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Comparison of trace element concentrations in livers of diseased, emaciated and non-diseased southern sea otters from the California coast

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Abstract

Infectious diseases have been implicated as a cause of high rates of adult mortality in southern sea otters. Exposure to environmental contaminants can compromise the immuno-competence of animals, predisposing them to infectious diseases. In addition to organic pollutants, certain trace elements can modulate the immune system in marine mammals. Nevertheless, reports of occurrence of trace elements, including toxic heavy metals, in sea otters are not available. In this study, concentrations of 20 trace elements (V, Cr, Mn, Co, Cu, Zn, Rb, Sr, Mo, Ag, Cd, In, Sn, Sb, Cs, Ba, Hg, Tl, Pb, and Bi) were measured in livers of southern sea otters found dead along the central California coast ($n = 80$) from 1992 to 2002. Hepatic concentrations of trace elements were compared among sea otters that died from infectious diseases ($n = 27$), those that died from non-infectious causes ($n = 26$), and otters that died in emaciated condition with no evidence of another cause of death ($n = 27$). Concentrations of essential elements in sea otters varied within an order of magnitude, whereas concentrations of non-essential elements varied by two to five orders of magnitude. Hepatic concentrations of Cu and Cd were 10- to 100-fold higher in the sea otters in this study than concentrations reported for any other marine mammal species. Concentrations of Mn, Co, Zn, and Cd were elevated in the diseased and emaciated sea otters relative to the non-diseased sea otters. Elevated concentrations of essential elements such as Mn, Zn, and Co in the diseased/emaciated sea otters suggest that induction of synthesis of metallothionein and superoxide dismutase (SOD) enzyme is occurring in these animals, as a means of protecting the cells from oxidative stress-related injuries. Trace element profiles in diseased and emaciated sea otters suggest that oxidative stress mediates the perturbation of essential-element concentrations. Elevated concentrations of toxic metals such as Cd, in addition to several other organic pollutants, may contribute to oxidative stress-meditated effects in sea otters.

Keywords: Sea otters; Trace metals; Cadmium; Marine mammals; Immune suppression

1. Introduction

The southern sea otter (*Enhydra lutris nereis*) population inhabits the central California coast including the Monterey Bay National Marine Sanctuary. This species is listed as 'threatened' under the Endangered Species Act. Southern sea otters have made a slower than expected recovery after a drastic decline in their population prior to the 20th century, due to hunting (Estes, 1990). After a decade of population growth from the mid 1980s to the mid 1990s, the population of southern sea otters exhibited a slow

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decline in the late 1990s. After a high of 2377 animals in 1995, the population declined to 2090 animals in 1999 (Hanni et al., 2003), increased to 2825 individuals in 2004 (Kannan et al., 2004), and then dropped back to 2735 in 2005 (USGS, 2005). Overall, this subspecies of sea otter has exhibited a sluggish population growth rate, averaging ~5% per year, while other populations of sea otters exhibited 17–20% growth per year (Estes, 1990). The decline observed in the late 1990s was attributed to high adult mortality rates, with infectious disease being the major cause of death (Thomas and Cole, 1996). Multiple pathogens were implicated in these deaths, including pathogens such as *Coccidioides immitis* and *Toxoplasma gondii* (Hanni et al., 2003). These findings suggest that the immune systems of mature animals in this population may be compromised (Schwartz et al., 2005).

The southern sea otter population is just one example, in an increasing trend worldwide, of reports of disease and mass mortality events affecting marine mammals (Harvell et al., 1999). The problems facing marine mammal populations, including southern sea otters, are likely multi-factorial (Schwartz et al., 2005) and include effects from habitat destruction, pollutants, municipal runoff, global climate change, and over-harvesting of marine resources. In attempts to elucidate the relationship between pollutants and health of southern sea otters, earlier studies reported exposure concentrations of several organic contaminants (Kannan et al., 1998, 2004; Nakata et al., 1998; Bacon et al., 1999). However, because the contaminants exist in complex mixtures and because interactions can potentially occur among contaminants, nutritional status, and other environmental factors, establishment of a link between contaminant exposure and large-scale mortality events in marine mammals is a challenging task. One approach is to carry out systematic postmortem investigations to establish the disease status of contaminated animals in a relatively large sample (when available) from a single species. In this study, concentrations of 20 trace elements were measured in livers of sea otters to compare exposure levels between diseased and non-diseased individuals. To our knowledge, this is the first report of trace element concentrations in livers of free-ranging sea otters.

