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Environmental Contamination and Toxicology

Heavy Metals in Tissues of Gray Whales *Eschrichtius robustus*, and in Sediments of Ojo de Liebre Lagoon in Mexico

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A significant die-off in the population of gray whales *Eschrichtius robustus* during 1999 has generated several hypotheses in trying to explain this phenomenon (e.g. Le Boeuf et al. 2000); among these hypotheses, chemical pollution has been mentioned as a possible cause of the occurrence of this event. Previously Tilbury et al. (1997) stated that chemical contamination might play a significant role on the presence of cetacean strandings. Along the Mexican coast, this higher than usual mortality during 1999 was even higher than other places along the migration corridor (Le Boeuf et al. 2000). Furthermore, during this particular year, an atypical behavior of the gray whales feeding occurred, they fed in unusual places along the southern part of the migration corridor. This behavior was recorded inside their breeding grounds (Le Boeuf et al. 2000), where traditionally gray whales do not feed while migrating southwards (Rice et al. 1984). Gray whales have a unique feeding strategy among mysticetes, because they mainly obtain their prey by filtering sediments, and eventually, they ingest the sediments along with their prey. Feeding of sediments close to the coast may expose the whales to different compounds as persistent organic pollutants and heavy metals (Varanasi et al. 1994). In order to diagnose metal toxic levels as a potential cause of illness or death of stranded gray whales, it is necessary not only to evaluate the metal levels in different parts of the whales' organism, but also in their surrounding environment.

Gray whales migrate every year from their feeding grounds, mainly in the Chukchi, Beaufort, and Bering seas in the Arctic, to their breeding grounds in the lagoons of the Baja California Peninsula in Mexico. Among these lagoons, it is in the Ojo de Liebre Lagoon where almost 50% of the population of gray whales are born (Rice et al. 1984). Ojo de Liebre Lagoon is located in the west coast of the Baja California Peninsula in Mexico, forming part of the Ojo de Liebre Lagoon Complex -along with Manuela and Guerrero Negro lagoons- (Fig. 1); it is located inside Sebastian Vizcaíno Bay. Ojo de Liebre is the largest among these lagoons, with 48 km length in its central part, a maximum depth of 24.5 m, and a surface of 571 km^2. Inside the lagoon there is a complex channel system, of which only 171 km^2 have a depth of more than 3 m, suitable for whale navigation (Sánchez 1990). The lagoon is located in a desert area with very dry and warm climate, with average annual temperature of 22°C, and winter precipitation of less than 36 mm, and there is no fresh water influx into the lagoon. The sediments of the lagoon are mainly due to a sedimentary transport by the California Current that washes the west coast of the peninsula and eventually reaches the lagoon (Carranza-Edwards et al. 1998). In 1988 the Mexican government protected the area, and in 1993 UNESCO declared it part of the world human heritage. However, in the

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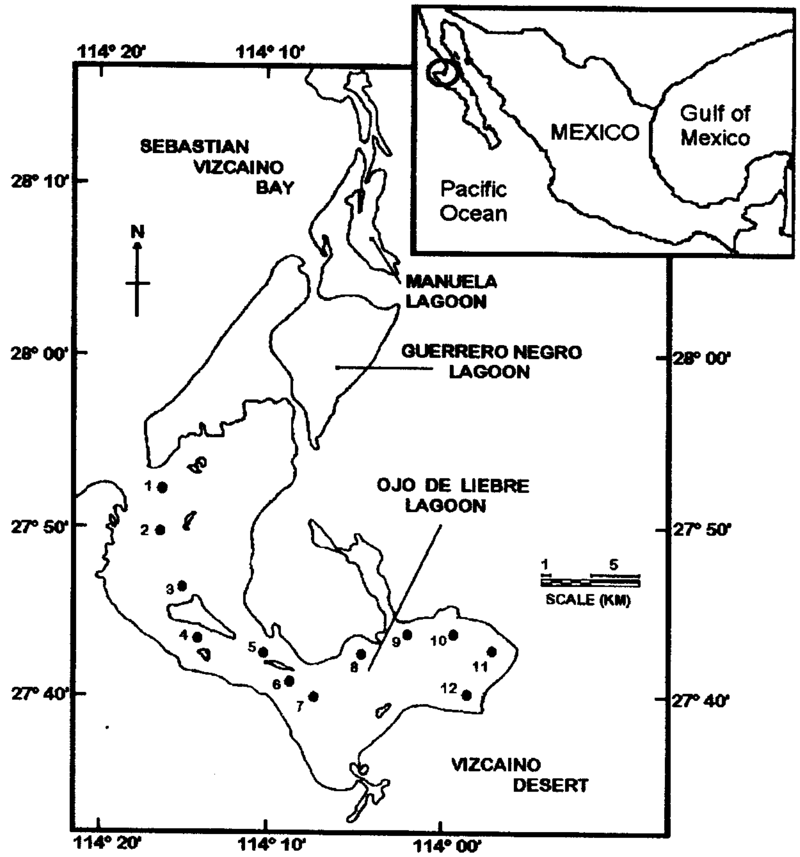

