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Methylmercury and Trace Element Distribution in the Organs of *Stenella coeruleoalba* Dolphins Stranded on the French Mediterranean Coast

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Abstract: The main objective of this study was to evaluate the contamination by mercury (**Hg**), methylmercury (**Me-Hg**), cadmium (**Cd**), selenium (**Se**), zinc (**Zn**), copper (**Cu**), iron (**Fe**) and manganese (**Mn**) in dolphins stranded on the French Mediterranean coast.

The distributions of these contaminants in the organs of dolphins have also been studied. Overall, contamination levels varied according to the following sequence: liver > kidney > lung > muscle, except for cadmium (kidney > liver > lung > muscle).

Size and sex of animals were also considered. Young dolphins were less impacted with trace elements than adults, except for copper. Among the studied parameters, the most important appeared to be the size of mammals. In addition, in the case of mercury and selenium, the sex of mammals seemed to be also relevant.

The correlations between the concentrations of trace elements suggest the existence of detoxification processes.

Since 1990s, using dolphins for tracing marine pollution, a slight reduction in the burden of the considered trace elements could be noted.

Keywords: Mediterranean sea, methylmercury, striped dolphins, *Stenella coeruleoalba*, trace elements.

1. INTRODUCTION

Pollution of the marine environment is, after overfishing, one of the main causes of the depletion of cetaceans, especially in the Mediterranean Sea. Some trace metals may be significant in contaminants for marine mammals who accumulate these pollutants primarily along the food chain. Dolphins therefore, tend to accumulate metals in their bodies forming toxic complex compounds. Zinc, copper, iron, and manganese are essential for mammals but they may become toxic when they exceed natural levels. In mammals, absorption, distribution, and excretion of these metals are regulated to maintain optimum levels in the body. Copper is absorbed mainly *via* the gastrointestinal tract and shows a wide distribution in the body. This metal is conveyed in blood in association with proteins, and is removed *via* the bile. Iron is involved in oxygen transport from lungs to tissues as hemoglobin, in oxygen storage as myoglobin. In the liver, Fe is stored intracellularly in the labile form such as ferritin and hemosiderin. The muscle of striped dolphins is abundant in hemoglobin and myoglobin, in which most of the Fe was bound to [1]. Manganese, is an essential element for proper functioning of the brain and the active respiratory system for protection against oxidative stress. It is also necessary for the production of vitamin B1 and vitamin E. Manganese acts as a cofactor of enzymes that facilitate various different metabolic processes [2]. Cadmium, mercury and selenium are known to be chronically toxic even when present at very low concentration in organisms.

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Cadmium is considered as one of the most toxic metals [3] with an acutely toxic dose of about 1 µg.kg^{-1} [4]. Against abnormally high levels of element contamination, marine organisms have developed detoxification mechanisms. This is especially true for mercury, with the demethylation of methylmercury and the formation of non-toxic tiemannite (HgSe) in the liver [5, 6]. For other elements such as cadmium, metallothioneins are supposed to bind with and then to reduce their toxic effects in the body [7- 8].

The objectives of this study were to analyze trace element in dolphins in order: (1) to evaluate current levels of contaminants in the tissues and organs, (2) to compare size groups and reveal possible differences between sexes, (3) to compare these results to the previous studies on the dolphins of the Mediterranean medium.

2. MATERIALS AND METHODS

2.1. Sampling

In this study, *Stenella coeruleoalba*, mostly fish and squid feeders, stranded on the Mediterranean coast from the French Riviera to the Spanish border were sampled. These dolphins were probably contaminated up stream in the general current bathing the western Italian coast as well the southern Sardaigna – Sicilia Sea areas (Fig. **1**). Fifty five specimens of (striped dolphins) *St. coeruleoalba* were studied in this work found stranded during the period 2002 - 2009 (Fig. **1**).

The collection of tissues (liver, kidney, lung, muscle) was performed by the French Mediterranean Cetacean Study Group (GECEM). Mammals were measured beforehand and weighed when possible. After dissection, the tissues and organs were sampled in polyethylene bags and kept frozen at -20°C. Before analysis, they were lyophilized and homogenized in a Teflon Ultra-Turrax T25 homogenizer (Janker and Kundel, Germany).

2.2. Age estimation

Usually, the age of cetaceans is determined from the dentition. However, some samples were severely deteriorated. For the dolphins studied in this work, the exact age could not be determined. The mammals were classified into two groups, following their sexual maturity that can be assessed from the size of the individuals [1, 9-10]:

- juvenile cetaceans with sizes smaller than 120 cm,
- mature cetaceans, with sizes greater than 120 cm.

Fifty five *St. coeruleoalba* were studied: 20 males, 20 females, 15 juveniles. Individual size and gender as well as date and place of stranding are shown in Table **1**.

2.3. Chemical Analysis

All equipments used in the sample processing were cleaned and subsequently soaked for at least 24 hours immersed in a 10% HNO_3 bath for 24 hours, and rinsed with Milli-Q water before use. For Cd, total Hg, Se, Zn, Cu, Fe and Mn analysis, 0.5 g of homogenized dried material was digested with 6 mL HNO_3 (65% ultrapur Normatom) in a 50 mL closed vessel by microwave digestion (Microdigest Model Microwave 3000, Anton Paar, Graz, austria) at 200°C for 30 min. The digested samples were cooled and brought to a final volume of 25 mL with Milli-Q water. Mercury was determined by cold vapor atomic absorption spectrometry (PSA 20400 Merlin Millenium, Orpington, UK) at 254 nm and methylmercury (Me-Hg) by gas chromatography/atomic emission spectroscopy as previously described [11]. Cu, Fe, Mn, and Zn were determined by flame atomic absorption spectrometry with Zeeman effect background correction (AA 220FS, Varian, Palo Alto, US), Cd, Se by graphite furnace Atomic Absorption Spectrometry, for the latter element using as hydride generator system (VGA 77, Varian).

2.4. Quality Assurance/Quality Control

The glassware used was cleaned with detergent, TDFD4 decFT30 (VWR-Prolabo, France), immersed in a 10% HNO_3 bath for 24 hours, and rinsed with Milli-Q water before use. Analytical quality was controlled using certified reference materials (CRM): DORM-2 from National Research Council Canada, IAEA (the International Atomic Energy Agency, Monaco), and SRM 2976 from NIST USA. For the analysis of Me-Hg, we used as certified control, the BCR 464. For analysis of each batch of extracts, a blank and a CRM sample was included. All results were in good agreement with the CRM (Table **2**).

2.5. Data Treatment

The different sets of data were examined for significant differences ($P < 0.05$) by the Wilcoxon's rank-sum test, and analytical data were subjected to Pearson product-moment correlation. Multiple regression analysis (R^2) was performed. All data were expressed as the mean value ± standard deviation (SD) (according to samples < 56 samples) based on dry weight.

3. RESULTS AND DISCUSSION

3.1. Zinc, Copper, Iron, Manganese

3.1.1. Average Concentrations

The levels of trace elements found in different tissues and organs are shown in Table **3**, together with their means, standard deviation, minimum and maximum values. All results are expressed on a dried weight basis. Zinc, copper, iron, and manganese, are essential elements. They showed the normal differential accumulation, function of organ. The highest levels measured were generally observed in liver, then in kidney or lung, while lowest levels were noted in muscle (Table **3**).