Unlike trace organic contaminants, many trace elements are essential for survival. However, both excesses and deficiencies of these trace elements lead to adverse effects. The essential elements include Co, Cr, Cu, Fe, Mg, Mn, Ni, Mo, Se, and Zn; the non-essential elements include As, Ag, Au, Be, Cd, Cs, Li, Hg, Pb, Sn, and Sr (Davis and Mertz, 1987). Certain heavy metals modify immune function via influences on a number of distinct and intriguing mechanisms (Lynes et al., 2006). While certain toxic metals (e.g., Cd, Pb) can diminish the adaptive capacity of exposed individuals, other heavy metals (e.g., Cu, Zn) are essential for effective immune functioning, and some metals (e.g., Hg, Be) can initiate inappropriate immune responses, leading to autoimmune disease (Lynes et al., 2006). High exposure to trace elements has been shown to affect immune parameters such as natural killer cell activity, phagocytosis, and lymphocyte proliferation (Bennett et al., 2001; Kakuschke et al., 2005). Studies of the mechanisms involved in different metal-mediated effects are needed to elucidate the consequences of environmental exposure to trace metals. In this study, we tested the hypothesis that exposure to elevated levels of certain toxic metals may compromise the immune system of sea otters and make them susceptible to pathogens. The goal of this study was to examine the association between hepatic trace element concentrations and pathological conditions in southern sea otters.

2. Materials and methods

2.1. Samples

A sample of adult female animals ($n = 80$) was selected from an archive of over 300 beached southern sea otters found freshly dead, between 1992 and 2002, along the central California coast (Fig. 1). We chose samples based on gender and age so as to eliminate these as confounding factors. Additionally, female sea otters were chosen because of their more localized movement patterns, which make them more suitable indicators of local sources of pollution (Ralls et al., 1996). Postmortem examinations were performed at the USGS National Wildlife Health Center (NWHC) in Madison, Wisconsin, for the determination of cause of death (COD). The COD was classified, based on necropsy findings, as one of four categories: emaciation, infectious disease, trauma, and other (Thomas and Cole, 1996). Each class is further divided into more specific subclasses. In this

Fig. 1. Sampling locations of dead southern sea otters from the central California coast.

study, we grouped animals that died of infectious diseases into a 'diseased' group ($n = 27$), and trauma and other into the 'non-diseased' group ($n = 26$). On the basis of body/nutritional condition at the time of necropsy, emaciated otters were grouped into a separate category ($n = 27$). Otters in the emaciation category include those that died in emaciated condition and had no evidence of other causes of death; these otters may have died from starvation or from debilitating physiological or functional abnormalities not apparent at necropsy. The emaciation category includes otters that died with evidence of recent pregnancy and mating ($n = 18$); dental diseases ($n = 4$); or no other co-factors ($n = 5$). Samples from the infectious disease category include those that died of acanthocephalan peritonitis ($n = 3$), protozoal encephalitis ($n = 2$), or fatal infections by bacteria ($n = 14$), fungi ($n = 3$), or parasites ($n = 1$). Also grouped in this category were other fatal cardiovascular ($n = 2$) and neurological ($n = 2$) infections. The category 'other' was comprised of animals that died of various gastrointestinal disorders such as intestinal torsions ($n = 5$), miscellaneous individual problems ($n = 3$), neoplasia ($n = 3$), or from undetermined ($n = 9$) causes. The category 'trauma' included otters that died from gun shot ($n = 3$) or shark bite ($n = 3$). In this study, we combined 'trauma' and 'other' into the other group.

2.2. Trace element analysis

Liver samples were collected from the carcasses at the time of necropsy, wrapped in aluminum foil, placed in sterile sampling bags (TWIRL'EM; Fisher Scientific International Inc., Hampton, NH, USA), and stored at −20 °C until analysis. Trace metals were analyzed following the method described elsewhere (Anan et al., 2002; Agusa et al., 2005). Prior to analysis, liver samples were freeze-dried and homogenized; an aliquot (~0.1 g) of the sample was weighed in a vial lined with Teflon® Liver samples were digested overnight in concentrated nitric acid (2 ml). Samples were then further digested in a microwave oven for 7 min at 200 W; this step was repeated three times. Concentrations of 19 trace elements (V, Cr, Mn, Co, Cu, Zn, Rb, Sr, Mo, Ag, Cd, In, Sn, Sb, Cs, Ba, Tl, Pb, and Bi) were determined by an inductively coupled plasma-mass spectrometer (ICP-MS) (Hewlett Packard-4500, Avondale, PA, USA), using yttrium (Y) as an internal standard. Concentrations of Hg were determined by a cold vapor atomic absorption spectrometer (Model HG-3000; Sanso, Tsukuba, Japan). The limit of detection for trace elements was 1 ng/g, dry wt, except for Sb and Cs (10 ng/g, dry wt) and Hg (50 ng/g, dry wt). Accuracy of the analysis was examined by analyzing Certified Reference Materials: dogfish muscle (DORM2; National Research Council, Ottawa, ON, Canada) and bovine liver (SRM1577b; National Institute of Standards and Technology, Gaithersburg, MD, USA) along with the samples. Recoveries of all the elements were in the range of 89–104%. The results are expressed on a dry weight basis.

