment, and through seafood, to man. Priority was given to cadmium, chromium, lead and mercury, since many surveys had shown that these elements occur in high concentrations in marine biota and hence these elements were considered especially harmful (UNEP/FAO/WHO, 1987; 1989; and UNEP, 1989).

To assess the trace metal contamination in Moroccan seafood products various species of marine organisms collected from different geographical areas of Mediterranean coast (Tanger, Tetouan, and Nador) were analyzed. Sampling and analysis were performed according to the UNEP/FAO/IAEA/IOC, reference methods for marine pollution studies.

2.1.1. Cadmium

Cadmium concentrations were found to range between 0.005 and 0.150 mg/kg f.w. with a mean concentration of all the samples analyzed of 0.044 mg/kg f.w. These concentrations are similar to those found in the same species in other Mediterranean locations; for example, the mean concentration for red mullet (*Mullus barbatus*) was 0.046 mg/kg f.w. for Mediterranean regions (UNEP/FAO/WHO, 1989). On the other hand, these concentrations are slightly higher than those found in fish species collected from Holland coasts and the North Sea coasts. In these two locations, cadmium concentrations were found to range between 0.002 and 0.006 mg/kg f.w. (Vos and Hovens, 1986) and 0.005 mg/kg f.w. in soles (Vyncke et al., 1984).

Cadmium concentrations in mussels (*Mytilus edulis*) collected from several locations along the Moroccan Atlantic coast varied between 0.222 and 0.375 mg/kg f.w. These concentrations are in the range of those found in mussels collected from the North Atlantic Ocean, where the reported values are between 0.09 and 0.33 mg/kg f.w. (ICES, 1980). High levels have been reported in the Oslo Commission area where the concentrations ranged between 0.043 and 12.6 mg/kg f.w. with a mean value of 1.040 mg/kg f.w. (UNEP, 1989). However the concentrations in mussel are difficult to compare in samples taken from different locations and seasons. This is due to the fact that mussels are sensitive to local pollution influences. Several countries have established legal limits in their fish and fishery product. In

the majority of cases limits range between 0.1 and 0.5 mg/kg f.w. (Nauen, 1983). The levels detected in this study are all within the established limits.

2.1.2. Chromium

Chromium concentrations in fish samples collected from the Mediterranean coastal waters varied between 0.023 and 0.568 mg/kg f.w. with a mean value for the most consumed species of 0.186 ± 0.118 mg/kg f.w. Very limited data on chromium concentration levels in marine organisms are available in the literature. Fowler and Oregioni (1979) reported a range value of 0.5-28.8 µg/kg dry weight in *mytilus* from the North-West Mediterranean (Ligurian Sea). Burns et al. (1982) reported a range of 0.3-3.1 mg/kg dry weight in shellfish tissue and a range of 3.2-3.4 mg/kg dry weight in fish muscle from a coastal area of Oman which is considered as non-polluted by heavy metals.

2.1.3. Lead

Concentrations of lead detected in this study are between 0.013 mg/kg f.w. for ray which is the less contaminated species and 0.770 mg/kg f.w. for longnose spurdof (*Squalus blainvillei*) with a mean value for the most consumed species of 0.195 ± 0.103 mg/kg f.w. In all cases, these concentrations were found to be slightly higher than those published for fishes collected in the North Sea and in the Irish Sea. Vos and Hovens (1986) reported concentrations of 0.04 mg/kg f.w. for ray, plaice and cod and 0.006 mg/kg f.w. for herring. Vynke et al. (1984) reported concentrations of 0.03 and 0.14 mg/kg f.w. in rays collected from North Sea and Irish Sea.

The mean concentration in *Mullus barbatus* from the Mediterranean regions is about 0.070 mg/kg f.w. (UNEP, 1989). Several other marine organisms have been analyzed from both the Mediterranean Sea and Atlantic Ocean. According to fish species and sampling locations the average levels varied from 0.040 for plaice to 1.17 mg/kg for *Thunnus*, which is the most contaminated species.