The highest values for the all organs were found for iron, ranging from a mean value of 493.5 µg.g^{-1} in muscle to 1018.8 µg.g^{-1}in the liver. Concentrations of Fe decreased according to: Liver ≈ Lung > Kidney ≈ Muscle. Zn ranged from a mean value of 37.9 µg.g^{-1} in muscle to 143.9 µg.g^{-1} in the liver; the levels in tissues decreased according to (liver > lung ≈ kidney > muscle). Level of copper ranged from a mean of 3.8 µg.g^{-1} in the lung to 33.7 µg.g^{-1} in the liver and decreased in the order: liver > kidney > muscle > lung. Manganese was present at much lower levels, ranging from a mean of 0.7 µg.g^{-1} in the muscle to 9.5 µg.g^{-1} in the liver, and decrease in the order: liver > kidney > lung ≈ muscle. Within the metal considered as toxic, the highest level was that of mercury, particularly in the liver with an average value of 514.2 µg.g^{-1}, compared with mean value of 61.9 µg.g^{-1} in the

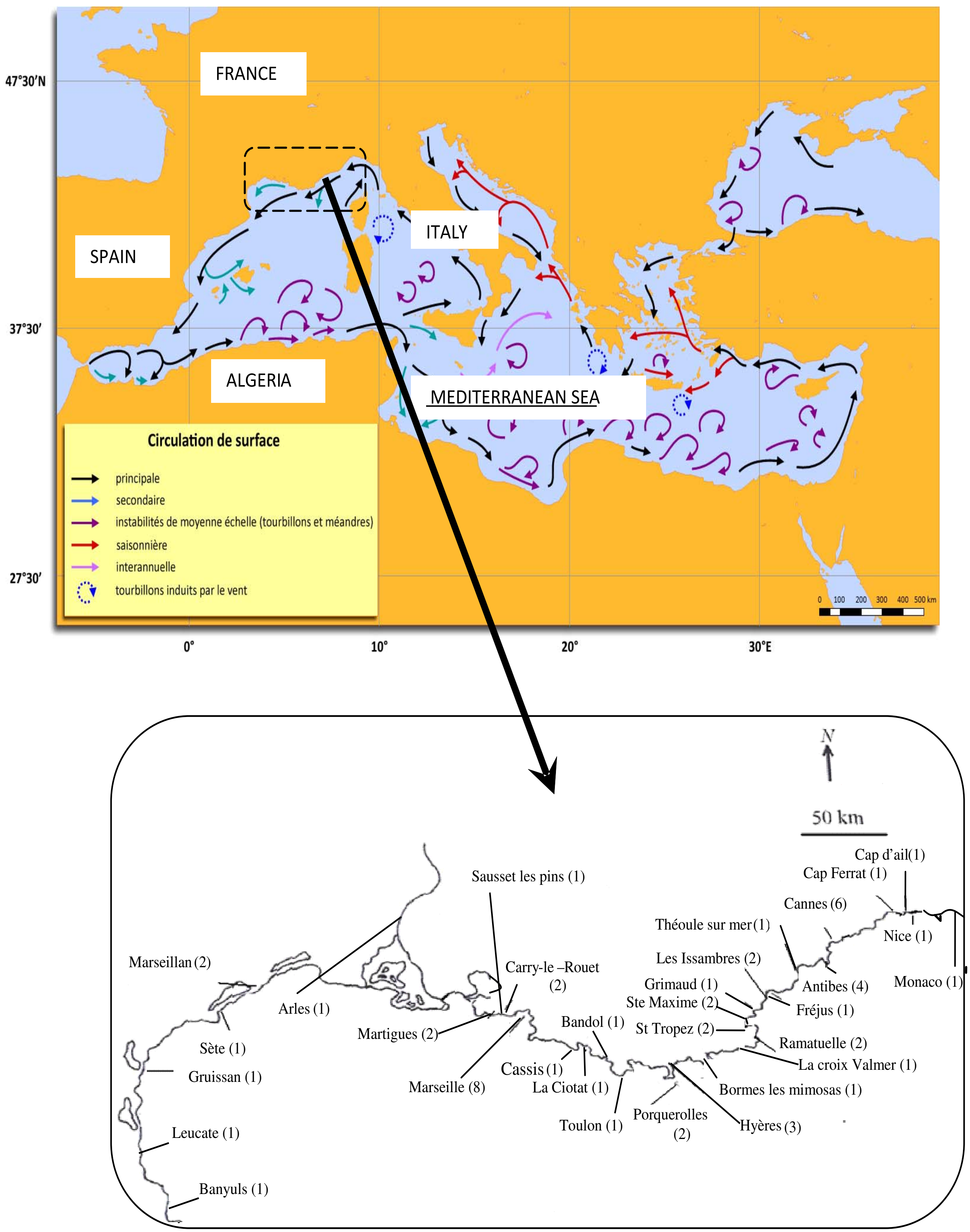

Fig. (1). Map of the sampling locations (main ocean currents according to Millot and Taupier-Letage, 2005).

Table 1. Codes and characteristics of the studied dolphins.

Reference	Stranding Date	Stranding location	N° Department	Sex	Length (cm)	Information's
11	09/05/06	Marseille	13	foetus	50	PU
XVI	20/07/04	Carry le Rouet	13	young	60	PU
4	09/08/05	Toulon Sud	83	young	87	VF
I	15/08/02	La Ciotat	13	young	94	-
VIII	12/10/03	Porquerolles	83	young	96	PU
5	17/08/05	Ramatuelle	83	young	98	FR
33	06/12/08	Marseille	13	young	99	FR
42	07/01/08	Grimaud	83	young	100	
1	06/11/03	Banyuls	66	young	101	FR
55	05/09/07	Ramatuelle	83	young	105	FR
X	03/03/04	Sausset les Pins	13	young	107	-
VI	21/10/02	Leucate	11	young	107	-
57	24/09/08	Ste Maxime	83	young	110	FR
30	17/04/08	Cassis	13	young	110	FR
II	17/09/02	Martigues	13	young	112	FR
38	20/11/07	Fréjus	83	female	130	-
58	17/09/07	Cannes	06	female	143	FR
IX	03/01/04	Marseille	13	female	145	FR
68	14/02/08	Saint-Jean Cap Ferrat	06	female	145	PU
61	01/12/07	Cannes	06	female	165	VF
IV	28/01/03	Saint-Tropez	83	female	150	-
69	25/02/08	Théoule-sur-mer	06	female	144	VF
40	09/12/07	Les Issambres	83	female	220	-
45	17/02/08	Les Issambres	83	female	190	-
62	14/12/07	Cap d'Ail	06	female	208	FR
36	23/07/07	Cannes	06	female	200	-
59	18/09/07	Cannes	06	female	212	FR
51	03/08/08	Saint-Tropez	83	female	190	-
34	16/11/06	Ste Maxime	83	female	194	-
XI	06/03/04	Marseille	13	female	195	FR
10	05/03/06	Marseille	13	female	206	FR
39	21/11/07	Hyères	83	female	200	-
V	20/05/03	Bandol	83	female	210	-
13	05/11/06	Martigues	13	female	151	FR
26	13/02/08	Sète	34	female	147	FR
70	27/10/08	Monaco	98	male	150	FR
63	19/12/07	Nice	06	male	163	PU
43	08/01/08	Cannes	06	male	155	-
71	27/12/08	Cannes	06	male	207	PU
2	15/01/05	Antibes	06	male	188	VF

(Table 1) contd…..

