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Report on research activities carried out for determination of Cadmium and Arsenic in tissues of different cetacean species

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Summary

Concentrations of Cd and As were determined in organs and tissues (muscle, heart, kidney, lung and liver) of two cetacean species (*Stenella coeruleoalba* and *Truncatus truncatus*) stranded along the Italian coasts during the period 2000–2009. Significant differences were found between Cd concentrations in the different analyzed tissues. Particularly, the kidney shows the highest concentrations of Cd in all analyzed specimens, followed by the liver. The heart shows the highest As concentrations with respect to the other tissues analyzed. Specimens of *S. coeruleoalba* show higher Cd concentrations in their tissue than specimens of *T. truncatus*, probably because of a higher proportion of squid in their diet.

Introduction

Trace element concentrations in the marine environment result from natural and/or anthropogenic sources (Law et al., 1996). Trace metals are natural components of the hydrosphere and many are necessary, in minute quantities, for the metabolism of organisms (e.g., arsenic, copper, iron, molybdenum, tin, etc.) (Ward, 1995). However, elevated concentrations in organisms' tissues induce toxicity through interference with enzymatic and metabolic activity (Ward, 1995). The ability of organisms to accumulate metals depends on their rates of uptake, metabolism and excretion. Influential factors include also the diet and the age of the organisms. Dolphins are at the top of the food chain and therefore accumulate metal loads from their prey. The large quantity of prey consumed and the long life span of dolphins further enhance their capacity to accumulate metals (Aguilar et al., 1999). Hence, marine mammals are very sensitive to environmental changes and have been considered good bioindicators of environmental contamination (Bellante et al., 2009). Cadmium and Arsenic are considered two of the most toxic elements to living organisms. However, most of the As in tissues of marine animals is in the form of various organo-arsenic species that are not toxic or carcinogenic to the marine animals themselves (Uthus, 1992) or to their consumers, including man. Therefore, it is not surprising that nearly all marine organisms contain measurable concentrations of inorganic and organic forms of As in their tissues. Marine mammals seem to depress arsenic contents at low levels in muscle and organ tissues and are able to excrete organic As ingested through their diet (Meador et al., 1993). Thus, As concentration, in the kidney and the liver, rarely exceeds 1.0 mg kg^{1} dw (Neff, 2002). However, anthropogenic and natural sources could enhance As concentrations in the environment and potentially influence dynamics of assumption in marine organisms. No data are available for As concentrations in marine mammal tissues from the Mediterranean Sea. Other metals, such as Cd, do not normally participate in metabolism and, at least in top predators, are accumulated throughout the entire life of an individual. While information on the levels of Cd in beached dolphins is available on several coasts, such data are almost non-existent for many Italian coastal areas, such as the Sicily Channel coast. In this study we present a new dataset of Cd and As concentrations in different tissues of two dolphin species (*S. coreuleoalba*, and *T. truncatus*) stranded along the Italian coasts during the period 2000–2009, in order to assess Cd and As distribution patterns in different tissues of cetaceans, particularly exploring potential bioaccumulation modes in the organs of the studied organisms.

Material and methods

Samples of muscle, liver, lung, kidney and heart were collected from specimens of striped dolphin (*S. coeruleoalba*, n=12) and bottlenose dolphin (*T. truncatus*, n=12) that were found stranded along Italian coasts during the period 2000–2009 (Table 1, Figure 1). Samples were wrapped in plastic bags and immediately stored at 20 C after collection. Plastic bags were acid-washed before use to prevent metal leakage. The total length of each specimen was measured before sample collection. Samples were dried at 60 C for 48 h and homogenized in an agate mortar. About 0.25 g of each air-dried and homogenized sample were digested under pressure in 10 ml of ultra-grade HNO_3 in Teflon liners using a microwave oven CEM MARS-5 (Figure 2) for 4h at 200 W and at T=160 C. Samples were prepared and analyzed with great caution to minimize contamination from air, glassware and reagents, all of which were of Suprapur quality. Metal concentrations were measured by ICP-AES Varian Vista MPX (Figure 2). Analyses were carried out by external calibration using standards in the same acid matrix of samples, prepared by dilution of ICP-MS High-Purity Standard Solutions. Reagent blanks and duplicated samples (about 10% of the total number of samples) were used to monitor appropriateness and reproducibility of preparation and analytical procedures. A reference standard material, Tort-2 (National Research Council of Canada), was used to assess the accuracy (estimated between 85 and 93%) and precision (ranging between 3 and 5%, RSD; n=10) of analyses. Results of quality control show an excellent agreement with certified data (Cd certified value: 0.2 ± 0.06 mg kg^{1} dw, Cd found value: 0.2 ± 0.07 mg kg^{1} dw; As certified value: 21.6 ± 1.8 mg kg^{1} dw, As found value: 20.4 ± 3.0 mg kg^{1} dw). All the individual data are calculated as an average of 3 duplicates. The analytical precision, measured as relative standard deviation, was routinely between 5 and 6%, and never higher than 10%. All results were calculated with respect to dry weight (dw). Detection limits of metal measurements were attested at 0.5 mg kg^{1} dw for As and 0.1 mg kg^{1} dw for Cd.

