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Accumulation of Contaminants in Fish from Wastewater Treatment Wetlands

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Increasing demands on water resources in arid environments make reclamation and reuse of municipal wastewater an important component of the water budget. Treatment wetlands can be an integral part of the water-reuse cycle providing both water-quality enhancement and habitat functions. When used for habitat, the bioaccumulation potential of contaminants in the wastewater is a critical consideration. Water and fish samples collected from the Tres Rios Demonstration Constructed Wetlands near Phoenix, Arizona, which uses secondary-treated wastewater to maintain an aquatic ecosystem in a desert environment, were analyzed for hydrophobic organic compounds (HOC) and trace elements. Semipermeable membrane devices (SPMD) were deployed to investigate uptake of HOC. The wetlands effectively removed HOC, and concentrations of herbicides, pesticides, and organic wastewater contaminants decreased 40−99% between inlet and outlet. Analysis of *Tilapia mossambica* and *Gambusia affinis* indicated accumulation of HOC, including *p,p′*-DDE and *trans*-nonachlor. The SPMD accumulated the HOC detected in the fish tissue as well as additional compounds. Trace-element concentrations in whole-fish tissue were highly variable, but were similar between the two species. Concentrations of HOC and trace elements varied in different fish tissue compartments, and concentrations in *Tilapia* liver tissue were greater than those in the whole organism or filet tissue. Bioconcentration factors for the trace elements ranged from 5 to 58 000 and for the HOC ranged from 530 to 150 000.

Introduction

Water is a scarce resource in many parts of the world and reclaimed wastewater is used to satisfy increasing demands (*1*). Treatment wetlands can provide additional polishing to wastewater treatment plant (WWTP) effluent while also creating habitat for a variety of plant and animal species (*2*, *3*). Organisms living in treatment wetlands can be exposed to contaminants through the water column, sediments, and successive food-web pathways. Thus, potential toxicity and ecosystem disruption from bioaccumulation of hydrophobic organic compounds (HOC) and trace elements are important considerations for treatment wetlands used to sustain a habitat function. Treatment wetlands effectively remove total suspended solids and nutrients (*3*) but the fate of trace-element and organic contaminants is less established (*4*−*11*). Although HOC and trace elements in WWTP effluents occur at low concentrations, potential ecological risks exist for treatment wetlands due to prolonged exposures in the 100% effluent maintained systems.

Bioconcentration occurs as water-borne chemicals accumulate in aquatic biota by diffusion through biological membranes in direct contact with water (*12*−*14*). Bioaccumulation describes uptake of contaminants by both diffusion and ingestion of food and sediment. Bioconcentration of HOC results from thermodynamically driven hydrophobic partitioning between water and lipid (*15*, *16*). Other factors influencing mass transfer include aqueous speciation, diffusion, metabolism, and environmental factors such as temperature and nutrient status (*17*, *18*). Bioconcentration can be described by the following (*12*, *13*):

$$\frac{dC_f}{dt} = k_u C_w - k_e C_f \quad (1)$$

where C_f is concentration in fish (ng/kg), C_w is concentration in water (ng/L), t is time, k_u is fish uptake rate ($^1/_t$), and k_e is fish elimination rate ($^1/_t$). The tendency of a chemical to accumulate in biota is described by the bioconcentration factor (BCF), a steady-state proportionality constant relating the concentration in fish tissue to the concentration in the aqueous phase:

$$\mathrm{BCF} = \frac{C_f}{C_w} = \frac{k_u}{k_e} \quad (2)$$

The BCF depends on species type and size as well as characteristics of the chemical in question. For HOC, the BCF can be estimated from physicochemical properties such as octanol/water partition coefficient, K_{ow} (*12*−*16*). Generally, BCF increases with increasing K_{ow}, although measured BCF can be lower than values estimated from chemical properties due to environmental factors. For example, compounds with large K_{ow} can have BCF values lower than predicted because partitioning of the compound into dissolved organic matter makes it unavailable for biouptake (*18*). Metabolism can affect the BCF by increasing the rate of elimination and lowering the amount of compound available for storage. In general, HOC preferentially partition into lipid-rich tissue, and normalizing BCF to lipid content can reduce variability of results. Although important from the perspective of habitat function, the complexity of evaluating bioaccumulation in food webs (*19*) is beyond the scope of this study.