Concentrations of lead in *Mytilus edulis* collected form several locations along the Moroccan Atlantic coast vary between 0.473 and 0.682 mg/kg f.w. These concentrations were found to be slightly lower than those published for *Mytilus galloprovincialis* collected from various Mediterranean areas where the levels were reported to vary between 0.6 and 1.8 mg/kg f.w. with an average of 0.8 mg/kg f.w. (UNEP, 1989).

2.1.4. Mercury

Mercury was detected in all fish samples analyzed. The concentrations were found to range between 0.190 and 0.785 mg/kg f.w. for the most consumed fish species with an average of 0.504 mg/kg f.w. These concentrations are rather high. However, they are still under the acceptable concentrations for human consumption since several countries have established limits for mercury in their seafood products (Nauen, 1983; USFDA, 1982) and in the majority of cases limits have been set between 0.5-1 mg/kg f.w. as total mercury.

Mercury was detectable at high concentrations in mussel samples from all sampling sites with concentrations ranging between 0.528 and 0.713 mg/kg f.w. For the Mediterranean Sea "Mussel Watch" program, levels of 0.004 to 7 mg/kg f.w. with an average of 0.232 mg/kg f.w. have been reported (UNEP/FAO/WHO, 1983).

These high levels confirm that mercury contamination in Moroccan coastal waters must be considered by authorities since rather high concentrations are detectable in some fish species. About 2% of the sample analyzed exceeded the upper limit of 1 mg/kg. Therefore, the high mercury levels in certain seafood species present a legal and possibly a sanitary problem in addition to any effects these levels may have on marine organisms and ecosystems.

3. Risk Assessment for High Fish Consumers

Human health risks for chlorinated hydrocarbons and trace metals in fish are evaluated in terms of comparison with acceptable levels set by FAO/WHO regulatory policy as there are no regulatory limits for chlorinated hydrocarbon in fish products in Morocco. Health risk is calculated for a population located in a coastal village area (M'diq) in the Mediterranean coast where people consume a large amount of fish daily. A survey of daily intake of seafood products was carried out for this population of fishermen and their families.

3.1. Chlorinated Hydrocarbons

Levels of chlorinated hydrocarbon pesticides and PCBs, detected in this study are not likely to cause any immediate toxicological problem. The daily intake of PCBs constitutes only a small percentage 2.8 % of the FDA's recommended consumption guideline for adults of 1μg/kg/day (Swain, 1988). However, most chlorinated hydrocarbon pesticides and PCBs showed limited evidence of carcinogenicity in humans but sufficient evidence of carcinogenicity for experimental animals and as such it is prudent to regard these organic contaminants as presenting a cacinogenic risk to humans (IARC, 1987). They may increase cancer risk not only for high fish consumers, but also for low fish consumers. Furthermore, it is assumed that

cancer initiating agents have no threshold dose and their effect is irreversible and additive (Barnes and Dourson, 1988; UNEP/FAO/WHO, 1990).

3.2. Trace Metals

Cadmium, could present an important toxicological problem in high fish consumers. Even though the daily consumption is only a couple of hundred micrograms, over a period of many years of exposure, the level may accumulate in the critical organ in humans (the kidney) to toxic levels (Friberg, 1988). Cadmium has been shown to be carcinogenic in animals, and although, there is no strong evidence for the cacinogenicity of cadmium to humans following oral exposure, a high incidence of cancers of the prostate and lung has been noted in cadmium smelters (USEPA, 1988c). In addition, smoking constitutes an important source of human exposure (Stoeppler, 1984). In the population group surveyed 70% of the adults were smokers which may elevate the PTWI above the acceptable level.

