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Heavy Metals in Hair Samples of the Mediterranean Monk Seal (*Monachus monachus*)

A. YEDILER*‡, A. PANOU† and P. SCHRAMEL*
**GSF-Forschungszentrum für Umwelt und Gesundheit GmbH, Institut für Ökologische Chemie, Ingolstädter Landstr. 1, D-8042 Neuherberg, Germany*
†Zoologisches Institut, Universität München, Seidlstr. 25, D-8000 München 2, Germany
‡To whom correspondence should be addressed.

For the first time the concentrations of copper, zinc, mercury, cadmium and lead in hair samples of the Mediterranean Monk Seal, one of the world's rarest mammals, were determined. The hair samples were collected in the absence of the animals from six caves located in the Ionian Sea, Greece. Our data suggest the importance of hair analysis as a useful indicator for heavy metals in these rare animals.

The Mediterranean Monk Seal (*Monachus monachus*, Hermann, 1779) has been classified by the Species Survival Commission of the World Conservation Union (IUCN) as strongly endangered since 1966. The estimated (Ronald & Duguy, 1984; Berkes, 1982; Mursaloglu, 1984) population size is now most likely less than 600. The main causes for continuing decline are probably decreased natality due to human disturbance (tourists, increased coastal popoulation), increased mortality due to deliberate killing (mainly by fishermen), entanglement in nets, and dynamite fishing. There is also a general loss of habitat with detrimental consequences for reproduction and survival (Ronald & Duguy, 1984; Sergeant *et al.*, 1978; Berkes *et al.*, 1979).

Significance of food shortage through overfishing and pollution is difficult to evaluate but they may contribute to decreased natality as well. Therefore attempts should be made to find out the influence of environmental pollution to the vitality of these rare animals.

Apparently organochlorines affect seal physiology, reducing the carrying capacity of the environment (Reijnders, 1980). Marine mammals, at the top of the food chain, are important indicator organisms of pollution in marine ecosystems. Contamination levels are best determined through direct analysis of organ tissues but this method is unsuitable for routine monitoring purposes in a rare and highly endangered species. Therefore in such cases interest should be focused on the analysis of hair samples (Airey, 1983; Paschal *et al.*, 1989; Takagi *et al.*, 1986; Wibowo *et al.*, 1986) as a nondisturbing way of sampling. The diagnostic value of hair samples, especially for human hair, is well established (Airey, 1983; Chatt & Katz, 1988; Petering *et al.*, 1971; Phelps *et al.*, 1980; Wilhelm *et al.*, 1991), but it is almost impossible from heavy metal analysis of hair to differentiate the endogenous from the exogenous contributions.

Very few studies on heavy metal accumulation in seal hair exist. Data on distribution of mercury in the tissues of four species of seals from individual localities in eastern Canada, in the marine food web, and in hair samples of two seal species (Harbour seal, *Phoca vitulina* and Hood seal, *Cystophora cristata*) have been reported by Sergeant and Armstrong (1973). Mercury concentrations in liver and hair samples increases with age and seem to be positively correlated. They also reported that the tissue concentrations for mercury vary with the position of the eaten organisms in the marine food web. In further investigations Sergeant *et al.* (unpublished data from 1974–1976) analysed levels of mercury in the hair of four Mediterranean Monk Seals from Turkey, and two from Madeira (Table 2). Further data are reported by Hyvärinen & Sipilä (1984). To find out the possible reason for the decrease of the ringed seal population, the authors measured concentrations of mercury, nickel, cadmium, lead and chromium in hair samples of Saimaa Ringed Seals (Lake Saimaa, Finland).

In the present study hair samples of the Mediterranean Monk Seal have been used to determine heavy metals (Cu, Zn, Hg, Cd, Pb).

Materials and Methods

Since the animals are so rare, and any kind of disturbance should be avoided, the hair samples were collected in the absence of the animals. Therefore we are not able to give information about the number of seals or the ages, sex, size of the seals in the sampling locations. Three hair samples were collected during the moulting period of the animals from four caves located in the northeastern part of Kefalonia Island and three in the southwestern part of Zakynthos Island in the Ionian Sea, Greece. The sampling sites are about 35 nautical miles apart. Samples from Kefalonia were collected in April 1986, March 1987, and March 1991. Samples from Zakynthos were collected in January–February 1989, and May 1991.

Each sample was washed with 0.1% detergent rinsed three times with deionized water, allowed to air dry for 12 h, and sealed in a clean plastic bag. Triplicates of each hair sample (0.1 g) were digested at 160°C for 12 h with 1 ml nitric acid using a teflon lined, high pressure decomposition vessel. After digestion Cu and Zn were analysed by inductively coupled plasma emission spectroscopy (Instruments S.A., JY-38+). Pb and Cd were analysed by atomic absorption spectrometer (Perkin Elmer, Zeemann/3030). Hg was determined by cold vapour atomic absorption spectroscopy (Perkin Elmer 2380).