Semipermeable membrane devices (SPMD) are in-situ samplers containing triolein inside low-density polyethylene tubing, and can mimic diffusive uptake of HOC into the lipid phase of aquatic organisms (*20*−*22*). The capacity of SPMD to accumulate HOC is related to the characteristics of the compound, demonstrated by the correlation between the triolein/water partition coefficients and BCF (*16*). Because SPMD are exposed to large volumes of water over extended periods of time, they provide an integrative approach to measuring low-level contaminants in aquatic environments. Uptake curves for SPMD have linear, curvilinear, and equilibrium regions, and the greatest uptake typically occurs in the linear region where C_w is greater than the concentration

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in the SPMD (C_{SPMD}, ng/kg). The uptake curve can be defined as (*22*):

$$C_{SPMD} = C_W K_{SPMD}\lfloor 1 - e^{-k_{uSPMD}t}\rfloor \quad (3)$$

where K_{SPMD} is the SPMD/water partition coefficient (C_{SPMD}/C_w) and k_{uSPMD} is the SPMD uptake rate ($^1/_t$):

$$k_{uSPMD} = \frac{R_S K_{SPMD}}{V_{SPMD}} \quad (4)$$

where V_{SPMD} is volume of lipid and membrane in the SPMD (L) and R_S is the sampling rate (L/d). Aqueous concentrations can be estimated from C_{SPMD} at equilibrium (typically deployed for 28 days) by:

$$C_w = \frac{C_{SPMD}}{K_{SPMD}} \quad (5)$$

Trace-element concentrations are defined in terms of soluble or total forms and can be introduced into organisms by diffusion from water or dietary consumption. In contrast to HOC, many trace elements are essential for organism health and aquatic biota have varying accumulation and excretion strategies for regulating internal concentrations (*23*, *24*). Essential elements are subject to regulation by limiting uptake at the total body concentration level, active excretion from the excess pool, or storage in an inert form. Nonessential element regulation involves excretion from the excess pool or internal storage. Body concentrations generally increase with increasing C_w, although the internal regulatory process can result in an inverse relation between C_w and BCF. For example, at low C_w organisms actively accumulate trace elements to achieve their metabolic needs, whereas at higher concentrations regulatory mechanisms limit additional uptake (*25*).

In this study we evaluate the bioaccumulation potential for a variety of inorganic and organic contaminants in a constructed treatment wetland supplied by WWTP effluent. Bioaccumulation potential was determined by measuring concentrations of major elements, trace elements, and HOC in aquatic and biota samples. In addition, SPMD were deployed to quantitatively evaluate HOC removal by the wetlands and the potential for uptake by biota.

Materials and Methods

Site Description. The Tres Rios Demonstration Constructed Wetlands, located near Phoenix, Arizona, consists of four 0.89−1.3 ha free-water-surface treatment wetlands having mixed deep-water and shallow-water zones (*26*, *27*). The wetlands operate under controlled flow conditions, receiving 7500 m^3/d (2 million gallons per day) of secondary-treated effluent. During the sampling period, hydraulic retention times ranged from 3−4 days at average water depths of 0.1−0.5 m and hydraulic loading rates of 7.5−15 cm/d. The wetlands provide habitat for a number of aquatic species including tilapia (*Tilapia mossambica*) and mosquitofish (*Gambusia affinis*). Vegetation is softstem bulrush (*Schoenoplectus tabernaemontai*), Olney's bulrush (*Schoenoplectus americanus*), duckweed (*Lemna* spp.), and marsh pennywort (*Hydrocotyle* spp.).

Sampling and Analysis. Detailed descriptions of sample collection and chemical analysis are presented elsewhere (*28*). Water samples were collected during summer (July and August 1998) and winter (February 2000) conditions and analyzed for dissolved organic carbon (DOC), pesticides, organic wastewater contaminants, HOC, major elements, and trace elements. *Tilapia* and *Gambusia* were collected at the same time. Individual *Tilapia* were weighed and measured, and *Gambusia* were collected as composites of approximately 100 fish. Samples were stored at −20 °C until analysis. For trace element analysis, individual *Tilapia* were dissected using ceramic instruments to give liver, filet, and other (bone, skin, nonliver organs) tissue samples. Whole-body *Gambusia* and *Tilapia* filet were minced and homogenized by ceramic knife, and *Tilapia* liver was homogenized with a Teflon probe. Similar processing was used for HOC analysis.

