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Elements in fish of Malibu Creek and Malibu Lagoon near Los Angeles, California

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Abstract

Our aim was to assess whether past discharges from a wastewater treatment plant increased metal pollutant loads in stream mobile species in a one-day baseline sampling study that included a coastal wetland. Mosquitofish (*Gambusia affinis*) of two sizes, black bullhead (*Ameiurus melas*), and crayfish (*Pacifastacus leniusculus*) were collected from Malibu Creek, and California killifish (*Fundulus parvipinnis*) of three sizes, as well as arroyo chub (*Gila orcutti*) were sampled from Malibu Lagoon near Los Angeles, California. Species from each locality were pooled by length, homogenized, digested by microwave wet ashing, and analyzed by simultaneous inductively coupled plasma atomic emission spectroscopy for 27 elements. Lagoon killifish 2.0–3.5 cm long contained levels of arsenic and lead above the levels for 95% of California fish, the EDL95. Black bullhead upstream of the discharge contained elevated levels of As, Cr and Se. Young mosquitofish <3 cm in length upstream of the discharge differed greatly in the order of abundance of their elements relative to larger mosquitofish and to other species collected. More sampling than this baseline study allowed was needed to determine if the wastewater treatment plant was a pollution source.

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1. Introduction

Environmental contamination by metals from anthropogenic sources can be hazardous to aquatic biota and human health (Nicola et al., 1987; Sadiq, 1992). Sediments are major sinks for many xenobiotics, including metals (Tessier et al., 1979; Elbaz-Poulichet et al., 1984; Nicola et al., 1987; Mason et al., 2000). Bottom fish often have large toxic metal body burdens (Nicola et al., 1987; Chou et al., 1999). Metals toxic to fish include Ag, As, Be, Cd, Cr, Cu, Fe, Pb, Hg, Ni, Se, Tl, and Zn. Concentrations of metals in tissues of sentinel species have been used to assess potential pollution hazards in California environmental monitoring pro-

grams (State of California Water Resources Control Board, 1990, 1993, 1995; NOAA, 1991a,b; Birge et al., 2000; Widianarko et al., 2000). Relatively nontoxic metals in fish are subdivided into essential elements, macroelements, and other (Reichenbach-Klinke and Landolt, 1973). The essential elements are B, Co, Cu, Fe, Mo, Se, V, and Zn. Macroelements include C, Ca, Mg, N, P, K, S, and Na. The elements of the “other” category include Al, Ba, Cl, Li, Mn, Si, Sr, and Ti.

The 280-km² Malibu Creek system is undeveloped except for the coastline near Malibu close to Los Angeles, California. It receives tertiary treated wastewater from the Tapia Water Reclamation Facility, Las Virgenes Municipal Water District (Fig. 1). Tapia has a 16 million gallons/day release capacity. At the time of this study in 1994, it discharged <3 million gallons/day, intermittently. There was concern that Tapia had polluted Malibu Creek and Lagoon, and the ocean beaches when the lagoon breached. Two endangered species were involved: the steelhead trout (anadromous *Onchorhynchus*