Figure 1. Localization of Ojo de Liebre Lagoon and the sampling sites.

eastern part of the lagoon millions of cubic meters of the lagoon seawater are pumped to concentrator and crystallizator ponds for industrial salt production. In the present study the physicochemical characteristics of the Ojo de Liebre Lagoon sediments were undertaken in order to obtain information of the natural environment where whales death by stranding takes place. The concentrations of heavy metals in tissues of gray whales stranded inside the lagoon were measured, and compared with metal concentration in the sediments of the lagoon.

MATERIALS AND METHODS

Sediment samples were collected manually using PVC tubes at 12 different sites inside the Ojo de Liebre Lagoon during the 2000 gray whale breeding season (Fig. 1), placed in plastic bags, and kept at 4°C until analysis. Sediments were dried at 110°C for 24 hours, homogenized and analyzed by X-ray Fluorescence Spectrometry in a Siemens sequential spectrometer SRS 3000. Two geochemical reference international samples (WS-E and PM-S) were used to estimate the accuracy of the method. The percentage of recovery was ±10% for all the elements studied (Zn: -12%), according to Verma et al. (1996) this is considered as acceptable.

Tissues from gray whales were collected during the 1999 gray whale breeding season. A total of eight gray whales were found dead inside the lagoon. The carcasses were measured with a metric tape to classify them following the criteria used by Sanchez (1998); calves, less than 600 cm in length; juveniles, length from 601 to 900 cm; adults, more than 901 cm. Samples of bone were collected cutting off from the right fin the cranial portion of a phalange. Samples were collected in sealed plastic bags and kept at 4°C while transporting, in the laboratory they were stored at –18°C until analysis. Tissue samples were dried at 60°C for 48 hours, homogenized, and 1g of sample was extracted with 10mL of HNO3 in a microwave oven (CEM 1994). Metal concentrations were measured in an Atomic Absorption Spectrophotometer-Graphite Furnace for Zn, Fe, Mn, and Pb; and in a hydride generator for Se and As. The accuracy of the methods was measured using certified reference material CRM DOLT-2 (Canadian National Research Council). Accuracy of recovery was Zn: -8.0 %, Fe: -4.4%, Mn: -5.0%, Pb: -6.5%, As: +4.0%, Se: +8.0%.

RESULTS AND DISCUSSION

Superficial sediments consist mainly of fine and very fine sand; only one sampling point (11) was constituted by very coarse sand. Organic carbon was low (0.1-0.5%) and carbonates ranged from 2.7-6.7%. An exception was sample 11 that had 1% and 75% of organic carbon and carbonates respectively (Table 1). Sample 11 showed a different chemical composition mainly due to the abundant presence of shell debris of bivalves; therefore it was not considered for the statistical analyses. The high correlation found ($p < 0.05$) between Al_2O_3 and SiO_2 (0.97), Na_2O (0.98), and K_2O (0.9) suggest that they are present as aluminosilicates; and the high correlation between Fe_2O_3 and TiO2 (0.8), MnO (0.8), Cr (0.8), and V (0.8) suggest the presence of heavy minerals with Cr and V associated. Heavy minerals are more abundant, apparently, in sampling site 8 that presents higher Zn, V, and Cr concentrations. A comparison of the chemical composition of the lagoon sediments with continental soils shows similar concentrations of SiO_2, MgO, K_2O and P_2O_5; and lower concentrations of Al_2O_3, Fe_2O_3, TiO_2, MnO and trace metals; this suggests natural values (Wedepohl 1991). Bellucci et al. (2002) reported higher concentrations of Zn and Pb (101-8295 and 21-929 $\mu g\ g^{-1}$) for the Venice Lagoon in Italy. Evans et al. (2003) found similar results on the concentrations of Cr, Ni, V, and Cu (42, 23, 69, and 35 $\mu g\ g^{-1}$ respectively) and higher values of Zn and Pb (116 and 95 $\mu g\ g^{-1}$) in the sediments of Ensenada de San Simon, Spain. Shumilin et al. (2001) also found similar concentrations of Zn and Cu (2.7-125 and 2.5-30.4 $\mu g\ g^{-1}$) in the sediments of La Paz Bay in Mexico.