The SPMD (Environmental Sampling Technologies, St. Joseph, MO) had a 0.2 triolein/membrane ratio (w/w), a membrane thickness of 75−90 μm, and a surface area/volume ratio of 450 cm^2/mL (*21*). During July and August 1998, SPMD were deployed for 4 weeks at the wetland inlets and outlets. An uptake-rate study (SPMD retrieved weekly or biweekly) was conducted during June and August 1999. Similar flow and temperature regimes existed between the wetland inlet and outlet during both deployments.

Field measurements were made at the time of sampling. Dissolved organic carbon was analyzed by ammonium persulfate/ultraviolet light oxidation with conductivity detection (*7*). Filtered water samples were analyzed for pesticides and metabolites (*29*, *30*) using C_{18} solid-phase extraction, elution with methylene chloride, and analysis by electron impact gas chromatography/mass spectrometry in the selected-ion monitoring mode (SIM GC/MS). Organic wastewater contaminants were extracted by continuous liquid−liquid extraction with methylene chloride and analyzed by SIM GC/MS (*31*). Ethylenediaminetetraacetic acid (EDTA) and nonylphenolethoxycarboxylic acids (NPEC) were isolated by evaporation, derivatized with acetyl chloride/propanol, and analyzed by SIM GC/MS (*31*).

Fish tissue was analyzed for HOC (*32*) using Soxhlet-extraction with methylene chloride, gel-permeation chromatography, alumina/silica chromatography, and dual-column gas chromatography with electron-capture detection (GC/ECD). The SPMD samples were extracted by hexane dialysis, fractionated by gel-permeation chromatography, and analyzed for HOC by GC/ECD.

Major cations were determined in filtered water samples by inductively coupled plasma/atomic-emission spectrometry (*33*) and trace elements were analyzed by inductively coupled plasma/mass spectrometry (*34*). Mercury was measured by cold-vapor atomic-fluorescence spectrometry (*35*). Fish tissue samples were microwave digested in closed Teflon vessels using high-purity nitric acid and analyzed for major cations and trace elements as described above.

Results

Comprehensive inorganic and organic chemical analyses were conducted on water, fish, and SPMD samples, and the complete data set is presented elsewhere (*28*). There was little difference in specific conductance and DOC between wetland inlets and outlets (Table 1). The metal complexing agent EDTA (*36*) was the most abundant (maximum concentration of 310 μg/L) organic contaminant measured in the wetland inflow (Table 1), and concentrations were attenuated 64−86% in the wetlands with the greatest removal in the summer. The next most abundant organic compounds in the wetland inflow were the degradation products of nonylphenolethoxylate (NPE) nonionic surfactants (*37*) including nonylphenolmonoethoxycarboxylic acid (NP1EC), nonylphenoldiethoxycarboxylic acid (NP2EC), nonyphenolmonoethoxylate (NP1EO), nonylphenoldiethoxylate (NP2EO), and nonylphenol. The NPEC compounds (140−160 μg/L) comprised over 95% of the total NPE degradates measured, and concentrations decreased 16−57% between inlet and outlet with greater removal in the summer. Nonylphenol concentrations decreased 37−42%, and NP1EO and NP2EO removal was greater than 90%. Other important wastewater indicator compounds (*38*, *39*), including triclosan and