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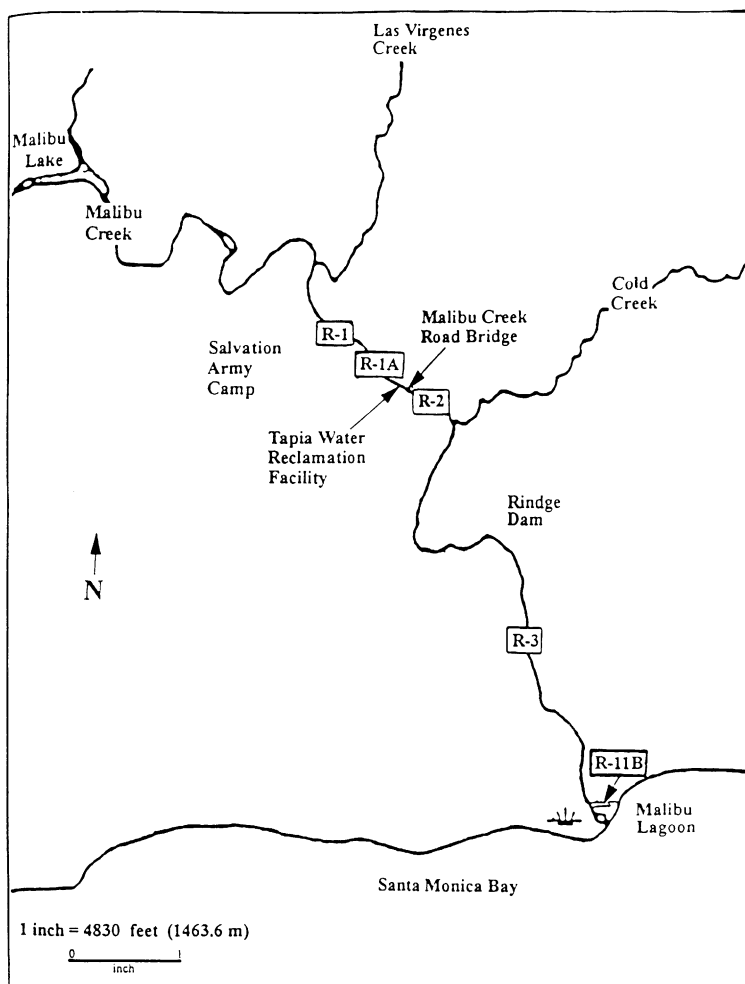


Fig. 1. Map of the Malibu Creek and Malibu Lagoon area showing the location of the sampling stations (R-1, R-1A, R-2, and R-11B).

mykiss) and the tidewater goby (*Eucyclogobius newberryi*). Lands near the lagoon are prime residential and tourist areas. During summers when berms form to close off the lagoon from the ocean, there is increased potential for contaminant buildup. We studied metal concentrations in fish as part of a baseline investigation that included analyses of organic and inorganic chemicals and viruses in water, as well as identification and enumeration of wetland biota (Ambrose et al., 1995).

2. Fish sampling and study area description

We sampled at stations R-1 and R-1A upstream of the Tapia discharge into Malibu Creek, and at one station R-2 that was 400 m below the discharge, but before Cold Creek emptied into Malibu Creek (Fig. 1). Station R-1A was 100 m upstream of the point of discharge. Station R-1 was near the Salvation Army Camp in Tapia County Park, 10 m downstream from where a state road crosses Malibu Creek. Station R-11B was on the east side of Malibu Lagoon, about 50 m south of the

Pacific Coast Highway bridge. Station R-3 was about halfway between the lagoon and the confluence of Malibu and Cold Creeks, but yielded no fish. These sampling locations had no stagnant pools on 1 October 1993. Fish and crayfish were collected with 4 mm-mesh non-tarred beach seines (10 m long \times 1.5 m high) equipped with lead weights and floating upper rings (Eaton et al., 1995). The nets, enclosing a 31 m² equilateral triangle, were swept at least five times through the enclosed area by two people; blocking nets were brought together and the whole assemblage pulled onto the shore. In the shallower lagoon, seines without blocking nets swept an area of 800 m² twice. Taxa were identified, counted, measured for length, and separated into seal-tight polyethylene bags on cold-packs for transport to the lab and storage.

3. Laboratory fish homogenization and analysis

On receipt, biota were washed with distilled water, blotted dry, weighed, and placed into polyethylene

seal-tight bags classified according to taxa and length. Whole taxa of a designated length range were cut into small pieces with Teflon-tipped scissors and then homogenized together in a ribbed acid-free pyrex bowl with a Biospec Products blender, Model Mi33/1281-0, Fisher Scientific, Pittsburgh, PA (Moeller et al., 2001). Each homogenate was then stored in 10-g lots in seal-tight polyethylene bags at -20°C .