Reference	Stranding Date	Stranding location	N° Department	Sex	Length (cm)	Information's
60	04/11/07	Antibes	06	male	177	FR
65	03/01/08	Antibes	06	male	208	FR
72	03/02/09	Antibes	06	male	160	-
35	04/07/07	La Croix Valmer	83	male	203	-
46	25/02/08	Bormes les Mimosas	83	male	150	-
37	05/09/07	Hyères	83	male	167	-
41	06/01/08	Hyères	83	male	163	-
31	09/08/08	Porquerolles	83	male	193	FR
16	22/08/07	Marseille	13	male	180	FR
21	19/12/07	Marseille	13	male	210	FR
24	19/01/08	Marseille	13	male	221	FR
25	06/02/08	Carry le Rouet	13	male	195	PU
56	18/07/08	Arles	13	male	197	FR
19	24/11/07	Marseillan	34	male	155	FR
29	26/02/08	Marseillan	34	male	197	FR
28	22/02/08	Gruissan	11	male	155	PU

VF = Very Fresh ; FR = Fresh ; PU = Putrefactive.

Table 2. Operating conditions for metal analyses; mean and standard deviation (in µg.g^{-1} dwt) for the replicate analysis of standard reference material; element quantification limits (QL in µg.g^{-1} dwt).

Element	Apparatus	λ (nm)	Standard Reference Material			QL
			Reference	Certified Value	Observed Value	
Cd	Flame AAS	228,8	IAEA 452	29.6±3.7	29.38±0.73	0.5
Cd	Furnace AAS	228,8	SRM 2976	0.82±0.16	0.78±0.2	0.01
Cu	Flame AAS	324,8	MESS 3	33.9±1.6	32±2	0.5
Cu	Furnace AAS	327,8	SRM 2976	4.02±0.33	3.53±0.12	0.01
Fe	Flame AAS	248,3	SRM 2976	171.0±4.9	177.8±14.1	5
Mn	Flame AAS	279,5	DORM-2	3.66±0.34	3.50±0.02	0.4
Hg	SFA	254	DORM-2	4.64±0.26	4.61±0.05	0.1
Se	Flame AAS Hydrures	196,0	IAEA 452	6.55±0.82	6.20±0.24	0.5
Zn	Flame AAS	248,3	SRM 2976	137±13	128.4±6.0	3
Me-Hg	GC/ AED	253,6	*BCR 464*	5.50 ± 0.17	4.81±0.55	0.05

kidney, 56.4 µg.g^{-1} in the lung and 26.4 µg.g^{-1} in the muscle. Relatively higher concentrations were also found for selenium with an average of 211.7 µg.g^{-1} in the liver. The mean level of 14.8 µg.g^{-1}of Me-Hg is found in liver. Consistently low mean values were found for cadmium, ranging from 0.1 µg.g^{-1} in the muscle to 3.1 µg.g^{-1} in the liver.

3.1.2. Variations in Relation with Sexual Maturity and Gender

Further division of both sexes was made into adult and juvenile (Fig. **2**).

Cu and Zn are essential elements participating in the formation or function of enzymes involved in metabolism; they concentrate primarily in the liver. Significant differences have been observed as a function of sex and between adults and juveniles with the exception of copper: Cu has appeared to concentrate mainly in the liver of juveniles [12-13]. The high levels of Cu in juveniles' livers can be explained from the transfer of this metal *via* the placenta of the mother. After birth, the young mammal eliminated Cu in the growth phase progressively [14]. Similarly, Kunito *et al*. [15] in their study of cetaceans have

Table 3. **Results in µg.g^{-1} dwt. Average concentrations (Mean), standard deviation (SD), minimum (Min) and maximum (Max) values, number of samples (N).**

		Cd	Hg	Me-Hg	Se	Zn	Cu	Fe	Mn
Liver	Mean	3.1	514.2	14.8	211.7	143.9	33.7	1018.8	9.5
	SD	3.3	903.1	12.5	398.6	73.7	30.2	531.5	4.3
	Min	0.01	9.9	0.4	1.4	43	10	265	2
	Max	11.4	5374	55	2350	370	193	2958	18.1
	N	50	50	49	50	50	53	45	45
Muscle	Mean	0.1	26.4	8	11	37.9	5.4	493.5	0.7
	SD	0.1	35.5	6.1	14.7	17.9	2.5	211	0.5
	Min	0.01	3.1	1.9	1.1	25	1.1	170	0.0
	Max	11.4	133	21	61.7	125	39.7	2262	2.1
	N	33	34	32	34	34	34	31	31
Lung	Mean	0.27	56.4	5.5	27.3	104	3.8	908.2	0.9
	SD	0.19	120	5.1	39.4	37	1.2	302.4	0.5
	Min	0.01	2	0.0	1.8	61	1.7	336	0.1
	Max	1.48	562	15.8	155.8	172	8.7	1010	2.4
	N	35	36	34	36	37	36	29	28
Kidney	Mean	10.3	61.9	7.4	28.7	94.2	11.3	718	2.0
	SD	11.6	63.2	6.4	26.3	16.8	5.0	231	0.9
	Min	0.01	5.4	0.1	3.0	52	8.2	80	0.7
	Max	45.4	268	29	121.5	132	37.3	1143	5.6
	N	48	50	46	50	50	52	44	44