Trace metals were analyzed in eight stranded whales (2 calves, 3 juveniles, and 3 adults). Carcasses were located within the area of sampling points 10, 11, 12 except one located close to sampling point 5. Concentrations of Zn, Fe, Mn, Pb, Se, and As were undertaken in different gray whale tissues; the results are shown in figure 2. Concentrations reported here are in dry weight. Higher Zn concentrations were observed in the kidney from a calf (57 $\mu g\ g^{-1}$), it is similar to the value reported by Thompson (1992) for the kidney of the northern bottlenose whale *Hyperoodon ampullatus* (54 $\mu g\ g^{-1}$) and the striped dolphin *Stenella coeruleoalba* (28-82 $\mu g\ g^{-1}$). The kidney has been reported to be, together with liver, one of the major reservoirs of Zn in cetaceans (Fujise et al. 1988, Varanasi et al. 1994). Zn concentrations in skin and muscle were lower in tissues from calves (Figure 2). Fe concentrations were higher in tissues from calves suggesting transport via the placenta as reported previously by

Table 1. Chemical composition of sediments of Ojo de Liebre Lagoon.

Site	SiO_2*	Al_2O_3*	Fe_2O_3*	TiO_2*	MnO*	MgO*	CaO*	Na_2O*	K_2O*	P_2O_5*
1	74.0	13.7	0.6	0.1	0.01	0.3	4.5	4.1	1.3	0.3
2	71.0	14.4	1.8	0.5	0.06	1.0	4.6	4.2	1.5	0.3
3	70.6	13.8	1.5	0.4	0.05	0.8	5.1	4.1	1.5	0.3
4	70.5	13.9	2.4	0.8	0.08	1.2	4.6	4.0	1.3	0.3
5	74.3	13.1	1.1	0.4	0.04	0.7	3.8	4.0	1.5	0.4
6	72.0	14.7	1.5	0.3	0.05	0.8	4.6	4.2	1.3	0.4
7	69.7	14.6	1.8	0.3	0.05	1.0	4.5	4.4	1.4	0.3
8	68.6	14.4	3.0	0.9	0.10	1.6	5.4	4.0	1.2	0.4
9	71.2	13.3	1.2	0.3	0.04	0.8	5.4	4.0	1.4	0.3
10	70.8	13.6	3.0	0.4	0.05	0.9	4.3	4.0	1.3	0.2
11	20.7	4.8	1.3	0.2	0.03	1.9	35.2	2.8	0.6	0.2
12	69.2	13.5	1.7	0.4	0.06	1.0	5.9	4.0	1.3	0.3
Avg.$^+$	71.1	13.9	1.8	0.4	0.05	0.9	4.8	4.1	1.4	0.3
SD$^+$	1.7	0.5	0.7	0.2	0.02	0.3	0.6	0.1	0.1	0.1
Cont. Soil	70.7	17.0	5.7	0.8	0.1	0.8	2.1	0.7	1.7	0.2

Site	LI*	OC*	$CaCO_3$*	Cu**	Zn**	Pb**	Ni**	V**	Cr **
1	1.8	0.1	4.4	47	19	10	12	19	17
2	1.6	0.2	6.7	39	27	8	56	45	47
3	2.4	0.2	4.8	42	26	14	8	35	29
4	1.4	0.2	4.3	35	30	10	24	54	41
5	1.2	0.3	3.6	46	14	11	23	28	26
6	0.9	0.3	3.5	40	24	11	14	43	29
7	2.1	0.5	2.7	39	40	10	12	43	34
8	1.3	0.2	3.9	34	37	10	14	76	48
9	2.7	0.3	5.2	42	18	11	17	33	35
10	2.2	0.4	3.0	41	21	8	20	41	23
11	33.1	0.1	75.0	<0.5	16	<5.0	<0.5	29	13
12	3.3	0.4	5.6	36	25	10	10	45	25
Avg.$^+$	1.9	0.3	5.5	40	25.6	10.3	19.1	42	32.2
SD$^+$	0.7	0.1	1.1	4.0	7.5	1.54	12.7	14.0	9.5
Cont. Soil	N/A	N/A	N/A	30	90	35	50	90	70

$^+$ Without sample 11 LI - Loss by Ignition
* In % OC - Organic Carbon
** In $\mu g\ g^{-1}$ dry weight SD - Standard Deviation

Fujise et al. (1988). The value found (445 $\mu g\ g^{-1}$) in the kidney of a calf is similar to the values (227-538 $\mu g\ g^{-1}$) reported by Mendez et al. (2002) in the kidneys of gray whales *Eschrichtius robustus*, and within the range reported by Varanasi et al. (1994) of 300-600 $\mu g\ g^{-1}$ for the same species. In muscle, concentrations between 342-460 $\mu g\ g^{-1}$ were found for the different organisms studied, similar to the values reported by Mendez et al. (2002) of 151-736 $\mu g\ g^{-1}$ for *E. robustus* and by Thompson (1992) of 424 $\mu g\ g^{-1}$ for the Cuvier's beaked whale *Ziphius cavirostris*. The high concentrations of Fe found in muscle in this study may be associated to the presence in this tissue of the protein myoglobin that allows cetaceans to dive for long periods of time. Tissues from calves