Three thawed 10-g homogenates of each species and length range were digested using an optimized microwave wet-ashing method involving nitric and perchloric acids (Moeller et al., 2001), a modification of the method of Que Hee and Boyle (1988). After acid evaporation, the digest residues were dissolved in 11.6% hydrochloric acid/2.8% nitric acid solutions for multi-elemental analysis by simultaneous inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Moeller et al., 2001).

The ICP-AES analytical conditions, quality assurance/quality control (QA/QC) measures, and detection limits (DLs) and least quantifiable limits (LQLs) of the 27 elements quantified have been described (Moeller et al., 2001). Data were corrected for interelemental and background corrections (Que Hee et al., 1985; Que Hee and Boyle, 1988). The results of the basic accuracy study for elemental concentrations in catfish fillet and whole fish homogenate are reported in Moeller et al. (2001).

The laboratory-related coefficients of variation (CV) calculated for each sample were (Moeller et al., 2001): nebulization intrarun (the relative standard deviation (RSD) in % of the four instrumental replicates of the same solution from one nebulization); intrarun (RSD in % relative to the overall average from the average data of three nebulizations on the same solution); and inter-run (RSD in % relative to the average from the three nebulized solutions from the set of three 10-g homogenates for each taxa and length classification). Spiking recoveries for the toxic elements As, Ba, Cd, Co, Cr, Mo, Ni, Pb, and Se were evaluated at 1 ppm above their actual homogenate values.

4. Results and discussion

Arithmetic mean \pm standard deviation (SD) of dry/wet weight ratios for triplicate samples were 0.27 ± 0.04 , crayfish (*Pacifastacus leniusculus*); 0.51 ± 0.02 , 2.0–3.5 cm California killifish (*Fundulus parvipinnis*); 0.51 ± 0.04 , 3.5–5.5 cm California killifish; 0.54 ± 0.01 , black bullhead (*Ameiurus melas*); 0.65 ± 0.11 , mosquitofish (*Gambusia affinis*) at station R-1; 0.68 ± 0.04 , <2.0 cm California killifish; 0.73 ± 0.04 , <3.0 cm mosquitofish; 0.77 ± 0.04 , arroyo chub (*Gila orcutti*); and 0.87 ± 0.04 , 3.0–5.5 cm mosquitofish. The highest CV was 17% for mosquitofish at station R-1. The overall average mean \pm SD of dry/wet weight ratios for mosquitofish

and California killifish independent of length were (0.75 ± 0.11 , CV 15%) and (0.567 ± 0.098 , CV 17%), respectively, significantly different at $p < 0.05$. Since intrarun and nebulization CVs for single elements above the LQLs were $\leq 10\%$, the inter-run CV cutoff was set at 20% to reflect dry/wet weight ratio variation. “Definite quantitation” was defined as an elemental concentration at least 10 times background (the LQL) with inter-run CV $\leq 20\%$. “Imprecise quantitations” were elemental concentrations \geq LQL with inter-run CV $> 20\%$. “Trace amounts” were concentrations between the DL and the LQL. Spiking recoveries for As, Ba, Cd, Co, Cr, Mo, Ni, Pb, and Se at 1 ppm above their actual homogenate values were 95–115% (Moeller et al., 2001).

The biota number caught were 40 black bullhead at station R-1; one crayfish and 33 arroyo chub at station R-2; 556 mosquitofish from stations R-1 and R-1A; and 284 California killifish from station R-11B. Table 1 presents the concentrations of the 27 elements for each taxa at each station in homogenate dry weight. These elements comprised 0.90–4.3% of the dry weight, and 0.66–2.2% of the wet weight. $\text{Ca} > \text{P} > \text{K} > \text{Na} > \text{Mg} > \text{Si} > \text{Zn} > \text{Fe} > \text{Al} > \text{Sr}$ was the usual order for each species, except for mosquitofish at station R-1A. The fish with the greatest number of toxic metal “definite quantitations” ($>$ LQL and inter-run CV $\leq 20\%$) were lagoon 2.0–3.5 cm California killifish (Ag, As, Cr, and Pb), then lagoon < 2.0 cm California killifish (As and Cr), and then < 3.0 cm mosquitofish above the Tapia discharge (Cr). Black bullhead above the Tapia discharge had the greatest Ag, As, Cd, Cr, Ni, and Pb, and the second largest Be and Se. Lagoon 2.0–3.5 cm California killifish had the greatest Be and Pb, the second largest Ag, As, and Cd, and the third highest Cr. Lagoon 3.5–5.5 cm California killifish had the greatest Ag, As, and Cd, and the second largest Cr and Pb. Se was greatest in the crayfish below the Tapia discharge point.