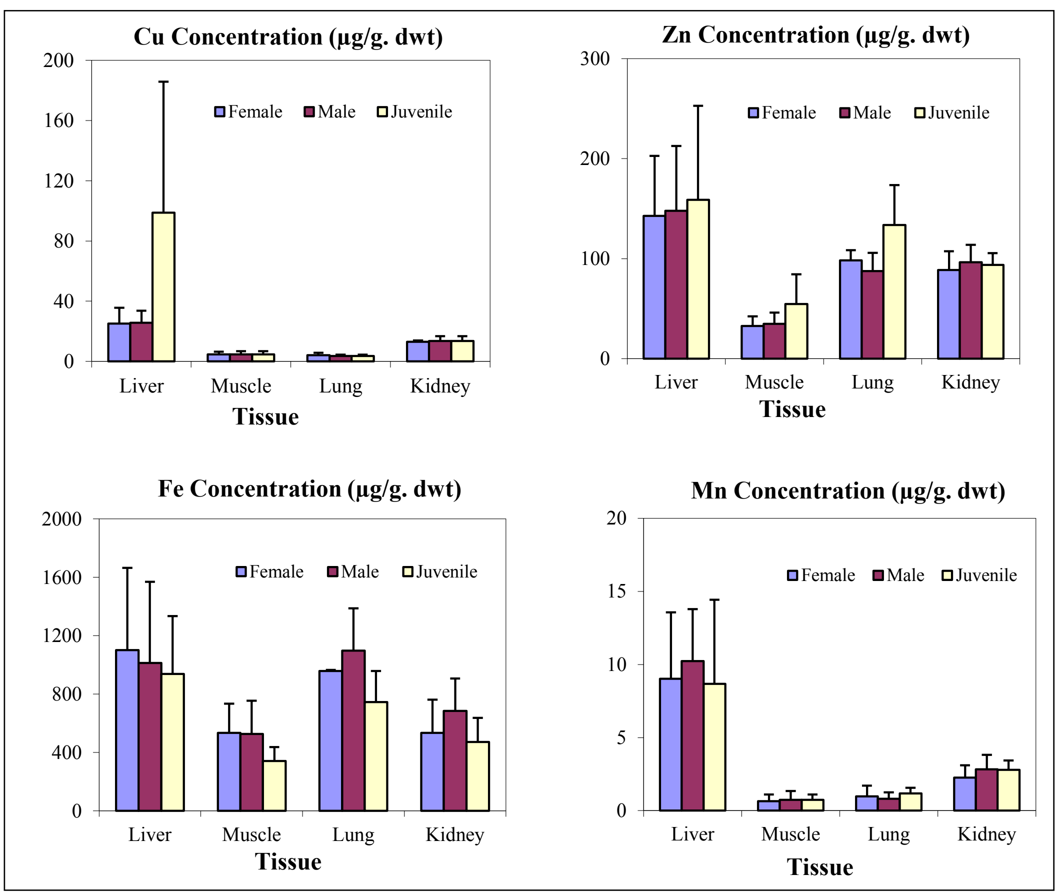

Fig. (2). Cu, Zn, Fe and Mn levels in the organs of *Stenella coeruleoalba* as a function of sexual maturity and sex.

found a negative correlation between copper levels in the liver and the age of estuarine dolphins. Conversely, Seixas *et al*. [16] have shown lower levels for the concentration of Cu for young *Sotalia guianensis* in comparison with adults (Brazil). In the other organs, copper concentrations seem not to depend on sex and size of the individuals. Mn results showed high concentrations in liver compared to other organs. In all tissues and organs, no significant differences between juveniles and adults were observed. No significant correlations were found between Mn concentration of liver, lung, kidney and muscle with size and sex. Bernhard and Andrea [17] have explained similar relationships in other fish due to these essential metals being regulated by the organism. Thus, the levels being maintained more or less constant in organs such as muscle. However, the level may vary in the excretory organs (lung, kidney). Zn accumulates in aquatic organisms, but the values of bio-concentration factors decrease with the tropic chain [18]. In mammals, absorption and excretion of this metal are regulated to maintain constant levels in the body. The Fig. (**2**) confirms this trend in all tissues and organs. However, the levels were slightly higher for juveniles (in the liver, lung and muscle). The higher levels of iron were obtained in liver and lung and relatively low concentrations were observed for juveniles in all organs. This distribution was similar to those reported by others author [18-21]. Bernard and Andreae [17] also noted similar relationship in some other aquatic species. The organisms allow a good regulation of the level of these essential metals and thus, their concentration in organs can be maintained more or less constant.

3.2. Cadmium, Selenium, Mercury and Methylmercury

3.2.1. Average Concentrations

According to the results presented in Table **3**, regardless of gender and sexual maturity, high levels of cadmium were found in the kidney of dolphins (10 ± 12 µg Cd.g^{-1} dry weight, n = 48) which is an organ of accumulation of this compound [4, 18, 19-20]. Levels of cadmium in the kidney were about four times higher than in the liver (3.1 ± 3.3 µg Cd.g^{-1} dry weight, n = 50). In lung, the measured concentrations were relatively low (0.3 ± 0.2 µg Cd.g^{-1} dry weight, n = 35), but the lowest contents were observed in muscle (0.1 ± 0.1 µg Cd.g^{-1} dry weight, n = 33). Cd level in the muscle in this study were 15-times lower than (0.4 µg.g^{-1} w.wt [22]; 0.9 µg.g^{-1} w.wt [7]; 0.28 µg.g^{-1} w.wt [23] and in the same order of (0.02 µg.g^{-1} w.wt [24]. On the other hand, liver level reported in Striped Dolphins from the Mediterranean: 1.5 µg.g^{-1} w.wt [22]; 3.7 µg.g^{-1} w.wt [7]; 1.6-5.39 µg.g^{-1} w.wt [23]) were higher to those determined in this study, however Bilandzic *et al*. 2012 [24] found 2.19 µg.g^{-1} w.wt in the Striped Dolphins from the Adriatic Sea. In this study Cd level in kidney were lower (2.5 µg.g^{-1} w.wt) than 6.10 µg.g^{-1} w.wt reported by Bilandzic *et al*. [24]. As has been found in others studies [24-25], Cd level decreased in tissues in the order: kidney > liver > lung > muscle.

The average concentrations of total mercury (Hg) were very high in liver (514 ± 903) µg Hg.g^{-1} dry weight, n = 50). These values were 8 times higher than in kidney and 9 times higher than in lung. Liver appeared as the target site of accumulation for this compound [18, 25]. Similarly, Roditi-Elasar *et al*. [7] have reported levels of 1.4 to 550 µg Hg.g^{-1} in the striped dolphins from Israeli Mediterranean coast. Overall, these values were higher than those reported by Holsbeek *et al*. [26], who have noted levels of 35 to 38 µg Hg.g^{-1} in the liver of *St. coeruleoalba* from the French Atlantic coast. The level of mercury in the different organs declined as follow: Liver >> Kidney > Lung > Muscle. This trend was similar to those regularly found in the literature [18, 27-29], however, another trend: Liver > Muscle > Kidney was found in dolphins stranded in Eastern Adriatic Sea [24].

The concentrations found in this study were relatively low considering that the tolerance limit of Hg in mammals lies between 100 and 400 µg.g^{-1} wet weight [24]. Level of 1,500 µg.g^{-1} wet weight was found in the liver tissue of **one** specimen of *Stenella coeruleoalba* from French Mediterranean coasts [30]. This value is higher to (181 µg.g^{-1} wet weight) reported by Roditi-Elasar *et al*. [7], in the same species stranded in Apulia. In this study, in the liver, the Hg level ranged from 9.9 to 5374 dry weight, with a mean value of 514.2 µg.g^{-1} dry weight or 128.6 µg.g^{-1} wet weight and fall within this range.

Note that concentration values in tissues expressed in dry weight, were converted to wet weight using the factor (ww/dw) of 0.25 establish for dolphins by Becker *et al*. [31].

For methylmercury (Me-Hg), concentrations ranged from 15 ± 13 µg.g^{-1} in the liver, to 5.5 ± 5.1 µg.g^{-1} in the lung. For this compound, the trend was: Liver > Muscle > Kidney > Lung. As total mercury, methylmercury concentrated preferentially in the liver with values about 2 times higher than in other organs. However, in the liver, the concentration of methylmercury was 20 to 50 times lower than the concentration of total mercury. Selenium concentrations varied in the same way as in the total mercury, with levels in the liver much higher than in the other organs (10 times higher for females and 5 for males). In most tissues and organs, selenium levels were significantly lower than those of mercury. These results are in contradiction with Kubota *et al*. [32], who have measured similar levels of Hg and Se, both in the liver and in the kidney of franciscana (Pontoporia blainvillei) of the Rio Grande (south of Brazil). These differences would be explained by the significantly dissimilar levels of exposition of dolphins to pollutants in the Mediterranean Sea compared to Rio Grande.

3.2.2. Variations in Relation with Sexual Maturity and Gender

Fig. (**3**) shows average results for Cd, Hg, Se, Me-Hg, in juveniles, adult females and adult males. All the concentrations are expressed in (mean ± Sd) µg.g^{-1} of dry weight.