Statistical analyses were performed using Statgraphics® 5 (Manugistics, Inc., Rockville, MD, USA). Concentrations of trace elements in sea otters did not follow a normal distribution, except Rb (Shapiro-Wilks W test; $p < 0.05$ for Rb). Therefore, the non-parametric Mann–Whitney U test was applied, to allow comparison of concentrations between two groups. Comparison of multiple groups was performed using ANOVA. Values below the limit of detection were assigned zero for the analysis.

3. Results and discussion

3.1. Residue levels and patterns

Those trace elements that were present at mean concentrations greater than 3 μg/g occurred in the following order, for both diseased/emaciated and non-diseased sea otters: Zn > Cu > Cd > Hg > Mn > Rb (Table 1, Fig. 2). Distribution of those trace elements that were present at less than 3 μg/g showed some differences among diseased, emaciated and non-diseased sea otters. Diseased and emaciated sea otters had a concentration pattern of Ag > Sr > Sn > Cr > Mo > Pb > V > Co > Ba > Cs > Sb > Bi > In > Tl, while non-diseased otters had relatively higher hepatic concentrations of Sr compared to Ag, Bi compared to Cs, and Sb compared to In (Sr > Ag > Sn > Cr > Mo > Pb > V > Co > Ba > Bi > Cs > Sb > In > Tl). Many of these elements (e.g., Mn, Co, Cu, and Zn) are essential for life and play important roles in enzyme chemistry. Concentrations of essential elements are regulated by homeostasis; therefore, the range of concentrations of essential elements in healthy individuals is expected to be small. Prior to this study, reports of normal concentration ranges of trace elements in healthy sea otters were not available. The only earlier study that measured trace elements in sea otter was for a captive aquarium specimen ($n = 1$) from Japan (Ninomiya et al., 2004). Hepatic concentrations of Cd, Pb, Zn, and Cu in this adult female sea otter were 0.36, 0.07, 31 and 17 μg/g, wet wt, respectively (Ninomiya et al., 2004). Based on an average moisture content of 70% in liver (as measured in our study), hepatic concentrations of Cd, Pb, Zn, and Cu expressed on a dry weight basis were 1.2, 0.24, 102 and 57 μg/g, respectively (Ninomiya et al., 2004). The mean concentration of Cd in our sea otter samples was approximately 100-fold higher, and those of Zn and Cu were 2- to 3-fold higher than those reported for the captive sea otter.

Concentration ranges for essential elements in livers of sea otters varied within an order of magnitude in our study (Fig. 3). The mean concentration of Zn was the highest among all of the trace elements analyzed, with a range of 95.0–542 μg/g, dry wt (mean ± SD: 230 ± 92.3 μg/g). Zn is an essential element, required for the functioning of enzymes that are involved in DNA and RNA synthesis. The concentration of Cu ranged from 26.3 to 401 μg/g (mean: 133 μg/g), and that of Mn ranged from 2.37 to 47.4 μg/g, dry wt (mean: 16.8 μg/g).

Table 1
Mean, median, and range of trace element concentrations (μg/g, dry wt) in livers of southern sea otters