In this particular population group of fishermen and their families, the amounts of fish and other seafood consumed, based on its mercury content could result in an intake exceeding acceptable levels. An assessment of the state of pollution of the Mediterranean Sea by mercury (UNEP/FAO/WHO, 1983) reached the conclusion that the general population did not appear at risk but there was evidence that specific population groups, particularly fishermen and their families in coastal fishing areas, could have an intake of mercury exceeding acceptable levels. A pilot study, which started in 1984, of selected coastal areas in three Mediterranean countries, did not confirm the previous assumption considering that the bulk of the total mercury was in form of methylmercury. In contrast, there was considerable variation in the proportion of the methylmercury content to the total mercury among the species analyzed (UNEP, 1989). In this specific case the methylmercury in percent of the total mercury in the species most consumed: *Trachurus trachurus*, *Boops boops*, and *Sardina pilchardus* was 48 %, 33 % and 28 % respectively (UNEP, 1989). These rather low percentages of methylmercury reduced the toxic risk for this group. However, special attention should be given to pregnant women who are at greater toxic risk of mercury, because of their lesser body weight and the greater sensitivity of the fœtus to mercury.

Assuming there is no dietary intake of lead from other sources than fish, the PTWI of 50 μg/kg body weight for adults population (3500 μg/person) is not exceeded for this group of population. For children, if we consider the same daily fish consumption established for adults of 190 g/day, the consequent daily intake of 49 μg/person will give a weekly intake of 334 μg/infant. Taking an infant weight of 30 kg, the calculated weekly intake is about 11.4 μg/kg body weight which is still below 50 % of the 25 μg/kg body weight established for children.

In assessing risk to human health one must consider that the levels of chlorinated hydrocarbons and heavy metals in seafood are only one part of the total exposure to these contaminants. If the intake from other sources (atmospheric, occupational exposure, drinking water, other food items) is near the hazardous level, contaminant amounts in seafood products may elevate the daily intake above the acceptable level.

The future viability of the ecosystem depends not only on the present situation but also on the trends of contamination. Assessment of human health risks in this situation cannot be achieved without continuous monitoring.

4. The Use of Mixed-Function Oxidase as Bioindicator of Fish Exposure to Pollutants

To determine if relationships might exist between contaminants in the fish and hepatic MFOs, cytochrome P450 and contaminant determinations were carried out from all individual fish. As contaminants we have dealt mainly with PCBs and DDTs and some trace metals such as cadmium, lead and mercury. The concentrations of DDTs varied between 0.22 and 26.19 μg/kg f.w. for all the species which are considered as a moderate contamination in comparison with other Mediterranean locations where the mean concentrations are about 25 μg/kg f.w. (UNEP/FAO/WHO/IAEA, 1990). The total PCB concentrations varied from 1.62 to 29.04 μg/kg f.w. These levels are not very high as compared to the other Mediterranean locations where the average concentration levels are in the range of 100 μg/kg f.w. (UNEP/FAO/WHO/IAEA, 1990).

There is no direct correlation between pollutants levels and concentrations of cytochrome P4501A1. This, could be the result of the poor cross-reactivity of the antitrout P4501A1 antibodies against P4501A1 from other fish species. However, Goksoyr et al (1991) have recently shown that the antibodies against trout P4501A1 readily cross-reacts with similar P450s from many marine species. Another explanation could be the effects of pollutants mixture such as oil and PCBs. Both polyaromatic hydrocarbons (PAHs) and PCBs induce P4501A1. Fish metabolize PAHs very rapidly and one of the only ways of showing environmental exposure is to look for its "fingerprint" i. e., P450 induction. By contrast PCBs are not readily metabolized and remain as residues in fish. The absence of correlation could also be attributed to pollutants inhibition or interference with P450 (MFO) systems, or it may be attributed to low levels of contaminant detected in this study in addition to the contaminant interactions particularly among heavy metals. Yoshiba and coworkers (1976) observed a decreased synthesis in cytochrome P450 in mice exposed to cadmium. Alvares et al. (1972) reported a 40 % to 50 % decrease in cytochrome P450 activity in hepatic microsomes of rats after an iv injection of 5 mg/kg $PbCl_2$ and an ip injection of 5 mg/kg CH_3HgCl. Similarly,

cadmium has been shown to inhibit ethoxyresorufin O-deethylase (EROD) and glutathione S-transferase (GST) activities in fish (George and Young, 1986).