TABLE 1. Summary of Concentrations and Percent Removals for Field Measurements, Dissolved Organic Carbon, Organic Wastewater Contaminants, and Pesticides Detected at the Tres Rios Wetlands during Summer (August 1998) and Winter (February 2000) Conditions[a]

compound	conc units	Summer			Winter		
		inlet[b]	outlet[b]	removal %[c]	inlet[b]	outlet[b]	removal %[c]
Field Measurements and Dissolved Organic Carbon							
temperature	°C	32.6	30.2	+7.4	24.6	15.1	+39
pH	std units	7.16	7.07	−1.2	6.94	7.27	+4.8
specific conductance	μS/cm	1722	1702	−1.2	1353	1365	+0.8
dissolved oxygen	mg/L	4.0	1.1	+72	3.4	4.2	−24
dissolved organic carbon	mg/L	8.0	8.4	+5.0	9.4	8.4	−10.6
Organic Wastewater Contaminants							
bisphenol A	ng/L	120	25	+79	120	104	+13
4-*tert*-butylphenol	ng/L	31	16	+48	21	35	−67
caffeine	ng/L	490	181	+63	650	87	+87
cholesterol	ng/L	39	300	>−1000	570	870	−53
2,6-di-*tert*-butyl-1,4-benzoquinone	ng/L	250	130	+48	470	106	+77
2,6-di-*tert*-butyl-4-methylphenol	ng/L	20	<5	- -	43	14	+67
1,4-dichlorobenzene	ng/L	180	31	+83	170	74	+57
17-*alpha*-estradiol	ng/L	<5	<5	- -	25	425	>−1000
ethylenediaminetetraacetic acid (EDTA)	μg/L	310	43.5	+86	240	86.5	+64
4-methylphenol	ng/L	32	<5	- -	11	<5	- -
4-nonylphenol	μg/L	0.66	0.42	+37	0.64	0.37	+42
4-nonylphenolmonoethoxylate (NP1EO)	μg/L	0.34	<0.05	- -	2.6	<0.05	- -
4-nonylphenoldiethoxylate (NP2EO)	μg/L	0.24	<0.05	- -	0.68	<0.05	- -
4-nonylphenolmonoethoxycarboxylic acid (NP1EC)	μg/L	140	60.5	+57	150	97.5	+35
4-nonylphenoldiethoxycarboxylic acid (NP2EC)	μg/L	150	99.5	+34	160	135	+16
4-*tert*-octylphenol	ng/L	45	21	+53	34	26	+24
4-*tert*-octylphenolmonoethoxylate	ng/L	46	<5	- -	26	<5	- -
4-*tert*-octylphenoldiethoxylate	ng/L	110	<5	- -	99	<5	- -
4-propylphenol	ng/L	<5	<5	- -	28	42	−50
triclosan	ng/L	81	86	−6	130	92	+29
Pesticides							
carbaryl	ng/L	5	<1	- -	na	na	na
chloropyrifos	ng/L	11	<1	- -	na	na	na
diazinon	ng/L	100	49	+51	na	na	na
3,4-dichloroaniline	ng/L	150	340	−127	na	na	na
prometryn	ng/L	12	13	−8	na	na	na
simazine	ng/L	13	<1	- -	na	na	na

[a] Conc, concentration; ng/L, nanogram per liter; mg/L, milligram per liter; μg/L, microgram per liter; μS/cm, microSiemen per centimeter; Removal, percent (%) concentration change between inlet and outlet, a positive value indicates decreasing concentrations and a negative value indicates increasing concentrations; <, less than; - -, not calculated because values were below detection limit; na, not analyzed. [b] Average concentration for replicate samples (inlet $n = 4$, outlet $n = 8$). [c] Average removal for all samples.

caffeine, were present at concentrations similar to those of nonylphenol and also were attenuated in the wetland. The degradate (3,4-dichloroaniline) of the herbicides linuron and diuron had outlet concentrations greater than inlet concentrations, but no parent compounds were detected. None of the HOC measured by the method used in this study were detected in the water samples (detection limit ~2 ng/L).

Concentrations of major cations and some trace elements, such as arsenic, boron, lead, and lithium were relatively constant between inlets and outlets (Table 2). The rare earth elements (REE), including gadolinium, increased between inlets and outlets. Other elements such as copper, iron, and mercury decreased across the wetlands.

Whole-body *Tilapia* collected during the winter of 2000 contained *p,p′*-DDE and *trans*-nonachlor, but the filet tissue did not have detectable levels of HOC (Table 3). The *Gambusia* collected in 2000 also contained *p,p′*-DDE and *trans*-nonachlor, as well as *cis*-chlordane, *trans*-chlordane, dieldrin, lindane, and PCBs. However, the *Gambusia* were collected from a different wetland unit than the *Tilapia*.