	Overall ($n = 80$)		Non-diseased ($n = 26$)		Emaciated ($n = 27$)		Infectious-diseased ($n = 27$)		Detectable observations
	Mean (range)	Median	Mean (range)	Median	Mean (range)	Median	Mean (range)	Median	
V	0.181 (0.03–2.8)	0.125	0.175 (0.03–0.53)	0.140	0.131 (0.038–0.73)	0.110	0.236 (0.032–2.8)	0.15	54/54; 26/26
Cr	0.532 (0.16–2.3)	0.470	0.604 (0.18–2.3)	0.475	0.563 (0.18–1.2)	0.520	0.433 (0.16–0.86)	0.41	54/54; 26/26
Mn	16.9 (2.4–47)	14.6	12.9 (2.37–29.5)	11.1	19.4 (8.31–45.9)	17.4	18.0 (6.06–47.4)	14.4	54/54; 26/26
Co	0.079 (0.02–0.25)	0.071	0.066 (0.016–0.17)	0.062	0.091 (0.033–0.25)	0.080	0.082 (0.036–0.19)	0.072	54/54; 26/26
Cu	133 (26.3–401)	112	124 (45.3–274)	115	161 (37.4–401)	126	115 (26.3–337)	107	54/54; 26/26
Zn	230 (95–542)	209	202 (95–376)	180	248 (135–440)	224	239 (117–542)	208	54/54; 26/26
Rb	2.88 (0.91–6.3)	2.94	3.09 (0.91–6.33)	3.14	2.78 (1.76–4.24)	2.69	2.76 (1.57–4.29)	2.92	54/54; 26/26
Sr	1.46 (0.08–23)	0.583	1.76 (0.12–19)	0.705	1.74 (0.161–22.9)	0.550	0.909 (0.079–8.3)	0.54	54/54; 26/26
Mo	0.52 (0.08–1.3)	0.479	0.55 (0.08–1.25)	0.484	0.475 (0.224–1.02)	0.457	0.518 (0.266–1.09)	0.518	54/54; 26/26
Ag	1.59 (0.16–5.8)	1.10	1.52 (0.17–5.1)	0.875	1.95 (0.2–5.8)	1.50	1.30 (0.16–5.3)	1.1	54/54; 26/26
Cd	91.9 (0.002–728)	58.4	63 (0.002–199)	47.8	123 (24.4–728)	82.1	89 (6.66–402)	54.8	54/54; 26/26
In	0.01 (<0.001–0.028)	0.002	0.01 (<0.001–0.028)	0.003	0.003 (<0.001–0.02)	0.002	0.007 (<0.001–0.019)	0.003	31/54; 14/26
Sn	1.11 (0.077–9.87)	0.381	1.61 (0.077–9.87)	0.404	0.628 (0.086–6.96)	0.322	1.096 (0.077–6.04)	0.399	54/54; 26/26
Sb	0.01 (<0.01–0.02)	0.010	0.02 (<0.01–0.02)	0.015	<0.01–0.01	0.01	0.015 (<0.01–0.02)	0.015	7/54; 4/26
Cs	0.02 (<0.01–0.03)	0.020	0.02 (<0.01–0.03)	0.020	0.016 (<0.01–0.02)	0.020	0.014 (<0.01–0.03)	0.01	45/54; 18/26
Ba	0.02 (0.006–0.16)	0.017	0.03 (0.007–0.1)	0.018	0.028 (0.007–0.16)	0.017	0.021 (0.006–0.12)	0.015	54/54; 26/26
Hg	17.8 (0.480–128)	12.0	19.3 (0.480–128)	11.0	18.4 (1.4–62)	13.0	15.6 (2.3–72)	12	54/54; 26/26
Tl	0.003 (<0.001–0.014)	0.002	0.002 (<0.001–0.013)	0.002	0.003 (<0.001–0.008)	0.002	0.003 (<0.001–0.014)	0.002	43/54; 17/26
Pb	0.22 (0.02–1.1)	0.118	0.31 (0.022–1.06)	0.120	0.169 (0.02–0.552)	0.122	0.200 (0.019–0.956)	0.116	54/54; 26/26
Bi	0.01 (<0.001–0.075)	0.007	0.01 (<0.001–0.075)	0.008	0.009 (<0.001–0.035)	0.008	0.013 (<0.001–0.072)	0.004	50/54; 25/26

Detectable observations represent samples with concentrations above the limit of detection. Values below the detection limit were assigned zero for calculating mean and median. n of 54 for diseased and emaciated groups and n of

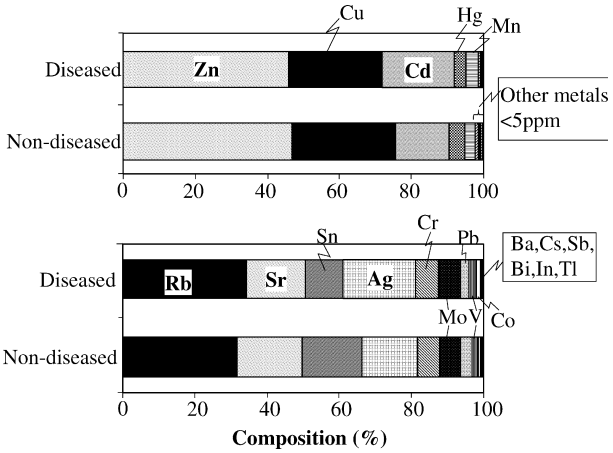

Fig. 2. Relative distribution of trace elements, for diseased (includes infectious-diseased and emaciated) and non-diseased sea otters. The upper panel represents trace metals with mean hepatic concentrations >3 μg/g, dry wt whereas the lower panel represents trace metals with mean hepatic concentrations <3 μg/g, dry wt.