Results and Discussion

The mean concentrations and the ranges from the triplicate measurements of the hair samples are shown in Table 1. Except for the sample no. 5 (cave no. 5) from Zakynthos the mean values for Cu, Zn, Hg, Cd, Pb are surprisingly homogeneous. This could indicate a homogeneously heavy metal distribution in the habitat of the species or else an endogenous regulation.

The copper and zinc concentrations are in the range from 9.93 to 20.4 μg Cu g^{-1}, and from 100 to 170 μg Zn g^{-1}. No literature data found for Cu and Zn in seal hair. However, our data are similar to Cu and Zn levels in human hair (Eads & Lambdin, 1973; Jamall & Jaffer, 1987; Wilhelm & Ohnesorg, 1990). This could be due to the fact that both in seals and humans Cu and Zn are regulated in organs as well as in the hair (Wagemann *et al.*, 1988; Wagemann, 1989).

The mercury levels range from 5 to 54 μg g^{-1} dry wt. Table 2 shows comparable high mercury concentration in one hair sample of monk seal from Alanya, Turkey (Berkes, sample from 1975), and in hair samples of a Saimaa ringed seal at an age of 2 yr or older (Hyvärinen & Sipilä, 1984).

Marine fish and invertebrates are probably the main source of heavy metals for the Mediterranean Monk Seal. In the present study, the small sample size and

TABLE 1

Means and ranges* of heavy metals in the hair of the Mediterranean Monk Seal from Kefalonia (K)- and Zakynthos (Z)-Island, Greece.

Samples	Cu	Hg	Zn	Cd	Pb
	μg g^{-1} dry wt			ng g^{-1} dry wt	
Cave 1^K (1986)	11.1 (11–11.4)	45.9 (44–48)	117.0 (109–122)	118.7 (111–130)	764.7 (672–865)
Cave 2^K (1987)	11.8 (11.4–12)	34.8 (32–38)	142.0 (139–146)	11.5 (9–14)	761.0 (693–988)
Cave 3^K (1991)	9.93 (9.4–10.1)	25.3 (25–26)	158.5 (150–167)	190.0 (165–230)	582.0 (365–799)
Cave 4^Z (1989)	10.5 (9.8–11.1)	54.3 (52–58)	109.0 (102–114)	198.7 (110–237)	767.0 (676–857)
Cave 5^Z (1989)	20.4 (20.1–21)	5.2 (4.8–6)	116.0 (112–119)	363.0 (326–434)	1420.0 (1310–1520)
Cave $6a^K$† (1991)	n.m.‡	20.3 (20–21)	127.0 (126–128)	230.0 (218–241)	800.0 (498–1170)
Cave $6b^K$ (1991)	n.m.	20.6 (19–22)	132.5 (129–136)	212.0 (202–222)	500.0 (430–628)
Means (SD)	12.6 ±4.3	22.43 ±15.4	128.9 ±17.2	202.56 ±83.9	784.67 ±310.6

*Triplicate analyses. †Same location, different resting places (a & b). ‡Not measured.

TABLE 2

Total mercury concentrations (μg g^{-1} dry wt) in the hair of Mediterranean monk seal from different sites, and in the hair of one Saimaa ringed seal, Finland.

Country	Locality	Body length	Year	Hg	Sampling
Greece	Kefalonia	Unknown	1986	45.9	A. Panou
	Kefalonia	Unknown	1987	34.8	
	Kefalonia	Unknown	1991	25.3	
	Zakynthos	Unknown	1989	54.3	A. Vlachoutsikou
	Zakynthos	Unknown	1989	5.2	
	Zakynthos	Unknown	1991	20.5	
Portugal	Madeira	238 cm	1957	7.0	G. Maul
	Madeira	235 cm	1961	7.5	
Turkey	Alanya	170 cm	1974	5.2	F. Berkes
	Alanya	188 cm	1975	27.5	
	Alanya	140 cm	1975	5.5	
	Bozyazi	90 cm	1976	6.9	
Finland	Lake Saimaa	Unknown	1984	20.7	Hyvärinen & Sipilä

small amount of background information regarding the population of the monk seal weakens, however, any attempt to draw firm conclusions from our few data. The data of Hyvärinen & Sipilä (1984) suggest that in older seals mercury concentrations are higher. In this respect the sample no. 5 from Zakynthos Island could belong to a young animal. Madeira in the Atlantic ocean does not seem to be influenced by industrialization. The low values of Hg in seals may be explained by low levels in the Atlantic.

Attempts have been made to correlate Hg levels in human hair to the diet. High human blood mercury concentrations, depending on the amount of seal eaten by Greenlanders, and an estimated hair/blood ratio to 289 have been reported by Hansen *et al.* (1983). Airey (1983) found a positive correlation between mercury concentration in hair and the consumption of fish contaminated with mercury. Kitamura *et al.* (1976) reported high concentrations of mercury in the urine, brain, liver, kidney and hair of patients suffering from Minamata disease, as well as in the fish and shellfish from Minamata Bay and in the mud from its bottom.