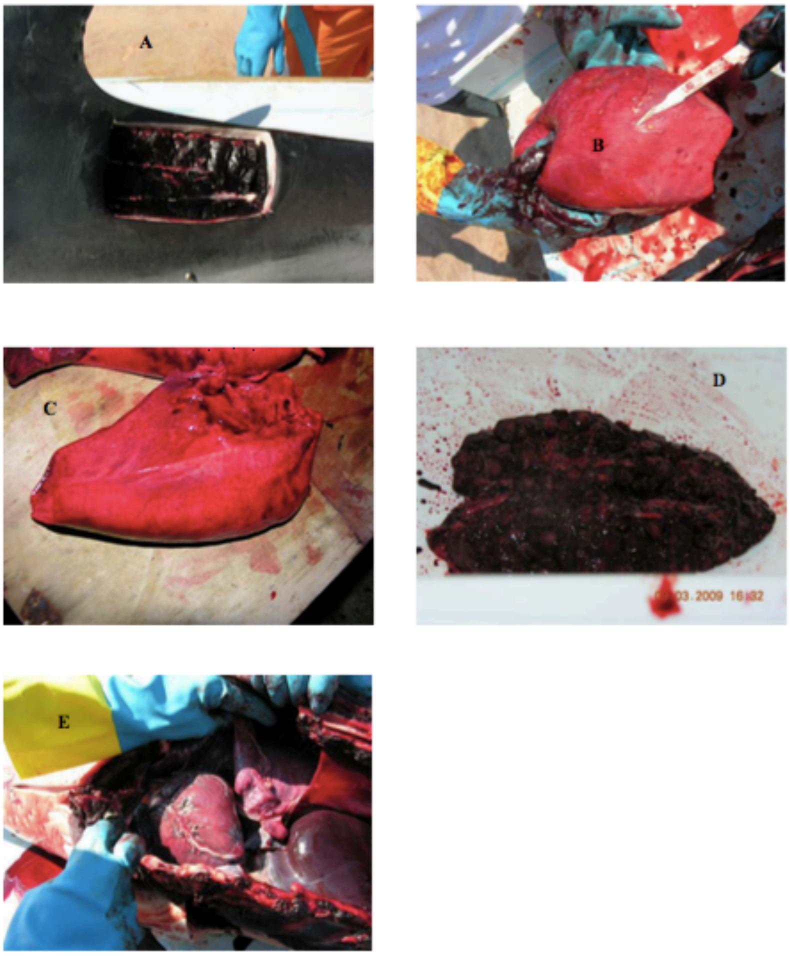
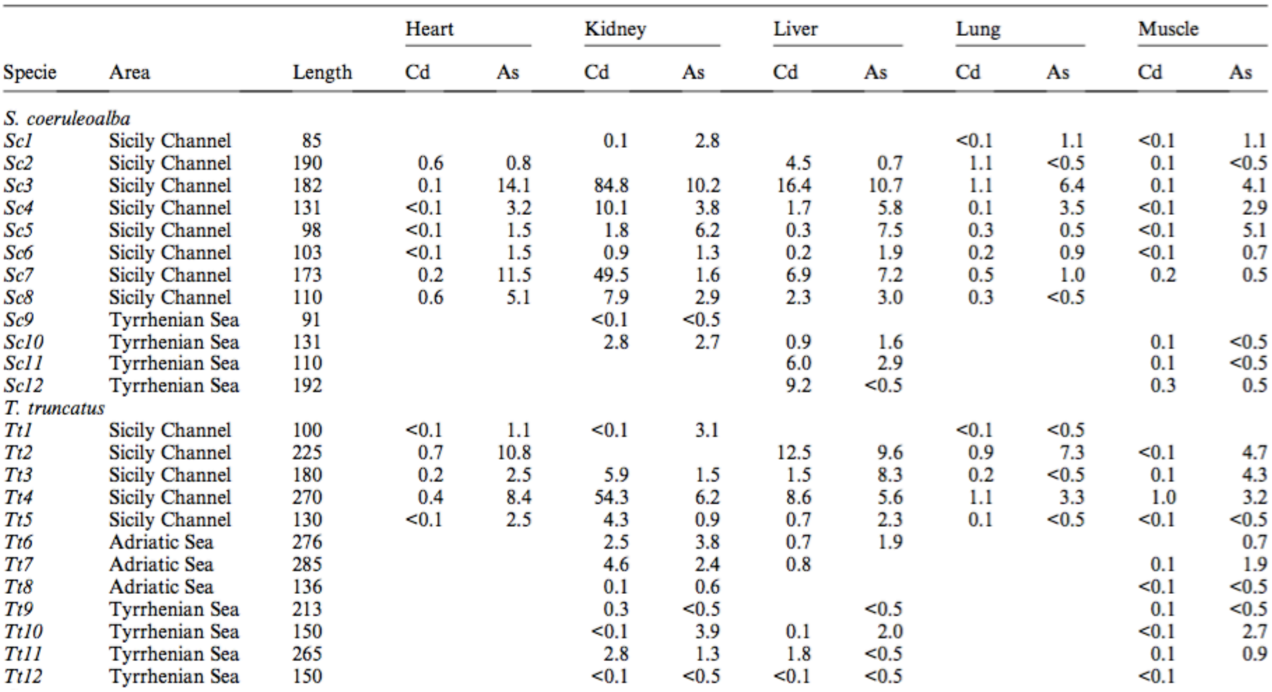