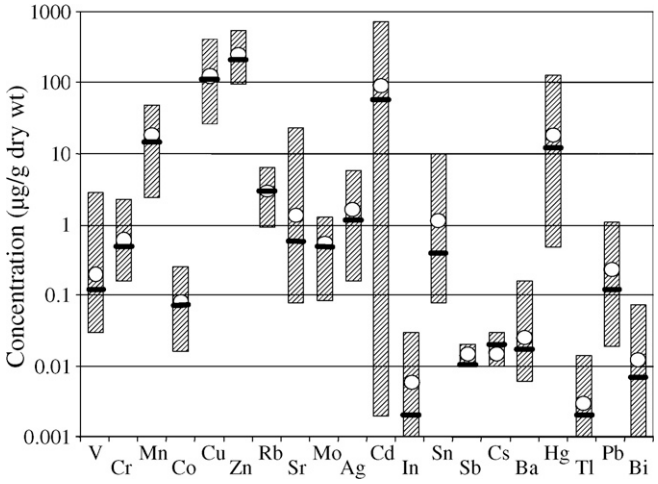

Fig. 3. Trace element concentrations in southern sea otter liver tissue ($n = 80$; all otters including diseased, emaciated and non-diseased). For each element, the vertical bar represents the range, the circle is the mean, and the horizontal line is the median (detection limit of Cs and Sb was 10 ng/g, dry wt, and that of In, Tl and Bi was 1 ng/g, dry wt).

Concentrations of non-essential elements (e.g., Cd, Hg, Pb, Sn, and Sr) varied considerably, across two to five orders of magnitude, in the livers of sea otters. Mean concentrations of Cd and Hg exceeded 15 μg/g. A concentration of 728 μg/g for Cd was the highest concentration measured for any metal in this study. Hg concentrations varied by three orders of magnitude, from 0.480 to 128 μg/g. Among the other known toxic metals, Pb was found in all of the samples analyzed, at concentrations ranging from 0.019 to 1.06 μg/g.

No earlier studies reported the concentrations of trace elements in free-ranging sea otters. In comparison with the concentrations reported for other marine mammals (e.g., Anan et al., 2002; Kunito et al., 2004), concentrations of Cu and Cd in the livers of the sea otters in our study

were elevated. For example, a general range of concentrations of Cu and Cd in livers of marine mammals was 5–50 μg/g and 0.5–25 μg/g, dry wt, respectively (we removed the outliers from the cited studies; Law et al., 2001; Anan et al., 2002; Kunito et al., 2004). Mean concentrations of Cu and Cd in the livers of our sea otters were greater than the highest concentrations reported for several marine mammal species (Anan et al., 2002; Kunito et al., 2004). When the concentrations were compared with those reported for marine mammals from the North American coasts, mean concentrations of Cu and Cd in the livers of our sea otters were 5- to 10-fold greater than those reported for spinner dolphins (*Stenella longirostris*), and 10- to 100-fold greater than those reported for gray whales (*Eschrichtius robustus*) from the southeast Gulf of California (Ruelas-Inzunza and Páez-Osuna, 2002). Hepatic Cu concentrations were 5-fold higher, and Cd concentrations were 100-fold higher in southern sea otters than those reported for bottlenose dolphins (*Tursiops truncatus*) from the Gulf of Mexico (Meador et al., 1999). Elevated concentrations of Cu in sea otters may be related to such sources as Cu-based antifouling paints used on ships and boats. Data collected from 1977 to 1990 by the California State Mussel Watch program showed an increase in Cu concentrations in mussels over that time period (Lauenstein and Daskalakis, 1998). Sea otters feed on mollusks, crustaceans, and various sessile and slow-moving benthic invertebrates, which are known to accumulate elevated levels of Cu. Similarly, high concentrations of Cd in sea otters may be related to their diet comprising of mussels and clams, which accumulate elevated levels of Cd in their tissues (Croteau et al., 2005). Furthermore, to maintain their high metabolism, sea otters consume 20% of their body weight daily and this high food intake rate can contribute to elevated exposure to trace metals (Kannan et al., 2004).