The highest levels of cadmium, selenium, mercury and methylmercury were found in the liver (and the kidney in the particular case of Cd), mainly in adults (male or female). Levels in the lung and muscle were relatively low independently of sex and sexual maturity. For each contaminant, the organs of adults are significantly more contaminated than those of juveniles with levels 5 to 500 times higher depending on organs and contaminants. The results seemed to confirm that the contamination essentially came from an accumulation over time from the diet. No transfer of contaminants, from the mother to their babies,

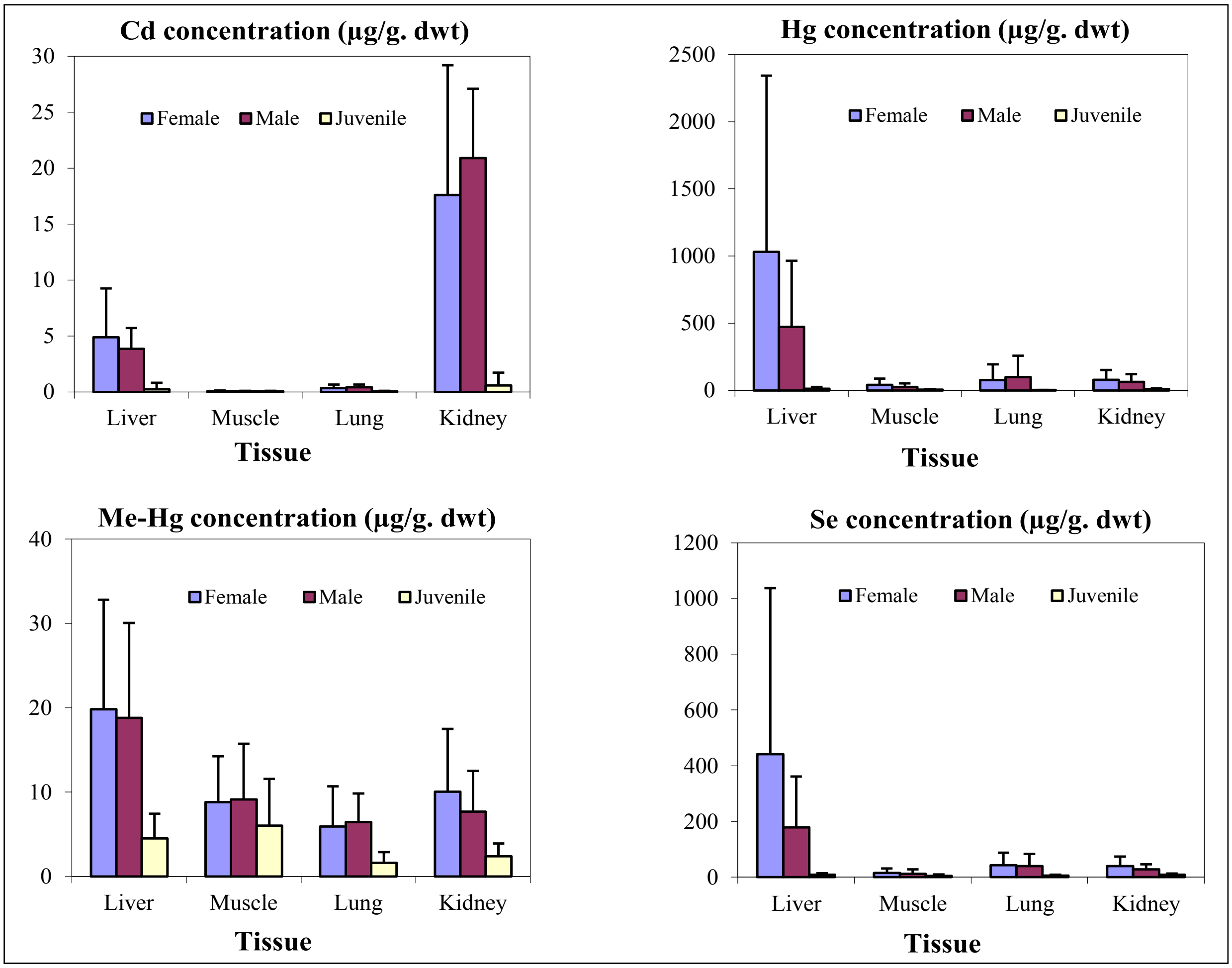

Fig. (3). Trace elements concentrations in the different organs of *Stenella coeruleoalba* for female, male, and juvenile respectively.

would occur during gestation or lactation [20]. The correlations between Cd-length were ($y = 0.47\ x - 0.91$, $R^2 = 0.47$, $P < 0.05$); ($y = 0.02\ x - 0.03$, $R^2 = 0.46$, $P < 0.05$) and ($y = 0.18\ x - 14.46$, $R^2 = 0.43$, $P < 0.05$) in liver, lung and kidney respectively, whereas no correlation were found in the muscle. A correlation ($y = 2.2\ x + 8$, $R^2 = 0.42$, $P < 0.05$) was observed between the levels of cadmium in liver and in the kidney. This result can be compared to those obtained by Cardellichio *et al*. [1] who have showed a correlation ($R^2 = 0.80$) for this metal in the same organs. Levels of Se and Hg increased significantly with the size of the mammals (Fig. **4**).

This phenomenon was even more dominant for females and may reflect a higher contamination in females compared to the males [33]. Regarding methylmercury, the increase of concentrations as a function of the dolphins'size was slow and gradual, independently of the gender of the dolphins. The percentage of methylmercury reported to total mercury decreased with the size of organisms (Fig. **5**).

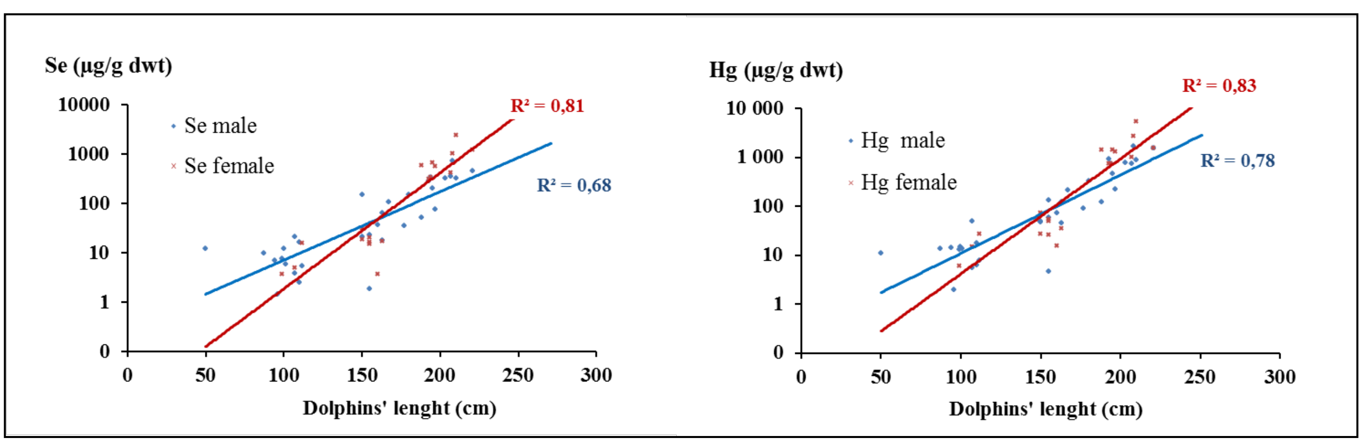

Fig. (4). Se and Hg concentration in the liver of dolphins as a function of size.