Trace-element concentrations in *Tilapia* liver usually were greater than those in *Tilapia* filet, and were similar to those in whole-body *Gambusia* (Table 2). Figure 1 presents data for select trace elements in whole-body *Gambusia* and *Tilapia* filet and liver. There was considerable variation in concentrations between individual *Tilapia*. Because the fish were introduced into the wetlands (*Gambusia* for vector control and *Tilapia* inadvertently) several years before the sampling, they had effectively lived their entire life in WWTP effluent and differences in concentrations between individuals can likely be attributed to age variations among the fish. The 2 fish species maintain a robust population in the treatment wetlands.

The SPMD accumulated HOC detected in the fish tissue as well as additional compounds, and concentrations declined between wetland inlets and outlets (Table 4). Several organic wastewater contaminants, including nonylphenol and NP2EO were detected in the SPMD. The SPMD uptake study (1999) indicated that at 28 days *cis*-chlordane, dieldrin, *trans*-nonachlor, and pentachloroanisole had greater than 70% removal between the inlets and outlets, lindane had 55% removal, and *p,p′*-DDE concentrations increased. The inlet SPMD uptake data exhibited linear behavior during the first 2 weeks of deployment, and approached equilibrium by 4 weeks, whereas the outlet SPMD took longer to reach steady state (Figure 2). Although flow and temperature were constant between the inlet and outlet, other factors including decreasing C_w and biofouling could influence the outlet SPMD uptake rate.

Discussion

The WWTP effluent supplying the water for the treatment wetlands is a complex mixture of inorganic and organic chemicals derived from the natural-water supply and domestic and commercial activity. Although contaminants experience daily concentration fluctuations, most chemical sources to the WWTP are relatively constant. The average hydraulic retention time (3.5 days; *27*) of the Tres Rios treatment wetlands is relatively short, resulting in a linear flow velocity of 65 m/d for the 228 m long wetlands. In

TABLE 2. Concentrations of Major Elements and Trace Elements in Water, *Gambusia*, and *Tilapia*, Their Associated Wetland Removals, and Their Bioconcentration Factors (BCF) for Samples Collected from the Tres Rios Wetlands, February 2000[a]