3.2. Comparison among diseased, emaciated and non-diseased groups

Differences in hepatic trace element concentrations among 'infectious-diseased' ($n = 27$), 'emaciated' ($n = 27$), and non-diseased ($n = 26$) sea otters were examined (Table 2). Hepatic concentrations of trace elements did not vary significantly between emaciated and infectious-diseased groups except for Cu in which emaciated otters had significantly higher concentrations than in diseased otters. Overall, concentrations of Mn and Co were significantly higher ($p \leqslant 0.05$) in both emaciated and infectious-diseased animals than in non-diseased animals (Table 2). Concentrations of Cd were significantly higher in emaciated sea otters than in non-diseased sea otters ($p < 0.05$). Similarly, Cd concentrations were marginally significant between diseased and non-diseased sea otters ($p = 0.07$). Concentrations of Zn in diseased and emaciated sea otters were marginally higher than those in non-diseased sea otters ($p = 0.06$).

Although both Mn and Co are essential for the activation of enzymes involved in protein and fat metabolism,

Table 2
Statistical significance in the concentrations of trace elements among infectious-diseased, emaciated, and non-diseased sea otters from the California coast

	M–W *U* test Emaciated versus non-diseased	M–W *U* test Emaciated versus diseased	ANOVA All three categories
V	$p > 0.05$	$p > 0.05$	$p > 0.05$
Cr	$p > 0.05$	$p > 0.05$	$p > 0.05$
Mn	$\mathbf{p < 0.05}$	$p > 0.05$	$\mathbf{p < 0.05}$
Co	$\mathbf{p < 0.05}$	$p > 0.05$	$\mathbf{p = 0.05}$
Cu	$p > 0.05$	$\mathbf{p < 0.05}$	$p > 0.05$
Zn	$p = 0.06$	$p > 0.05$	$p = 0.07$
Rb	$p > 0.05$	$p > 0.05$	$p > 0.05$
Sr	$p > 0.05$	$p > 0.05$	$p > 0.05$
Mo	$p > 0.05$	$p > 0.05$	$p > 0.05$
Ag	$p > 0.05$	$p > 0.05$	$p > 0.05$
Cd	$\mathbf{p < 0.05}$	$p > 0.05$	$p = 0.06$
In	$p > 0.05$	$p > 0.05$	$p > 0.05$
Sn	$p > 0.05$	$p > 0.05$	$p > 0.05$
Sb	NA	NA	NA
Cs	$p > 0.05$	$p > 0.05$	$p > 0.05$
Ba	$p > 0.05$	$p > 0.05$	$p > 0.05$
Hg	$p > 0.05$	$p > 0.05$	$p > 0.05$
Tl	$p > 0.05$	$p > 0.05$	$p > 0.05$
Pb	$\mathbf{p < 0.05}$	$p > 0.05$	$p > 0.05$
Bi	$p > 0.05$	$p > 0.05$	$p > 0.05$

M–W *U* test = Mann–Whitney *U* test; ANOVA: analysis of variance.

chronic overdose or prolonged exposure to Mn can cause severe disruption of the mammalian central nervous system (Pearson and Greenway, 2005). Mn is a constituent of manganese superoxide dismutase (MnSOD), the principal antioxidant enzyme present in mitochondria. Levels of MnSOD increase in response to oxidative stress and free-radical production (Pal et al., 1999). Oxidative stress is perceived as a secondary phenomenon, a consequence of tissue injury. For example, excess production of $O_2^{\cdot-}$, H_2O_2, and other reactive species by phagocytes, at sites of chronic inflammation, can cause severe damage. Tissue injury can release metal ions from their storage sites within cells, leading to $OH^{\cdot}$ generation. Malnutrition, as in emaciated otters, or infectious diseases, as observed in diseased sea otters, can elevate MnSOD levels (Berger et al., 2004). High hepatic Mn levels in diseased sea otters may be attributed to oxidative stress-mediated production of MnSOD. Another explanation for the high concentrations of Mn in diseased otters is decreased excretion due to impaired liver function. Mn accumulation in individuals with liver dysfunction has been shown to contribute to neurological problems and Parkinson's disease-like symptoms (Pal et al., 1999; Berger et al., 2004). Nevertheless, we do not know whether the high concentration of Mn in the diseased and emaciated sea otters was a cause or a consequence of disease.

The higher concentrations of Co in diseased and emaciated sea otters compared to that in non-diseased sea otters further support the hypothesis of oxidative stress-mediated perturbation of essential element homeostasis. Co is a constituent of vitamin B_{12} (cobalamin), which is essential for

the metabolism of homocysteine. Homocysteine levels in the blood increase in diseased mammals, and ultimately induce lipid peroxidation and oxidative stress (Zock and Katan, 1998). To protect tissues from oxidative stress, synthesis of vitamin B_{12} (and therefore, hepatic concentrations of Co) increases (Stampfer and Malinow, 1995). Vitamin B_{12} synthesis is regulated by homeostasis.