Figure 1: Sample collection of muscle (A), liver (B), lung (C), kidney (D) and heart (E) from cetacean body.

Figure 2: microwave oven CEM MARS-5 and ICP-AES Varian Vista MPX used for Cd and As determination.

Results and discussions

Cadmium and arsenic concentrations in tissues of the analyzed species are shown in Table 1.



			Heart		Kidney		Liver		Lung		Muscle	
Specie	Area	Length	Cd	As	Cd	As	Cd	As	Cd	As	Cd	As
S. coeruleoalba												
Sc1	Sicily Channel	85			0.1	2.8			<0.1	1.1	<0.1	1.1
Sc2	Sicily Channel	190	0.6	0.8			4.5	0.7	1.1	<0.5	0.1	<0.5
Sc3	Sicily Channel	182	0.1	14.1	84.8	10.2	16.4	10.7	1.1	6.4	0.1	4.1
Sc4	Sicily Channel	131	<0.1	3.2	10.1	3.8	1.7	5.8	0.1	3.5	<0.1	2.9
Sc5	Sicily Channel	98	<0.1	1.5	1.8	6.2	0.3	7.5	0.3	0.5	<0.1	5.1
Sc6	Sicily Channel	103	<0.1	1.5	0.9	1.3	0.2	1.9	0.2	0.9	<0.1	0.7
Sc7	Sicily Channel	173	0.2	11.5	49.5	1.6	6.9	7.2	0.5	1.0	0.2	0.5
Sc8	Sicily Channel	110	0.6	5.1	7.9	2.9	2.3	3.0	0.3	<0.5		
Sc9	Tyrrhenian Sea	91			<0.1	<0.5						
Sc10	Tyrrhenian Sea	131			2.8	2.7	0.9	1.6			0.1	<0.5
Sc11	Tyrrhenian Sea	110					6.0	2.9			0.1	<0.5
Sc12	Tyrrhenian Sea	192					9.2	<0.5			0.3	0.5
T. truncatus												
Tt1	Sicily Channel	100	<0.1	1.1	<0.1	3.1			<0.1	<0.5		
Tt2	Sicily Channel	225	0.7	10.8			12.5	9.6	0.9	7.3	<0.1	4.7
Tt3	Sicily Channel	180	0.2	2.5	5.9	1.5	1.5	8.3	0.2	<0.5	0.1	4.3
Tt4	Sicily Channel	270	0.4	8.4	54.3	6.2	8.6	5.6	1.1	3.3	1.0	3.2
Tt5	Sicily Channel	130	<0.1	2.5	4.3	0.9	0.7	2.3	0.1	<0.5	<0.1	<0.5
Tt6	Adriatic Sea	276			2.5	3.8	0.7	1.9				0.7
Tt7	Adriatic Sea	285			4.6	2.4	0.8				0.1	1.9
Tt8	Adriatic Sea	136			0.1	0.6					<0.1	<0.5
Tt9	Tyrrhenian Sea	213			0.3	<0.5		<0.5			0.1	<0.5
Tt10	Tyrrhenian Sea	150			<0.1	3.9	0.1	2.0			<0.1	2.7
Tt11	Tyrrhenian Sea	265			2.8	1.3	1.8	<0.5			0.1	0.9
Tt12	Tyrrhenian Sea	150			<0.1	<0.5	<0.1	<0.5			<0.1	