CHAPTER XII
GENERAL CONCLUSION

Trace metal contamination of Moroccan seafood products are high but they remain under the acceptable limits. These levels are comparable with those found in the other Mediterranean countries and to some other locations of the world. Mean concentrations of lead, chromium, mercury and cadmium in the most consumed species are: 0.195 μg/kg f.w., 0.186 μg/kg f.w., 0.504 μg/kg f.w., 0.030 μg/kg f.w., respectively.

Mercury is of special importance because about 2% of fish samples analyzed exceed the limits set by several countries. Therefore, the high mercury levels in certain seafood species present a legal and possibly a sanitary problem in addition to any effects these levels may have on marine organisms and ecosystems.

Risk assessment of trace metals of population of fishermen and their families revealed that the amounts of fish and other seafood consumed, based on its mercury content could result in an intake exceeding acceptable levels for this metal. This should be confirmed by more specific survey of levels of mercury in blood and hair before more definite judgment is stated for this group of population. However, special attention should be given to pregnant women who are at greater toxic risk of mercury, because of their lesser body weight and the greater sensitivity of the fœtus to mercury. The percentage of cadmium and lead intake, through the consumption of seafood, to the acceptable daily intake is about 11.9-14.9 % for cadmium and 9.96 % for lead. No special problems are likely to occur even with this high level of fish consumption (190 g/person/day) as far as cadmium and lead. Special attention should also be given to children and infants because they are more vulnerable to lead toxicity.

In assessing risk to human health one must consider that the levels of toxic trace metals in seafood, are only one part of the total exposure to these contaminants. If the intake from other sources (atmospheric, occupational exposure, drinking water, other food items, etc.) is near the hazardous level, contaminant amounts in seafood products may elevate the daily intake above the acceptable level.

The use of mussels (*Mytilus edulis*) to assess trace metals contamination in coastal waters provides a good indicator of environmental contamination. Despite some variations due to size of the mussels and seasonal effects, this tool can be optimized by using the whole soft tissue of a composite sample of mussels of similar size (5-6 cm) collected at different periods during the year.

All the species analyzed showed moderate contamination levels by chlorinated hydrocarbon pesticides. p,p'DDT and its analogs (p,p'DDD and p,p'DDE) and lindane were

detected at the highest concentrations for most of the species. HCB, heptachlor epoxide, aldrin, dieldrin and endrin were present at relatively lower concentrations.

There were no seasonal variations for the compounds analyzed except for p,p'DDT which shows an increase during spring season which, at least partly, could be accounted for by local agricultural and pest control practices during this season in spite of the official ban of chlorinated hydrocarbon pesticides for agricultural purposes. Also no difference in contamination between sampling sites could be observed.

No immediate health risks are likely to be generated through the consumption of seafood products because of chlorinated hydrocarbons since the percent of the daily intake for high fish consumers is only 0.24 to 1.71 of the acceptable daily intake.

PCB contamination of seafood products showed very limited levels as compared with other locations in the world. No problems are likely to occur with respect to human consumption of fishery products since the levels are below acceptable limits in fish and fishery products as established by several countries and the daily intake for high fish consumers constitute only about 2.8% of the acceptable daily intake.

The use of mixed function oxidase enzymes as an index of fish exposure to pollutants is difficult to establish with environmental samples. This is likely because fish are exposed to a mixture of contaminants and the interaction(s) between these contaminants and the MFO systems make correlation between pollutant levels and the increase of MFO activity rather uncertain.

There is an increasing need for the monitoring of both toxic metals, chlorinated hydrocarbons and organic and inorganic pollutants in seafood products as well as in other food items. Establishment of a priority pollutants list as well as national regulatory limits is urgently needed to insure both human and environmental health.

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Appendix Table. Nomenclature of the analyzed marine organisms.