Sample preparation effects on metal concentrations have been reviewed in the literature (Schmitt and Finger, 1987; Lambie and Hill, 1998). The effect of dissolved solids may be important in ICP-AES analysis because our homogenates with the highest total analyzed elemental mass had the highest CVs. Similar effects were reported by Engman and Jorhem (1998) for As, Cd, Co, Cr, Cu, Mn, Ni, Pb, Se, and Zn in digests of fish fillet. At least three homogenate replicates should be nebulized to define the laboratory imprecision component of biological variation.

Table 2 provides the various guidelines for the toxic elements of the present study. Local area people use whole small fish in stews, soups, and for canning so that the consumption standards, Mearne Fish Fillet International Standards (MFS) (Nicola et al., 1987) and the Median International Standards (MIS) (Nauen, 1983), are applicable. The major reference ecology thresholds

Table 1
Elemental concentrations in homogenates (H) of various species at different sampling locations (R)

Element	DL	Black Bullhead (R-1, H-3)	Mosquitofish			Arroyo chub (R-2, H-6)	Crayfish (R-2, H-4)	California killifish		
			R-1A, <3.0 cm, H-1a	R-1A, 3.0–5.5 cm, H-1b	R-1, <5.5 cm, H-2			R-11B, <2.0 cm, H-5a	R-11B, 2.0–3.5 cm, H-5b	R-11B, 3.5–5.5 cm, H-5c
Ag	0.02	6 (5)	<0.02	0.08 (0.09)	2 (4)	<0.02	<0.02	<0.02	5 (1)	6 (5)
Al	10	41 (40)	31 (3)	14 (3)	30 (40)	15 (3)	27 (7)	36 (2)	86 (8)	70 (60)
As	0.02	6 (3)	0.2 (0.1)	2 (1)	2 (2)	1.0 (0.5)	4.1 (0.9)	1.1 (0.2)	5 (1)	6 (3)
B	0.3	40 (30)	6 (1)	10 (2)	10 (20)	9 (5)	10 (10)	7 (5)	50 (10)	30 (20)
Ba	0.05	1.8 (0.8)	1.5 (0.4)	0.8 (0.2)	0.8 (0.2)	0.8 (0.2)	2.6 (0.8)	1.1 (0.2)	1.9 (0.1)	1.7 (0.8)
Be	0.04	0.05 (0.03)	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	0.06 (0)	<0.04
Ca	3.0	22000 (9000)	5900 (3000)	6100 (3200)	13000 (7000)	7600 (1000)	15000 (9000)	12000 (2000)	22000 (2000)	20000 (14000)
Cd	0.02	0.2 (0.1)	0.04 (0.02)	0.09 (0.03)	0.07 (0.06)	0.09 (0.04)	0.17 (0.04)	0.04 (0.01)	0.18 (0.07)	0.2 (0.1)
Co	0.2	2 (1)	0.4 (0.2)	0.5 (0.2)	0.6 (0.6)	0.5 (0.2)	0.7 (0.4)	0.5 (0.2)	2.0 (0.5)	2 (1)
Cr	0.02	3 (1)	0.34 (0.03)	0.4 (0.1)	1.0 (0.4)	0.27 (0.09)	1.4 (1)	0.43 (0.06)	1.6 (0.2)	2 (1)
Cu	0.1	2 (1)	2.0 (0.6)	2.5 (0.4)	2.2 (0.5)	1.7 (0.5)	2.0 (10)	2.8 (0.4)	2.6 (0.2)	6 (5)
Fe	1.0	70 (40)	36 (2)	24 (3)	50 (40)	23 (6)	30 (40)	58 (6)	90 (7)	90 (60)
K	10	2500 (900)	54 (7)	1400 (70)	2100 (2000)	1600 (400)	8700 (5000)	3600 (200)	4800 (200)	5400 (800)
Li	0.04	1 (1)	0.15 (0.03)	0.26 (0.08)	1.3 (0.7)	0.15 (0.06)	0.6 (0.1)	0.30 (0.03)	1.40 (0.03)	1.1 (0.8)
Mg	1.0	730 (400)	140 (5)	300 (100)	490 (400)	270 (40)	1400 (600)	600 (70)	1100 (100)	1100 (300)