Table 1: Sites of stranding, Specie, Area, Length (cm) and Cd and As concentrations in the analyzed tissues (mg kg ^{1} dw)

Cadmium shows a wide range of concentrations in kidney samples (<0.1 to 84.8 mg kg^{-1} dw for *S. coeruleoalba* and <0.1 to 54.3 mg kg^{-1} dw for *T. truncatus*). Narrower ranges of concentrations were found in the other organs. As shown in Figure 3, significant differences were found between Cd concentrations in the different tissues for both species. Particularly, the kidney shows the highest concentrations of Cd in all analyzed specimens followed by the liver. Low values of Cd were found in heart, lung and muscle samples. Positive correlation was found between Cd concentrations and length in all tissues of *S. coeruleoalba* and *T. truncatus*. Thus, the over-time storage of Cd confirm that this toxic metal cannot be excreted in all tissues of these specie. A narrow range of As concentrations (<0.5 to 14.1 mg kg^{-1} dw for *S. coeruleoalba*; <0.5 to 10.8 mg kg^{-1} dw for *T. truncatus*) for all tissues is evident. The highest values of As were recorded in heart tissue followed by liver samples (Figure 1). Positive significant correlation with length emerges only for As in heart samples ($R = 0.9$). No statistically reliable ($p < 0.05$ for all the estimated Spearman correlation coefficients) differential accumulation pattern with length emerges for As in the other tissues. This suggests that these tissues perform an excretory function for this toxic metal. Further analyses are required to definitively assess preferential As accumulation in heart. As shown in Table 1, Cd seems to be more concentrated in tissues of the species *S. coeruleoalba*. Dolphins accumulate Cd mainly through the diet. It is well known that squids are toxic metal accumulators and a source of Cd to their predators (Koyama et al., 2000). So the higher Cd concentrations found in tissues of *S. coeruleoalba* can be explained by different relative diet compositions with a great proportion of squids. No clear difference in As contents was detected between the two species. The lack of statistical difference between As concentration in the same organs of *S. coeruleoalba* and *T. truncatus* specimens suggests undifferentiated dynamics of physiological and metabolic pathways which control arsenic incorporation in the cetacean body. This study offers the first survey of As distribution in cetaceans from the Mediterranean Sea.