Higher concentrations of Zn in diseased/emaciated sea otters than in non-diseased sea otters may be related to an increase in Zn-containing SOD (CuZn-SOD), which is produced as a consequence of oxidative stress. Oxidative stress can be caused by exposure to organic contaminants and/or toxic metals. Concentrations of organic contaminants such as polychlorinated biphenyls, tributyltin, and DDT have been found previously to be elevated in southern sea otters, particularly in diseased individuals (Kannan et al., 1998, 2004; Nakata et al., 1998). The occurrence of high concentrations of Zn in diseased marine mammals (seals and porpoises) has been reported (Anan et al., 2002; Bennett et al., 2001). The association between Zn concentration and disease status was thought to result from Zn redistribution and disturbances in homeostatic regulation of Zn, in response to diseases (Bennett et al., 2001; Anan et al., 2002). Overall, elevated levels of essential elements in diseased sea otters indicate that diseased otters have altered homeostasis of essential elements.

Elevated exposure to toxic metals such as Cd and immunotoxic organic pollutants could be contributing to the initiation of cascades of complex reactions that alter the homeostasis of essential elements. As mentioned above, hepatic concentrations of Cd were significantly higher in emaciated otters and marginally higher in diseased sea otters than in non-diseased sea otters. Furthermore, mean concentrations of Cd in our sea otters were higher than those reported previously for other marine mammals. Due to the preferential enrichment of Cd in kidney over liver, concentrations of Cd are expected to be much higher in kidneys of sea otters than the concentrations that we found in livers. For example, the concentration of Cd in the kidney of a captive sea otter was 13-fold higher than that in its liver (Ninomiya et al., 2004). Although kidney was not analyzed in our study, these results suggest that Cd is a critical toxic metal in sea otters. Accordingly, elevated concentrations of Cd in diseased and emaciated individuals suggest that this metal played some role in disease. Cd stimulates metallothionein synthesis and interferes with Cu and Zn metabolism. Metallothionein synthesis is induced in humans following exposure to metals such as Cd, or as a result of malnutrition or oxidative stress (Min, 2000). Despite the protective role of metallothionein, this compound becomes less effective in protecting animals from Cd toxicity in malnourished animals such as the emaciated and diseased sea otters in our study (Shimizu and Morita, 1990). Differences in the concentrations of trace elements other than Mn, Co, Cd, and Zn were not statistically significant between the diseased and the non-diseased groups.

High concentration of Cd in livers of sea otters is a cause for concern. The critical limit suggested for damage to kidney cortex of humans and laboratory animals vary from 50 to 200 μg/g, wet wt (Elinder and Järup, 1996; Sonne-Hansen et al., 2002). Approximately, 15% of the sea otters analyzed had liver concentrations >50 μg/g, on a wet weight basis (calculated based on a moisture content of 70% in liver, in this study). However, kidneys of sea otters may contain higher concentrations of Cd because of its preferential accumulation, and further studies should analyze kidneys.

The results of this study suggest that elevated concentrations of essential elements such as Mn, Co, and Zn in diseased and emaciated otters may be a consequence of oxidative stress. Elevated exposure to toxic metals such as Cd may have contributed to this condition. It is believed that exposure to stressors, including organic contaminants and toxic metals, causes oxidative stress, which leads to the synthesis of metallothionein and SOD involved in cellular defense mechanisms. Although SOD is important for scavenging free radicals, an excess of SOD can be deleterious, as found in studies with mice (Amstad et al., 1991; White et al., 1991), and presumably in other mammals as well. To investigate the association of elevated trace elements with SOD, it would be valuable to measure superoxide dismutase in sea otter liver tissue.

3.3. Relationships among trace elements

We examined relationships among trace metals in the livers of sea otters by non-parametric Kendall's Tau correlation analysis (Table 3). Trace element concentrations below the limit of detection were assigned a value of zero for this analysis. Among the essential elements, Zn, Cu, Co, and Mn were significantly correlated with each other and with several other trace metals. It is interesting to note that all four of these metals are components of SOD or are involved in oxidative stress-related defense. All four of these essential elements were significantly correlated with concentrations of Cd in both diseased/emaciated and non-diseased otters. Concentrations of Ag and Cs were correlated with Cu, Mn, and Zn concentrations.