Mn	0.02	4 (2)	5.4 (0.3)	4 (2)	11 (7)	2.1 (0.4)	13 (7)	10 (2)	22 (2)	15 (10)
Mo	0.06	6 (5)	0.30 (0.04)	1.2 (0.3)	2 (4)	0.5 (0.2)	1.0 (0.7)	<0.06	7.9 (0.6)	6 (5)
Na	4.0	870 (300)	81 (6)	540 (40)	980 (1000)	360 (70)	4000 (2000)	2100 (100)	2300 (100)	2500 (400)
Ni	0.3	1.2 (0.7)	0.4 (1.0)	<0.3	<0.3	1 (2)	0.7 (1.0)	0.3 (1.0)	0.6 (0.6)	0.6 (0.6)
P	1.0	12000 (5000)	2600 (200)	4400 (2000)	7200 (4000)	4700 (700)	13000 (3000)	7400 (1000)	12000 (800)	12000 (6000)
Pb	0.05	4 (3)	0.1 (0.1)	0.5 (0.3)	1 (2)	0.3 (0.2)	0.3 (0.9)	0.3 (0.1)	4.1 (0.5)	3 (2)
Se	0.2	8 (5)	0.3 (0.2)	3.0 (0.4)	6 (2)	1.1 (0.2)	13 (5)	1.2 (0.3)	2 (1)	4 (1)
Si	30	60 (50)	90 (20)	60 (30)	50 (30)	57 (3)	110 (60)	80 (30)	70 (20)	80 (40)
Sr	0.05	40 (20)	10 (1)	12 (5)	20 (30)	12 (2)	30 (10)	42 (6)	77 (6)	70 (50)
Ti	0.07	1.1 (0.5)	1.4 (0.1)	0.40 (0.06)	1 (1)	0.6 (0.1)	0.9 (0.5)	2.01 (0.08)	2.6 (0.2)	3 (2)
V	0.04	0.5 (0.3)	0.15 (0.03)	0.08 (0.07)	0.3 (0.2)	<0.04	0.2 (0.4)	0.26 (0.05)	0.6 (0.2)	0.6 (0.6)
Zn	0.02	43 (20)	19 (1)	44 (6)	35 (7)	36 (7)	66 (30)	32 (3)	49 (3)	40 (20)
Average ^a		58 (19)	25 (24)	34 (22)	87 (54)	36 (38)	70 (64)	24 (22)	16 (20)	59 (24)

Data are interrune arithmetic means and standard deviations (in brackets) of triplicate 10-g wet weight homogenates, in µg element/g dry weight.

^a Average coefficient of variation in % and its standard deviation in % for all elements for a given species.

in California are the elevated data levels (EDL95 or EDL85) for whole fish (State of California Water Resources Control Board, 1993). EDLs indicate the upper 95% and 85% threshold concentrations respectively, for all fish in California waters from 1978 to 1989 caught at specific sampling stations. Species reference values are lost, and a “reference average fish” is created. Table 3 gives the homogenates that exceed the MFS, MIS, and EDL95_{whole fish}. Homogenate 5b for killifish (2.0–3.5 cm) exceeds EDL95_{whole fish} thresholds for Ag, As, Cr, and Pb, and also the MFS/MIS guidelines for As and Pb. Others with elemental concentrations of interrune CV ≤ 20% that exceed EDL95_{whole fish} are homogenates 1a, 2, and 5a for Cr. Bottom feeders tend to accumulate metals from sediments (Nicola et al., 1987), although more recent work which involved 21 elements in Nova Scotia lake winter flounder (*Pseudopleuronectes americanus*) reported positive correlations only with sediment Cd, Cu and Mn (Chou et al., 1999). Fish from upstream of the Tapia discharge at Stations R-1 and R-1A had

higher concentrations of As and Cr in general than those from below the discharge at Station R-2. However, the greatest toxic metal concentrations were found in lagoon fish.