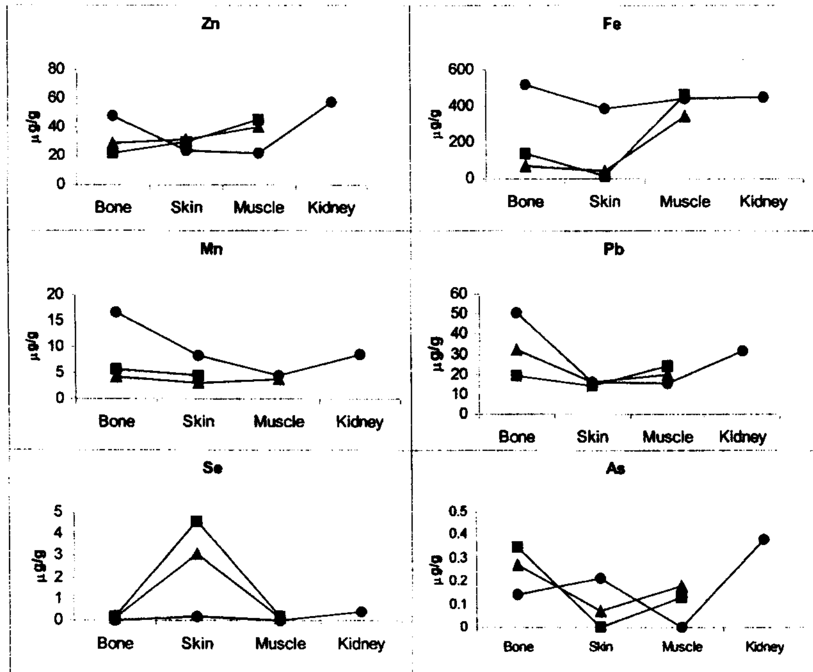

Figure 2. Concentration of heavy metals in tissues of gray whales (dry weight).
● Calves, ■ juveniles, ▲ adults.

showed the highest manganese concentrations (avg. 16.1 µg g^{-1} in bone, 8.3 µg g^{-1} in skin, 4.5 µg g^{-1} in muscle, and 8.5 µg g^{-1} in kidney). Mendez et al. (2002) reported manganese values of 0.12-4.08 µg g^{-1} in kidney and 0.1-0.6 µg g^{-1} in muscle for *E. robustus*. Higher Pb concentrations were found in bone (avg. 18.6 µg g^{-1}) and kidney (avg. 31.6 µg g^{-1}) from calves; Parsons et al. (1999) reported 15.9 µg g^{-1} in kidney from a Bryde's whale *Balaenoptera edeni*, and Mendez et al. (2002) reported that on seven gray whales studied one had 4 µg g^{-1} and another 6.12 µg g^{-1} in kidney. Kemper et al. (1994) found that the highest lead levels concentrate in bone of marine mammals. Gray whales tend to accumulate Zn, Fe, Mn, and Pb in early ages, as the whales grow, the concentration of these elements decreases notably. Lead concentrations found in tissues of gray whales are higher when compared with other studies on the same species and with other cetacean species. This higher Pb levels found in tissues of gray whales could cause serious deleterious effects. Shlosberg et al. (1997) reported the death of a bottlenose dolphin *Tursiops truncatus*, which had a kidney concentration of 3.6 µg g^{-1} wet weight. The potential toxic effects of these high concentrations of Pb in the whales need further research. Selenium showed a high affinity for the skin (avg. 2.7 µg g^{-1}). The highest concentrations of this element were found in this tissue. The concentrations of selenium reported in this study for bone (0.04-0.3 µg g^{-1}) and muscle (0.04-0.16 µg g^{-1}) are similar to those reported previously (Varanasi et al. 1994). Arsenic concentrations were higher in bone of juveniles (0.35 µg g^{-1}) and adults (0.27 µg g^{-1}). The concentrations of As reported in this study are similar to the values reported elsewhere (Varanasi et al. 1994).

There was no significant correlation when comparing tissues against sediments. The lack of correlation between these variables could be explained by the low number of samples, the high variation shown by the concentrations of these metals in tissues, and finally, because gray whales are organisms that migrate long distances, and they are in permanent movement inside the lagoon. Further research, mainly in their feeding grounds, and along the migration corridor is paramount. Nevertheless this study provides valuable information on the burden of metals in tissues of gray whales and in the sediments of a breeding lagoon.

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