element	inlet[b] C_w µg/L	RSD %	outlet[b] C_w µg/L	RSD %	removal %	*Gambusia* whole[c] C_f mg/kg	RSD %	*Tilapia* filet[d] C_f mg/kg	RSD %	*Tilapia* liver[e] C_f mg/kg	RSD %	*Gambusia* whole BCF[f] L/kg	*Tilapia* filet BCF[f] L/kg	*Tilapia* liver BCF[f] L/kg	BCF_{liver}/BCF_{filet}
Major Elements															
Ca	61000	(1)	66000	(4)	−8	53000	(4)	1200	(120)	5600	(63)	870	20	92	4.7
K	17000	(1)	17000	(5)	0	12000	(5)	15000	(20)	11000	(83)	710	880	650	0.7
Mg	30000	(2)	31000	(4)	−3	1900	(5)	1200	(18)	2400	(110)	63	40	80	2.0
Na	179000	(9)	177000	(7)	+1	3700	(6)	5100	(22)	4300	(77)	21	28	24	0.8
P	4900	(25)	4300	(62)	+12	29000	(2)	8300	(23)	6100	(101)	5900	1700	1200	0.7
S	63000	(1)	67000	(2)	−6	12000	(8)	12000	(22)	5500	(99)	190	190	87	0.5
Si	8900	(0)	8100	(6)	+9	< 250	- -	< 300	- -	680	(190)	- -	- -	76	- -
Trace Elements															
Al	6.7	(7)	6.0	(71)	+11	22	(21)	< 0.9	- -	200	(120)	3200	- -	30000	- -
As	5.1	(10)	5.4	(4)	−5	0.46	(25)	0.46	(33)	1.7	(120)	89	89	330	3.7
B	590	(10)	560	(2)	+5	< 9	- -	< 10	- -	< 7	- -	- -	- -	- -	- -
Ba	17	(13)	12	(27)	+28	9.4	(0)	< 0.06	- -	9.3	(130)	570	- -	570	- -
Be	< 0.007	- -	< 0.009	- -	- -	< 0.3	- -	< 0.3	- -	< 0.2	- -	- -	- -	- -	- -
Bi	0.017	(5)	0.006	(58)	+66	< 0.005	- -	< 0.006	- -	0.24	(120)	- -	- -	- -	- -
Cd	0.012	(46)	0.011	(310)	+5	< 0.07	- -	< 0.07	- -	< 0.2	- -	- -	- -	- -	- -
Ce	0.012	(9)	0.046	(95)	−287	0.092	(5)	< 0.003	- -	0.39	(130)	7800	- -	33000	- -
Co	0.13	(90)	0.26	(33)	−107	< 0.05	- -	< 0.06	- -	0.52	(130)	- -	- -	4100	- -
Cr	< 0.2	- -	< 0.4	- -	- -	< 2	- -	< 2	- -	< 1	- -	- -	- -	- -	- -
Cs	0.04	(260)	< 0.04	- -	- -	< 1	- -	< 2	- -	< 1	- -	- -	- -	- -	- -
Cu	1.78	(2)	1.57	(9)	+12	8.3	(6)	3.2	(47)	51	(15)	4600	1800	28000	16
Dy	0.0014	(17)	0.0039	(64)	−182	0.007	(120)	< 0.008	- -	0.01	(510)	5000	- -	4900	- -
Er	0.0015	(25)	0.0033	(48)	−127	< 0.01	- -	< 0.01	- -	< 0.01	- -	- -	- -	- -	- -
Eu	< 0.0002	- -	0.0012	(95)	- -	< 0.003	- -	< 0.004	- -	0.004	(430)	- -	- -	- -	- -
Fe	83	(3)	55	(41)	+33	180	(11)	13	(80)	1600	(120)	2200	150	19000	130
Gd	0.046	(85)	0.093	(30)	−103	< 0.009	- -	< 0.009	- -	0.021	(190)	- -	- -	460	- -
Hg	0.0011	(41)	0.0005	(29)	+51	0.064	(0)	0.008	(52)	0.054	(110)	58000	7700	49000	6.3
Ho	0.0007	(9)	0.0012	(36)	−85	< 0.002	- -	< 0.002	- -	0.005	(180)	- -	- -	7600	- -
La	0.004	(24)	0.016	(104)	−311	0.045	(2)	< 0.003	- -	0.16	(130)	11000	- -	42000	- -
Li	59	(16)	56	(8)	+5	0.3	(10)	0.1	(470)	0.5	(190)	5	2	8	5.0
Lu	0.0004	(44)	0.0007	(38)	−67	< 0.002	- -	< 0.002	- -	< 0.001	- -	- -	- -	- -	- -
Mn	23	(3)	16	(51)	+32	18	(7)	1.1	(83)	6.4	(110)	770	47	280	5.9
Mo	12	(11)	16	(11)	−39	< 0.1	- -	< 0.1	- -	4	(49)	- -	- -	360	- -
Nd	0.006	(9)	0.018	(94)	−216	0.038	(30)	< 0.01	- -	0.11	(120)	6700	- -	20000	- -
Ni	5.2	(28)	6.0	(8)	−15	< 0.2	- -	< 0.1	- -	1.8	(120)	- -	- -	350	- -
Pb	0.19	(15)	0.19	(14)	0	0.05	(330)	< 0.04	- -	0.44	(140)	240	- -	2300	- -
Pr	0.0013	(3)	0.0043	(93)	−229	0.013	(13)	< 0.001	- -	0.037	(120)	9600	- -	28000	- -
Rb	12	(6)	12	(10)	−2	11	(6)	9.0	(21)	10	(120)	890	750	860	1.1
Re	0.074	(32)	0.061	(7)	+18	< 0.006	- -	< 0.006	- -	< 0.01	- -	- -	- -	- -	- -
Sb	0.43	(1)	0.53	(8)	−24	< 0.04	- -	< 0.05	- -	< 0.03	- -	- -	- -	- -	- -
Se	0.7	(73)	0.5	(54)	+26	5.2	(7)	4.7	(43)	10	(85)	7700	6900	15000	2.2
Sm	0.0013	(7)	0.0052	(80)	−296	0.011	(27)	< 0.008	- -	0.034	(170)	8500	- -	26000	- -
Sr	725	(2)	759	(6)	−5	170	(6)	4	(130)	31	(89)	230	5	43	8.8
Ta	< 0.002	- -	< 0.002	- -	- -	0.019	(78)	0.019	(56)	0.02	(190)	- -	- -	- -	- -
Tb	0.0005	(23)	0.0009	(30)	−100	< 0.002	- -	< 0.002	- -	0.002	(440)	- -	- -	5200	- -
Te	0.024	(24)	0.020	(18)	+18	< 0.1	- -	< 0.1	- -	< 0.3	- -	- -	- -	- -	- -
Th	0.0014	(22)	0.0042	(51)	−199	< 0.008	- -	< 0.009	- -	0.034	(170)	- -	- -	25000	- -
Tl	< 0.003	- -	< 0.007	- -	- -	< 0.06	- -	< 0.07	- -	< 0.1	- -	- -	- -	- -	- -
Tm	0.0003	(20)	0.0005	(60)	−79	< 0.002	- -	< 0.002	- -	< 0.001	- -	- -	- -	- -	- -
U	2.3	(24)	2.3	(7)	−4	0.035	(30)	< 0.006	- -	0.21	(120)	15	- -	94	- -
V	3.6	(18)	5.1	(25)	−42	< 0.8	- -	< 0.9	- -	2.7	(180)	- -	- -	760	- -
Y	0.015	(23)	0.032	(40)	−111	0.028	(27)	0.008	(74)	0.088	(120)	1900	560	5900	11
Yb	0.0021	(19)	0.0043	(40)	−99	< 0.008	- -	< 0.008	- -	< 0.01	- -	- -	- -	- -	- -
Zn	18	(32)	14	(33)	+21	180	(0)	49	(24)	110	(110)	9900	2700	6000	2.2
Zr	0.045	(23)	0.040	(11)	+13	0.13	(14)	0.07	(40)	1.13	(120)	2900	1400	25000	17