Previous State of California monitoring data in Malibu Creek fish are limited. Bullhead livers sampled on 04/30/1985 contained 1.4 µg/g wet weight of Se, well below the EDL95 fish liver threshold of 5.92 µg/g (State of California Water Resources Control Board, 1993). The elemental concentration ranges in µg/g wet weight in the fillets of ocean-going fish taken in the nearby Santa Monica Bay for topsmelt (*Atherinops affinis*) and adult striped mullet (*Mugil cephalus*) common in Malibu Lagoon were (NOAA, 1991b) Ag, <0.002–0.003; As, 0.15–0.70; Cd, 0.001–0.029; Cr, 0.005–0.030; Cu, 0.13–0.85; Se, 0.38–1.3; and Zn, 3.0–20. If these are multiplied by the ratio of EDL95_{whole fish} to EDL95_{fillet} from Table 2, these ranges become: Ag, <0.004–0.006; As, 0.33–1.5; Cd, 0.017–0.49; Cr, 0.065–0.39; Cu, 0.74–4.8; Se, 0.36–1.2; and Zn, 3.6–24. The corresponding ranges for the

Table 2

Elements (E) of the present study with MFS (Nicola et al., 1987), MIS (Nauen, 1983), elevated data levels for 1978–1987 fish fillet at the upper 95% threshold EDLF95 and elevated data levels for 1978–1987 whole fish at the upper 95% threshold EDLWF95 (State of California Water Resources Control Board, 1993), market basket (MB) data (Gartrell et al., 1985, 1986), US FDA guidelines FDA (Gartrell et al., 1985, 1986), and World Health Organization guidelines (WHO) (HSDB, 1997)

E	MFS (µg/g)	MIS (µg/g)	EDLF95 (µg/g)	EDLWF95 (µg/g)	FDA (µg/day)	WHO (µg/kg bw)	MB (µg/g)
Ag	None	None	<0.02	0.04	None	None	None
As	1.0	1.5	0.36	0.79	None	2 ^a	0.02–0.61 ^a
Cd	1.5	0.3	0.01	0.17	57–72	None	Tr–0.062
Cr	3.5	1.0	<0.02	0.25	None	None	None
Cu	10	20	0.69	3.96	None	None	None
Ni	0.2	None	<0.10	0.42	None	None	None
Pb	2.0	2.0	<0.10	0.53	429	None	Tr–0.14
Se	2.0	2.0	3.40	3.27	50–200	None	Tr–0.52
Zn	50	45	33.20	40.6	15,000	None	5.6–38

Concentrations in µg/g are as wet weights.

^a As arsenic trioxide; Tr, trace; MB values are for meat, fish and poultry combined.

lagoon specimens of the present study were Ag, <0.003–4.4; As, 0.11–3.1; Cd, 0.032–0.11; Cr, 0.25–1.7; Cu, 1.2–6.4; Se, 0.21–4.5; and Zn, 14–38. The measured ranges for these elements observed in the present study agree reasonably well with the predicted values from ocean-going fish that are known to be present in Malibu Lagoon. Only Ag had an observed upper limit higher than an order of magnitude than predicted. In a later study by our research team (Cohen et al., 2001), California killifish 4.5–6.0 cm long taken in March and April of 1995 from the mouth of the Malibu Lagoon showed the following trace element concentrations in µg/g dry weight for $n = 11$: Ag, <0.02; As, <0.02; Cd, 0.20 ± 0.02 ; Cr, 2.8 ± 0.2 ; Cu, 3.5 ± 0.8 ; Ni, <0.3; Pb, <0.05; Se, <0.2; and Zn, 132.0 ± 9.6 . The corresponding concentrations