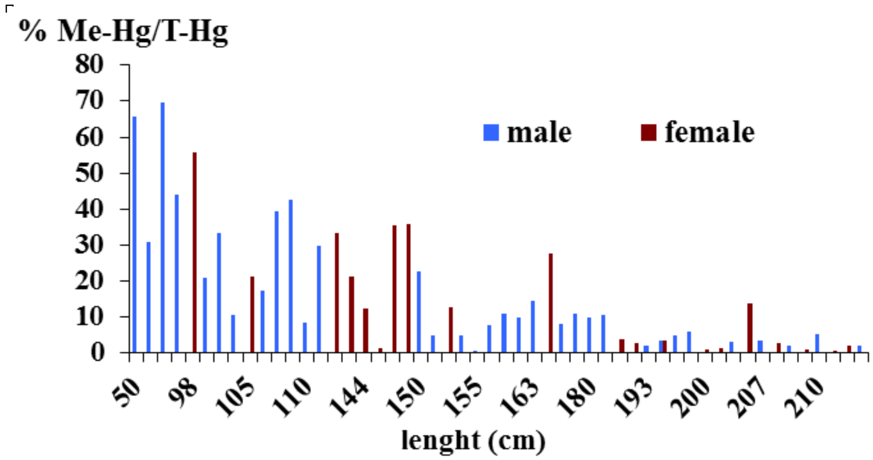

Fig. (5). Molar ratios MeHg/ Hg total as a function of the dolphins'size, in the liver.

There was a critical size around 150 cm from which the ratio methylmercury/mercury decrease drastically. This would denote that detoxification processes occurred and would be more efficient for mature organisms. These processes would also occur when the level of methylmercury reaches a critical value.

3.2.3. Relationship Between Elements Concentrations

Compounds such as selenium, mercury and cadmium had a similar repartition in the different organs studied. So, in the following paragraph some correlations have been analyzed concerning these elements.

3.2.3.1. Hg to Se relationship

Correlations between Hg and Se in the organs of mammals have already been described in the literature [29, 34 -37]. In the present study, the significant relationships $R^2 = 0.98$ ($y = 3.02x - 26.64$, $P < 0.05$); $R^2 = 0.94$ ($y = 2.2x - 50.21$, $P < 0.05$); $R^2 = 0.78$ ($y = 1.88x + 0.49$, $P < 0.05$); $R^2 = 0.55$ ($y = 3.09x - 31.17$, $P < 0.05$); were found respectively between the concentrations of Se and Hg in lung, liver, muscle and kidney. Correlation coefficients between the levels of inorganic mercury and the level of selenium were $R^2 = 0.93$ ($y = 0.36\ x -23.76$, $P < 0.05$) for adults and $R^2 = 0.59$ ($y = 0.6x + 3.22$, $P < 0.05$) for juveniles. Similar results have been obtained by Cardellicchio *et al*. [1]. The high correlation between Hg and Se in the organisms could reflect a direct coupling between these two elements in the liver and would be explained by the existence of a detoxification mechanism involving both elements. Methylmercury is the dominant form of mercury in the food chain. Generally, most of the accumulated Hg in the liver is inorganic mercury (I-Hg), suggesting a demethylation process of methylmercury in the liver probably in order to protect organisms [35, 38].

Many studies on marine mammals have shown that there is a close correlation between inorganic mercury and selenium [39, 40]. In order to underline possible correlations between the levels of methylmercury, inorganic mercury and selenium in the different organs, concentrations of inorganic mercury were calculated. The common trends appeared in inorganic mercury (I-Hg) and selenium in the liver. Correlation coefficients between the levels of inorganic mercury and selenium in the liver were ($R^2 = 0.93$, $y = 2.1\ x + 64$, $P < 0.05$) for adults and ($R^2 = 0.56$, $y = 0.7\ x + 1$, $P < 0.05$) for juveniles.

The Fig. (**6**) illustrates the I-Hg/Se molar ratios in dolphins of different sizes and sexes in the liver.

In the kidney, a correlation of ($R^2 = 0.94$, $y = 0.21\ x - 21.19$, $P < 0.05$) was obtained between the levels of selenium and inorganic mercury for males and ($R^2 = 0.98$, $y = 0.93\ x - 77.5$, $P < 0.05$) for females (Fig**. 7**).

The I-Hg/Se molar ratio in dolphins of different sizes and sexes in kidney is included on Fig. (**8**).

In the lung, concentrations of inorganic mercury and selenium significantly increased with the size of dolphins in the same way for both compounds. A correlation ($R^2 = 0.99$, $y = 3.05\ x - 18.28$, $P < 0.05$) was obtained between the level of total mercury and the level of selenium in lungs, taking into account all the individuals (Fig. **9**).

The I-Hg/Se molar ratio in dolphins of different sizes and sexes in the lung is illustrated in Fig. (**10**).

In many studies treating this subject, the molar ratio Hg/Se, measured in the organs of dolphins was greater than 1 [37]. In this work ratios were often close to 1 (especially for the adult livers). Detoxification processes would be achieved by the transformation of methylmercury in inorganic mercury *via* the selenide (HgSe) formation [16, 35, 41-42]. These transformations would essentially occur when the level of organic mercury in the liver is high, and more commonly in the oldest organisms. This process must be the main detoxification process for mercury compared to detoxification processes involving metallothioneins (MTs), a family of cysteine-rich proteins with low molecular weight [37, 43].

3.2.3.2. Se to Cd Relationship

Bibliographical studies have reported strong correlations between Se and Cd levels in the livers of cetaceans [1, 16]. This relationship has already been observed in the liver of Globicephala melas (pilot whales) [38]. Monaci *et al*. [16] have reported an average molar ratio Se/Cd of 107 in the liver of *St. coeruleoalba* of western Mediterranean. In the present study, the average molar ratios Se/Cd was found to

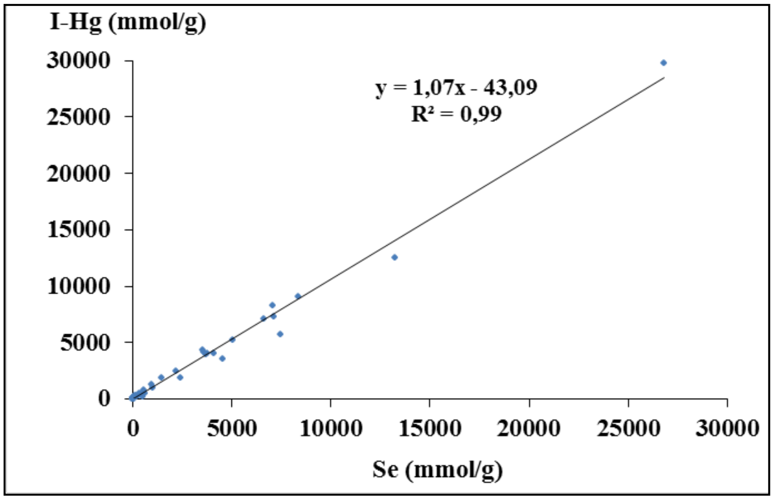

Fig. (6). Relationship between molar concentrations of inorganic mercury (I-Hg) and Se in liver.