Code	Scientific Name	English name	French name
1	*Auxis thazard*	Frigate tuna	Auxide
2	*Belone svetovidovi*	Ganfish	Aiguille
3	*Boops boops*	Bogue	Bogue
4	*Campogramma glaycos*	Vadigo	Liche lirio
5	*Chelidonichthys cuculus*	Red gurnard	Grondin
6	*Chelidonichthys lucerna*	Tub gurnard	Grondin
7	*Chelidonichthys trigloporus*	Steakhead gurnard	Grondin
8	*Citharus linguatula*	Spotted flounder	Limande
9	*Dentex macrophtalmus*	Large eye dentex	Denté à gros yeux
10	*Dentex maroccanus*	Morocco dentex	Denté du maroc
11	*Diplodus bellottii*	Senegal seabream	Sar
12	*Diplodus puntazzo*	Sharpsnout seabream	Sar à museau pointu
13	*Diplodus vulgaris*	Common two-banded seabream	Sar à tête noire
14	*Eledone cirrosa*	Curled octopus	Poulpe blanche
15	*Helicolenus dactylopterus*	Rock fish	Rascasse de fond
16	*Illex coindetii*	Short fin squid	Encornet rouge
17	*Lepidotrigla dieuzeidei*	Spiny gurnard	Grondin
18	*Loligo forbesi*	Forbes squid	Encornet de Forbes
19	*Lophius budegassa*	European anglerfish	Baudroie rousse
20	*Merluccius merluccius*	European hake	Merlu
21	*Merluccius senegalensis*	Senegalese hake	Merlu du Sénégal
22	*Microchirus theophila*	Bastard sole	Sole- perdrix juive
23	*Micromesistrus poutassou*	Bleue whithing	Merlan bleu
24	*Mullus barbatus*	Red mullet	Rouget
25	*Mullus surmulletus*	Stripped red mullet	Rouget barbet de roche

Appendix Table. Nomenclature of the analyzed marine organisms.

Code	Scientific Name	English name	French name
26	*Mytilus galloprovincialis*	Meditarranean mussel	Moule
27	*Nephros norvegicus*	Norway lobster	Langoustine
28	*Octopus macropus*	While-spotted octopus	Poulpe tâcheté
29	*Octopus vulgaris*	Common octopus	Pieuvre
30	*Pagellus acarne*	Axielary seabream	Pageot acarné
31	*Pagellus bogaraveo*	Blackspot	Dorade rose
32	*Pagellus belottii*	Red pandora	Pageot à tache rouge
34	*Pagellus mormyrus*	Striped seabream	Marbré
35	*Palaemon serratus*	Common prawn	Crevette/Bouquet
36	*Panaeus melicertus*	Caramote prawn	Langoustine
37	*Parapenaeus longirostris*	Deep wach roses-shrimp	Crevette rose
38	*Parapandalus narval*	Narval shrimp	Crevette narval
39	*Peristedion cataphractum*	Malarmats	Malarmats
40	*Phycis blennoïdes*	Greater fork-beard	Phycis de fond
41	*Pomadasys incisus*	Bastard grunt	Grondeur métis
42	*Psetta maxima*	Turbot	Turbot
43	*Raja montagui*	Spotted ray	Raie douce
44	*Raja naevus*	Cuckoo ray	Raie fleurie
45	*Sardina pilchardus*	European pilchard	Sardine commune
46	*Sardinella aurita*	Madeiran sardinella	Grande Allache
47	*Scomber japonicus*	Chub mackerel	Maquereau espagnol
48	*Scropaena scrofa*	Red scorpion fish	Rascasse rouge
49	*Scyliorhinus canicula*	Small spotted catashark	Roussette
50	*Sepia officinalis officinalis*	Common cuttle fish	Seiche commune
51	*Sepia orbignyana*	Pink cuttle fish	Seiche rosée

Appendix Table. Nomenclature of the analyzed marine organisms.