for these elements for California killifish of 3.5–5.5 cm length in the present study were Ag, 6 ± 5 ; As, 6 ± 3 ; Cd, 0.2 ± 0.1 ; Cr, 2 ± 1 ; Cu, 6 ± 5 ; Ni, 0.6 ± 0.6 ; Pb, 3 ± 2 ; Se, 4 ± 1 ; and Zn, 40 ± 20 . The respective concentrations for these elements in Malibu Lagoon topmelt at Station R-11B that were 8.5–9.7 cm in length in 1995 were for a homogenate of $n = 15$ fish: Ag, 0.40 ± 0.10 ; As, <0.02; Cd, 0.30 ± 0.10 ; Cr, 14.0 ± 2.2 ; Cu, 2.7 ± 1.4 ; Ni, 8.1 ± 1.8 ; Pb, 0.80 ± 0.11 ; Se, <0.2; and Zn, 85.0 ± 11 . National Contaminant Biomonitoring Program (NCBP) data of the US Fish and Wildlife Service data for these elements for 1986/1987 in 319 freshwater fish samples included the geometric mean, highest value, and 85th percentile in µg/g wet weight (Schmitt et al., 1999): As, 0.083, 1.53, 0.24; Cd, 0.011, 0.32, 0.04; Cu, 0.794, 11.0, 1.7; Se, 0.417, 3.41, 0.66; and Zn, 21.2, 94.5, 31.7. Our results for As and Cd are higher than the corresponding NCBP elemental data.

Juvenile mosquitofish <3 cm had low Na and K, and an atypical K/Na ratio (w/w) of 0.66 relative to 2.2–2.8 for other mosquitofishes and 1.7–4.4 for other specimens of the present study. The Ca/P ratio of 2.4 also differed relative to 1.4–1.8 for other mosquitofish, and 1.2–1.8 for the other fish. Mortality of the fathead minnow (*Pimephales promelas*) has been correlated to whole body Na depuration after toxic chemical challenge (Grippe and Dunson, 1991).

The elements measured in fish of the present study were not unique markers for the wastewater treatment plant effluent. The same fish species could not be compared above and below the Tapia discharge as sampling collected only what was available at a single day in time. Comparatively few fish species were also caught. Only a single time 1993 sampling was attempted for economic, weather, and logistical reasons. Subsequent sampling in 1995 resulted in fish metal concentrations that agreed within an order of magnitude. Sampling of unique markers of the effluent is necessary to prove effluent pollution.

Table 3

Homogenates and elements (E) in Table 1 fish whose arithmetic mean wet weight concentrations exceed the MFS, MIS, and the State of California elevated data level 95% threshold for whole fish (EDL) in Table 2

E	Guideline	Homogenates (H) exceeding the guideline threshold
Ag	EDL	H-1b, -2, -3, -5b, ^a -5c
As	EDL	H-1b, ^b -2, -3, ^b -4, ^b -5b, ^b -5c, ^b -6
	MFS	H-1b, -2, -3, ^b -4, -5b, ^a -5c
	MIS	H-1b, -3, -5b, ^a -5c
Cr	EDL	H-1a, ^a -1b, ^b -2, ^a -3, ^b -4, -5a, ^a -5b, ^a -5c, ^b
	MIS	H-3 ^b
Cu	EDL	H-4
Ni	EDL	H-3, -6
	MFS	H-1a, -3, -4, -5a, -5b, -5c, -6
Pb	EDL	H-2, -3, -5b, ^a -5c ^b
	MFS/MIS	H-3, -5b ^a
Se	EDL	H-2, -3, -4
	MFS/MIS	H-1b, ^a -2, ^b -3, ^b -4, ^b -5c

^a Concentration of CV < 20%.

^b Concentration of CV > 20% and mean \pm SD exceeds guideline.

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