The relationships of toxic metals (e.g., Hg, Pb) with essential elements such as Mn, Co, Cu, and Zn varied between the diseased/emaciated and the non-diseased groups. No significant correlation was found between Hg and Mn, Co, Cu, or Zn in non-diseased otters. However, in diseased and emaciated sea otters, Hg was significantly correlated with Mn, Co, Cu, and Zn. Similarly, Pb was not correlated with Mn, Cu, and Zn in non-diseased sea otters, but it was significantly correlated with Mn, Cu, and Zn in diseased and emaciated sea otters. These results suggest that in diseased and emaciated animals, several toxic metals (e.g., Cd, Hg, Pb) act in concert to alter the homeostasis of essential elements. The association between toxic and essential elements in diseased individuals may reflect sequestration of the metals by binding proteins such

Table 3
Matrix of Kendall's Tau correlation coefficients among trace element concentrations in livers of southern sea otters

	V	Cr	Mn	Co	Cu	Zn	Rb	Sr	Mo	Ag	Cd	In	Sn	Sb	Cs	Ba	Hg	Tl	Pb	Bi
V	1.00	−0.06	0.03	0.06	0.07	0.03	−0.14	0.06	0.07	−0.06	−0.14	**0.26****	**0.28****	−0.05	−0.06	0.07	0.09	0.13	**0.36****	0.05
Cr		1.00	0.14	−0.04	0.14	0.09	**0.22****	0.14	0.11	**0.16***	**0.16***	0.06	0.14	0.12	**0.32****	**0.22****	−0.03	−0.03	0.07	0.03
Mn			1.00	**0.29****	**0.37****	**0.55****	**0.17***	−0.09	**0.21****	**0.33****	**0.38****	**0.20***	**0.21****	0.15	**0.47****	**0.16***	**0.19***	−0.03	**0.20***	0.07
Co				1.00	**0.16***	**0.30****	0.11	−0.09	0.08	**0.19***	**0.28****	−0.13	−0.13	0.08	0.12	−0.01	**0.16***	0.15	−0.04	0.13
Cu					1.00	**0.38****	**0.24****	−0.05	**0.18***	**0.37****	**0.17***	0.14	**0.19***	**0.23***	**0.32****	0.15	**0.17***	0.07	**0.21****	**0.26****
Zn						1.00	0.14	−0.12	0.12	**0.41****	**0.36****	0.04	0.09	0.09	**0.36****	0.11	**0.15***	0.06	0.13	**0.17***
Rb							1.00	−0.02	**0.35****	**0.17***	0.09	0.06	**0.16***	0.13	**0.52****	0.02	0.02	−0.08	**0.18***	0.06
Sr								1.00	0.02	−0.02	0.02	−0.06	0.04	0.04	−0.02	**0.19***	−0.02	−0.11	−0.03	0.04
Mo									1.00	0.09	0.02	**0.19***	**0.22****	0.13	**0.35****	0.11	0.07	0.00	**0.28****	0.03
Ag										1.00	**0.41****	−0.14	−0.08	**0.28****	**0.31****	**0.21****	0.03	0.07	−0.04	0.10
Cd											1.00	−0.02	0.08	**0.26****	**0.30****	0.15	0.04	0.12	−0.02	−0.01
In												1.00	**0.74****	0.07	**0.23***	0.03	0.02	0.07	**0.62****	−0.09
Sn													1.00	0.01	**0.27****	0.05	0.10	−0.09	**0.60****	−0.03
Sb														1.00	**0.31****	0.13	0.00	**0.21***	0.04	0.08
Cs															1.00	**0.18***	0.13	−0.09	**0.21***	0.07
Ba																1.00	−0.04	−0.03	0.02	0.01
Hg																	1.00	**−0.17***	−0.00	**0.32****
Tl																		1.00	0.07	0.05
Pb																			1.00	−0.05
Bi																				1.00

* Correlation is significant at 0.05 level (two-tailed).
** Correlation is significant at 0.01 level (two-tailed).

as metallothioneins, which play a major role in regulating the availability of metals for metal-dependent proteins.

The results of this study indicate that concentrations of several trace elements are increased in diseased and emaciated sea otters. We do not know whether these increases are a cause or a consequence of the pathological condition. Increases in the concentrations of essential elements such as Mn, Co, and Zn appear to be a consequence of oxidative stress. It is probable that infection by microorganisms, as well as exposure to toxic contaminants, induces oxidative stress. The production of reactive oxygen species (i.e., oxidative stress) may have an adverse effect on the immune system. Further studies are needed to assess the role of toxic metals, both alone and in concert with organic contaminants, in suppressing the immune system in marine mammals. Elevated concentrations of Cd in sea otters appear to be a particular concern. Furthermore, our study suggests that trace elements provide potential markers by which we can understand oxidative stress-mediated perturbations to the health of marine mammals.

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