Code	Scientific Name	English name	French name
52	*Serranus cabrilla*	Comber	Serran chèvre
53	*Serranus scriba*	Painted comber	Serran écriture
54	*Solea vulgaris*	Sole	Sole
55	*Sparus caeruleostictus*	Blue spotted seabream	Pagre à points bleus
56	*Sparus pagrus pagrus*	Common seabream	Pagre commun
57	*Sphyraena sphyraena*	European barracuda	Bécune européenne
58	*Squalus blainvillei*	Longnose spurdog	Aiguillat coq
59	*Todarodes sagittatus sagittatus*	European flying squid	Tetenou commun
60	*Todaropsis eblanae*	Lesser flying squid	Encornet souffleur
61	*Trachinus draco*	Greater weever	Grande vive
62	*Trachurus mediterraneus*	Mediterranean horse mackerel	Chinchard à queue jaune
63	*Trachurus trachurus*	Atlantic horse mackerel	Chinchard d'europe
64	*Zeus faber*	John dory	Saint-Pierre

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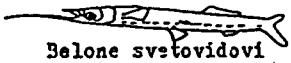
Auxis thazard



Belone svetovidovi

Boops boops

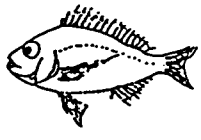
Campogramma glaycos

Chelidonichthys cuculus

Chelidonichthys lucerna

Chelidonichthys trigloporus

Cithrus linguatula



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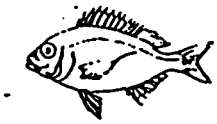
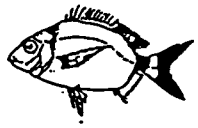


Dentex maroccanus

Diplodus bellottii

Diplodus puntazzo



Diplodus vulgaris

Eledone cirrosa

Helicolenus dactylopterus

Illex coindetii

Lepidotrigla dieuzeidei

Loligo forbesi

Lophius budegassa

Merluccius merluccius

Merluccius senegalensis

Mullus barbatus

Mullus surmulletus

Microchirus thoophila

Micromesistrus poutassou

Mytilus galloprovincialis

Nephros norvegicus

Octopus macropus

Octopus vulgaris

Pagellus acarne

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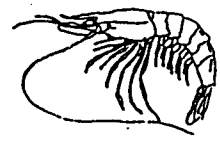
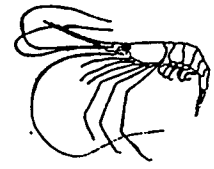
Pagellus bellottii

Pagellus erythrinus

Pagellus mormyrus



Parapenaeus longirostris



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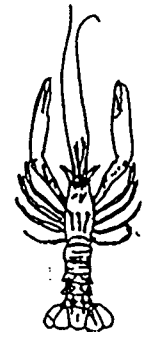
Palaemon serratus

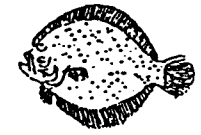


Panaeus melicertus

Peristedion cataphractum

Phycis blennoides

Pomadasys incisus



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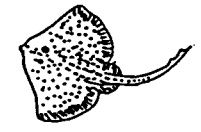

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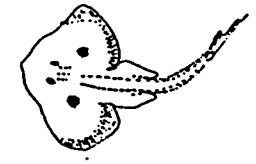
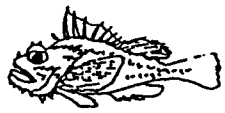


Raja naevus

Sardina pilchardus

Sardinella aurita

Scomber japonicus

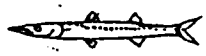


Scorpaena scrofa

Sparus pagrus pagrus

Zeus faber

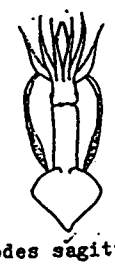
Scyliorhinus canicula



Sphyraena sphyraena

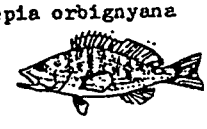
Squalus blainvillei

Sepia officinalis officinalis



Sepia orbignyana

Todarodes sagittatus sagittatus



Serranus cabrilla

Todaropsis eblanae

Serranu scriba

Solea vulgaris

Trachinus draco

Trachurus mediterraneus

Sparus caeruleostictus

Trachurus trachurus