Concentrations of cadmium and lead were less than 1 $\mu g\ g^{-1}$ (except Pb in sample no. 5), which should be considered to be rather low. Hyvärinen & Sipilä (1984) reported a mean lead concentration of 6 $\mu g\ g^{-1}$ in hair samples of still-born pups and adults of 1–2 yr old Saimaa Ringed Seals. No significant differences were found for Cd and Pb in different age classes. It is known that Pb accumulates in human hair and blood depending on levels of external exposure (Hansen *et al.*, 1983; Jamall & Allen, 1990; Yongxian *et al.*, 1990).

There are no data from the Mediterranean region about the influence of diet, age, body size, or pollution on the heavy metal concentrations in seals. Data from seals in Canada (Sergeant & Armstrong, 1973), in England (Heppleston & French, 1973) and the Netherlands (Van de Ven & Koeman, 1979) suggest that the tissue concentrations of mercury increase with age, and are most pronounced in the liver. An increase with age of the cadmium, zinc and mercury in livers and kidneys of harbour seals (*Phoca vitulina*) was also reported by Tohyama *et al.* (1986). However, it is not known to what extent the environmental contamination with heavy metals (especially with mercury) might contribute to the vitality of the animals.

Our data can only give a first hint of heavy metal burden of Mediterranean Monk Seals. A continuous monitoring of contamination levels, and correlations with known sources of pollution might show that hair samples are a useful indicator of the heavy metal burden of these rare species. Presently it is unknown whether the seemingly inadvertant decline of the population could be connected to heavy metal contamination. Larger sample sizes and comparative data from polluted and unpolluted regions might help to assess the possible contribution of pollution to the vitality of Monk Seals.

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Heavy Metals and Organochlorine Residues in Ganges River Dolphins from India

K. KANNAN*, R. K. SINHA†, S. TANABE*‡, H. ICHIHASHI* and R. TATSUKAWA*
**Department of Environment Conservation, Ehime University, Tarumi 3-5-7, Matsuyama 790, Japan*
†Environmental Biology Laboratory, Department of Zoology, Patna University, Patna, 800 005, India
‡To whom correspondence should be addressed.

The Ganges River dolphin, *Platanista gangetica*, is predominantly found in the Ganges River and its tributaries in India. The Ganges is heavily polluted by the annual usage and discharge of about 2500 t of pesticides and 1.2 million t of fertilizers in its catchment area (Mohan, 1989). Despite this, little is known about the concentrations of toxic micropollutants such as organochlorines and heavy metals in the Ganges ecosystem. Since small cetaceans in marine environments lack the ability to metabolize persistent organochlorines, they are reported to be at greater risk from environmental contamination by organochlorine chemicals (Tanabe *et al.*, 1988). It is of particular interest to examine whether freshwater dolphins exhibit a specific accumulation pattern of persistent pollutants and are thus at risk. A recent survey documented declining trends in the populations of river dolphins, including the Ganges dolphin (Mohan, 1989). The monitoring of contaminant levels in river dolphins may therefore be an important aspect of their conservation.

The present study provides information on the levels of major organochlorines and heavy metals in Ganges River dolphins collected from Patna (25°N, 85°E), India. Four specimens found entangled in fishing nets or drowned were used for analysis. Blubber, muscle, kidney and liver tissues were obtained from animals collected during January 1988–March 1992; these were preserved in 10% formalin and stored at 4°C until analysis. Data on sex, length and age of the individuals studied are shown in Table 1. According to Kasuya (1972), the length of the Ganges dolphin at the time of birth is 70 cm and attains 199 cm at 28 yr. The age determinations for dolphins collected in this study were based on the above estimates. We also collected the gut contents of the dolphins studied, to analyse the contaminant levels in their diet. The diet consisted mainly of fish living in muddy environments, such as *Chela laubuca*, *Colisa fasciatus*, *Mastacembelus pancalus* and *Puntius sophore* (the length of each fish was less than 6 cm). All four species of fish were pooled to obtain sufficient quantities of material for chemical analysis.

For heavy metal analysis, the samples were dried for 12 h at 80°C and the dried samples of about 0.5 to 1.0 g each were digested in a mixture of nitric, sulphuric and perchloric acid to produce a transparent solution. These solutions were diluted to 50 ml with distilled water and the concentrations of Fe, Mn and Zn were measured directly by atomic absorption spectrophotometry (Shimadzu AA-680 atomic absorption/flame emission spectrophotometer connected to a Shimadzu PR-5 graphic printer). Levels of Cu, Pb, Ni, and Cd were measured after diethyl dithiocarbamate–methyl isobutyl ketone extraction (Honda *et al.*, 1982).

The determination of organochlorine pesticides and PCBs in dolphin tissues and their fish diet followed the procedure of Tanabe *et al.* (1991). The method consists of Soxhlet extraction, florisil dry column chromato-

TABLE 1

Details of the samples analysed. See text for comments on age estimates.

Specimen no.	Sex	Growth stage	Length (cm)	Age (yr)	Collection date
1	Male	Neonatal	70.4	Few days	24 January 1988
2	Male	Immature	104	1.0	6 October 1991
3	Female	Immature	115	1.3	21 July 1991
4	Female	Adult	233	30	27 March 1992