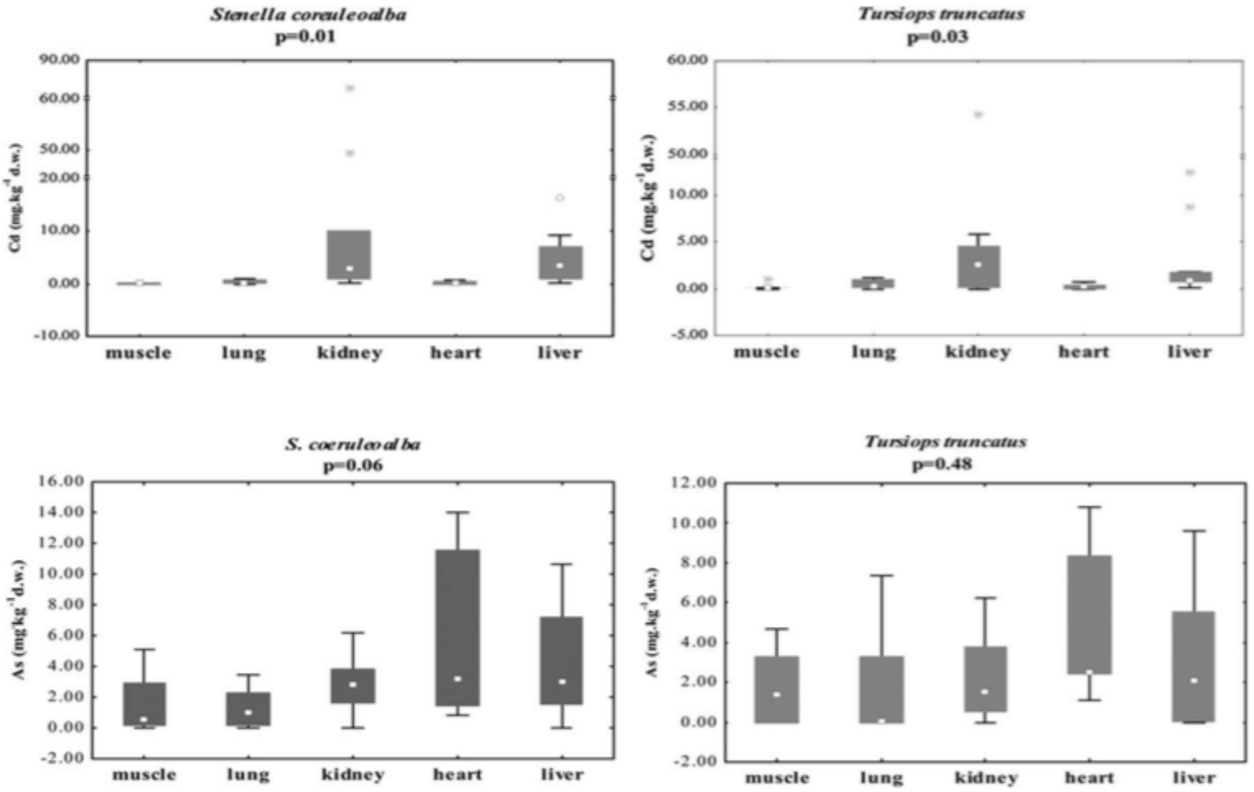


Figure 3: Cd and As distribution in the different tissues of *S. coeruleoalba* and *T. truncatus*

References

Aguilar A., Borrell A., Pastor T. Biological factors affecting the variability of persistent pollutant concentrations in cetaceans, J. Cetacean Res. Manage., 1999, 1, 83–116.

Bellante A., Sprovieri M., Buscaino G., Salvagio Manta D., Buffa G., Di Stefano V., Bonanno A., Barra M., Patti B., Giacoma C., Mazzola S. Trace elements and vanadium in tissues and organs of five species of cetaceans from Italian coasts, Chem. Ecol., 2009, 25, 311–323.

Koyama J., Nanamori N., Segawa S. Bioaccumulation of waterborne and dietary cadmium by oval squid, Sepioteuthis lessoniana, and its distribution among organs, Mar. Pollut. Bull., 2000, 40, 961–967.

Law, R.J. Metals in marine mammals, in Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations, ed. W. N. Beyer, G. H.Heinz and A. W.Redmon-Norwood, CRC Press, Boca Raton, FL, 1996, pp. 357–376.

Meador J.P., Varanasi U., Robisch P.A., Chan S.L. Toxic metals in pilot whales (Globicephala melaena) from strandings in 1986 and 1990 on Cape Cod, Massachusetts, Can. J. Fish. Aquat. Sci., 1993, 50, 2698–2706.

Neff J.M. Bioaccumulation in Marine Organisms: Effect of Contaminants from Oil Well Produced Water, Elsevier, Oxford, 1st edn, 2002.

Uthus E.O. Evidence for arsenic essentiality, Environ. Geochem. Health, 1992, 14, 55–58.

Ward N.I. Environmental Analytical Chemistry, ed. F. W. Fifield and P. J. Haines, Chapman and Hall, London: Blackie Academic and Professional, 1995.