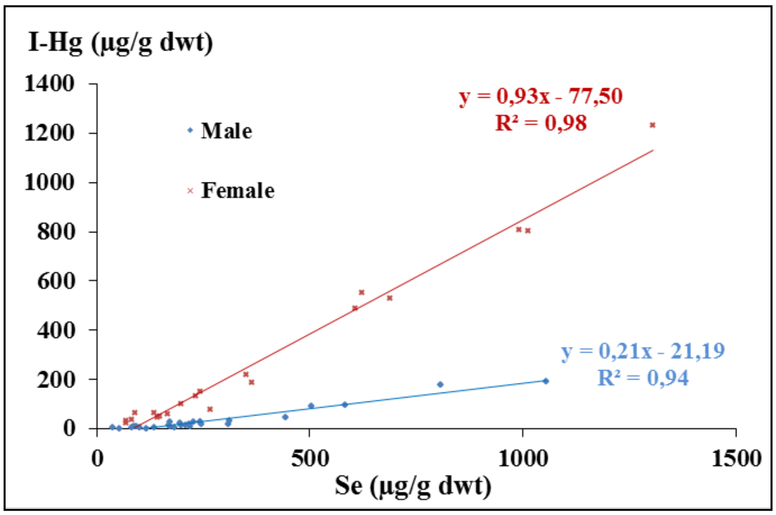

Fig. (7). Correlation between Inorganic Hg and Se concentrations in the kidney (for males and females).

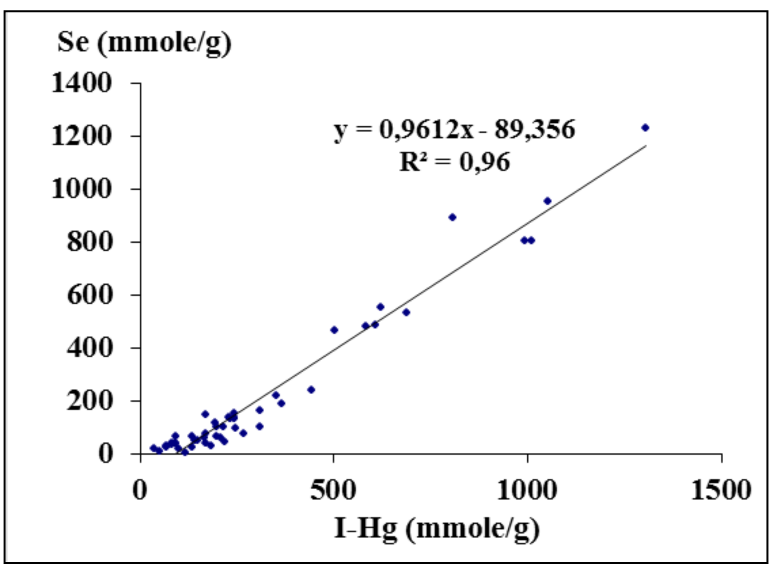

Fig. (8). Relationship between molar concentrations of inorganic mercury (I-Hg) and Se in kidney.

be 80, 57, and 37 for females, males, and juveniles, respectively in the liver.

In the kidney, the average molar ratios were 13, 3, and 2 in juveniles, females and males, respectively. We observed a

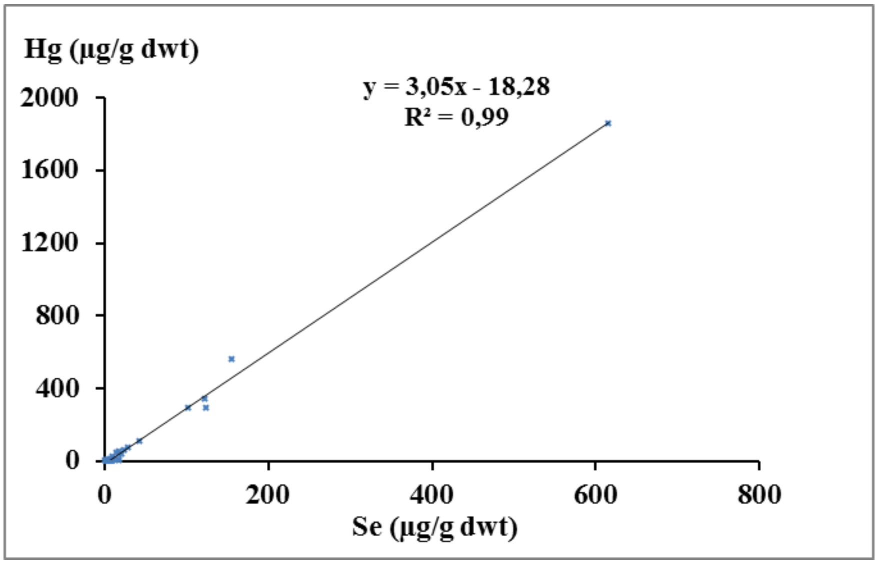

Fig. (9). Relationship between Hg and Se concentration in the lungs (all samples).

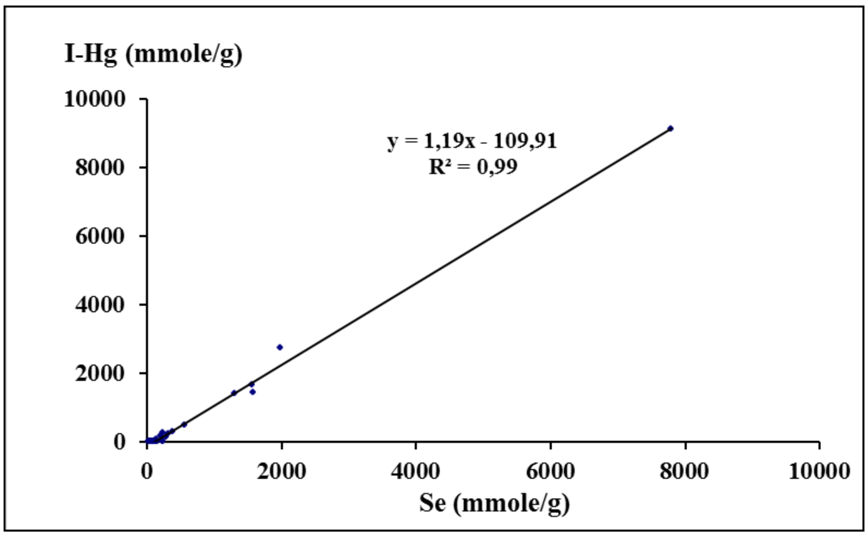

Fig. (10). Relationship between molar concentrations of inorganic mercury (I-Hg) and Se in lungs.

low correlation ($R^2 = 0.39$, $y = 1.46\ x+5.4$, $P < 0.05$) between Se and Cd levels. Similarly, Monaci *et al*. [16] have not obtained significant correlations in the kidney for these compounds. In marine organisms, selenium may act as a protection against the toxic effects of Cd as in the case of Hg [14]

3.2.3.3. Hg to Cd Relationship

A correlation ($R^2 = 0.50$, $y = 127\ x$, $P < 0.05$) was obtained between Hg and Cd concentrations in the livers of male. By contrast, in females, no correlation was observed. In the kidney, Seixas *et al*. [16] have not found any correlation between these compounds but other authors [21, 38] have reported positive correlations between these two compounds in the liver and in the kidney. In contrast, negative correlations have been obtained in the *St. coeruleoalba* and the *Tursiops truncatus* [7]. This could suggest that a competition mechanism might exist between Hg and Cd and would explain the negative correlations found [34].

4. TEMPORAL VARIATIONS IN THE MEDITERRANEAN AREA

The differential accumulation of toxic metals in dolphins is dependent on many factors including the speciation of metal ions, routes of uptake, quality of food and biochemical regulations. The ecological conditions vary from one marine environment to another and the dolphins feeding patterns differ from one ocean to another. Thus, we only compare our results to those obtained in the literature in the Western Mediterranean area. The different temporal trends of the compounds are displayed in Fig. (**11**).

Concentrations of Hg and Cd seemed to decrease over time while Cu and Zn concentration seemed to remain relatively constant throughout the studied period. The input of trace element waste in the Mediterranean Sea is commonly believed to have decreased or at least stabilized over the last decade. However, reducing pollutant input does not necessary lead to a rapid decrease for their concentrations in the marine environment [16, 44].

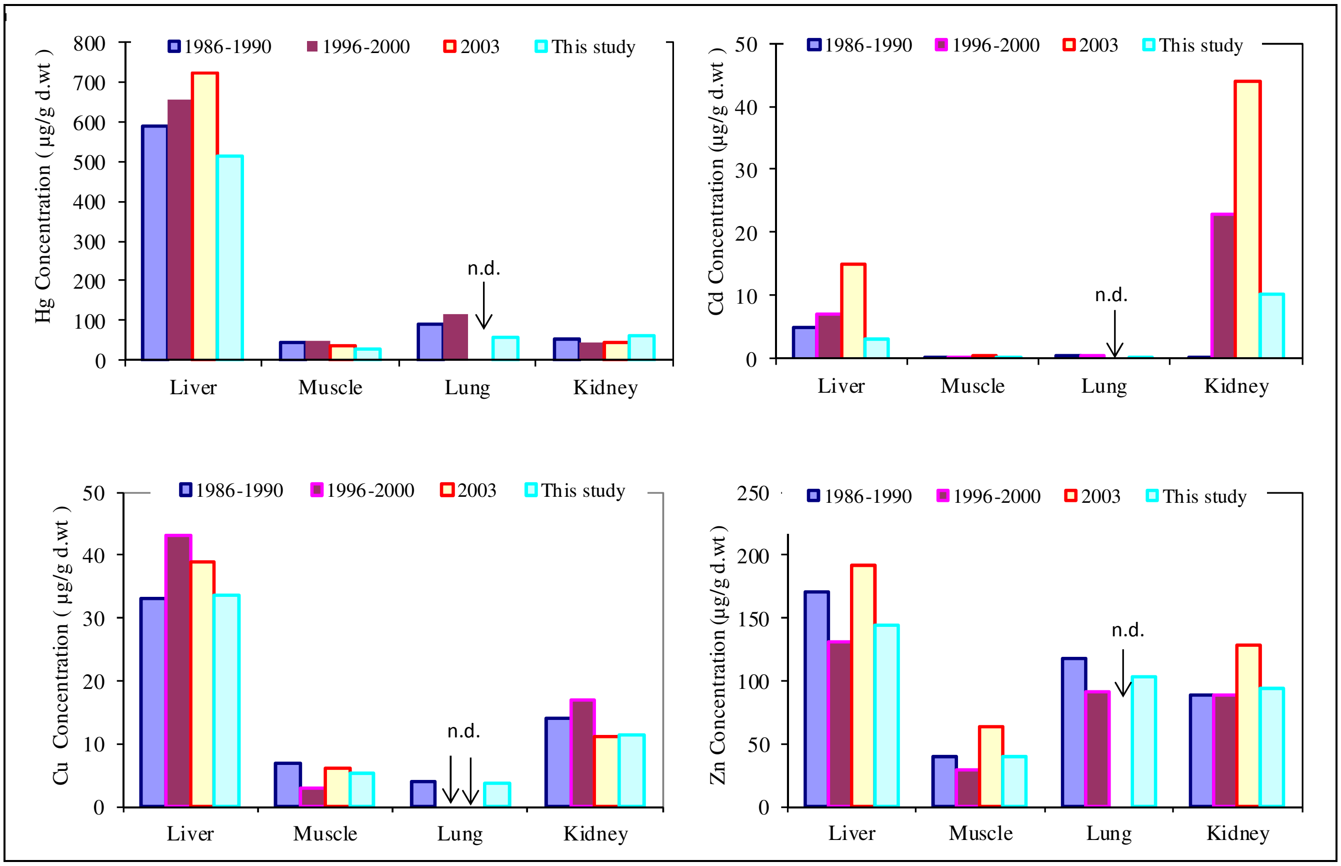

Fig. (11). Mean values for trace elements in the liver, muscle, lung, and kidney of *Stenella coeruleoalba* from the western Mediterranean Sea, by year (n.d. = not determined).

CONCLUSION

In the present study, the levels of contaminants showed normal variation between organs and from one individual to another. In all the samples studied, all the compounds analyzed were in the same range than those found by the other authors, particularly in the case of mercury. In this case, the mean for all liver samples = 514.2 $\mu g.g^{-1}$ d.wt, and were in line with other reports from the same geographical area [7, 22, 30, 45-47].

Among the biological parameters of variability for the dolphins, age (linked to size) and weight appeared to be most important [48]. In the case of mercury and selenium, sex also appeared to be a parameter of significant influence.

The liver was in general the most contaminated organ (the kidney also in the particular case of Cd). Contaminations in the muscle and the lung were generally relatively low. The statistical analysis of the trace element contamination of *St. coeruleoalba* suggested that the female was more contaminated than the male. For Se and Hg (and contrary to Cd-Se; Cd-Hg), contamination level increased significantly with the length and the age of the dolphins, regardless of the tissue analyzed. It is well known, the Hg accumulates with age, accompanied by increase in Se levels *via* formation of Hg-complex [13]. Contamination of juveniles was generally low except in the particular case of the Cu in the liver which could be explained by a transfer of this contaminant from mother to foetus during gestation and lactation. Some relationships between elements Hg-Se were demonstrated in this study which could be linked with detoxification processes. Interaction of Se with Hg is well known [49], and thus Se might detoxify Hg *via* formation of insoluble complexes in the liver of the striped dolphins, because high positive correlation was found between Se and Hg. The Hg/Se molar ratios increased with levels in the livers of dolphins. In this work ratios are often close to 1. Detoxification processes would be achieved by the transformation of methylmercury in inorganic mercury *via* the selenide (HgSe) formation. Regarding relationships between metals, the essential elements Cu and Zn are commonly related to Cd [41], which would suggest induction of metallothionein. In this study, neither Cd-Cu, nor Cd-Zn relationships were significant.

The comparison of our results to previous studies on dolphins from the Mediterranean Sea seems to confirm the reduction or at least stabilized over the last decade of the dolphins' contamination by trace elements over time. However, reducing stable pollutant does not necessarily lead to rapid decrease in concentration in the marine environment, but the trend has already been observed concerning organic contamination by polychlorinated biphenyls and pesticides [50]. These results must be linked with the regulation of elements waste in the Mediterranean pool initiated in 1975 with the "Mediterranean Action Plan" and validated in 1995 with the "Barcelona Convention, 1995" [51].

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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