[a] C_w, dissolved aqueous concentration; C_f, concentration in fish tissue; µg/L, microgram per liter; mg/kg, milligram per kilogram; RSD, percent (%) relative standard deviation; removal, percent concentration change between inlet and outlet, a positive value indicates decreasing concentrations and a negative value indicates increasing concentrations; <, less than; - -, not calculated because water or tissue values were below the detection limit; tissue concentrations reported on a dry weight basis. [b] Median concentration for replicate analysis (inlet $n = 4$, outlet $n = 8$) of samples analyzed in triplicate. [c] Median concentration for triplicate analysis of single composite sample prepared from ~100 individual specimens. [d] Median concentration for 7 individual samples each analyzed in triplicate. [e] Median concentration for 5 individual samples each analyzed in triplicate. [f] BCF calculated from eq 2 using inlet C_w values.

contrast, streamflow velocities are on the order of tens of kilometers per day (*38*). However, even in wastewater dominated systems with high flow velocities, the potential for bioconcentration exists due to the constant nature of the WWTP effluent source. Because the hydraulic regime in the Tres Rios treatment wetlands is maintained exclusively by WWTP effluent, dilution by mixing with water of a different chemical character is not a significant attenuation factor.

The presence of EDTA and NPEC in the wetlands at µg/L concentrations reflects their high use, high water solubility, and relative recalcitrance. Although EDTA is not attenuated by WWTP processes due to resistance to biodegradation and low affinity for sorption, it is susceptible to photolytic degradation (*36*) which likely accounts for the removal in the solar irradiated treatment wetlands. Concentrations of NPEC also were attenuated in the wetlands although removal was less than for EDTA. The mechanisms for removal of the NPE derived compounds are complex and include photolysis, volatilization, biodegradation, and sorption (*37*).

Attenuation of trace elements in the wetlands (Table 2) can result from precipitation, sorption, and uptake by biota. Removal for the winter 2000 samples varied for different trace

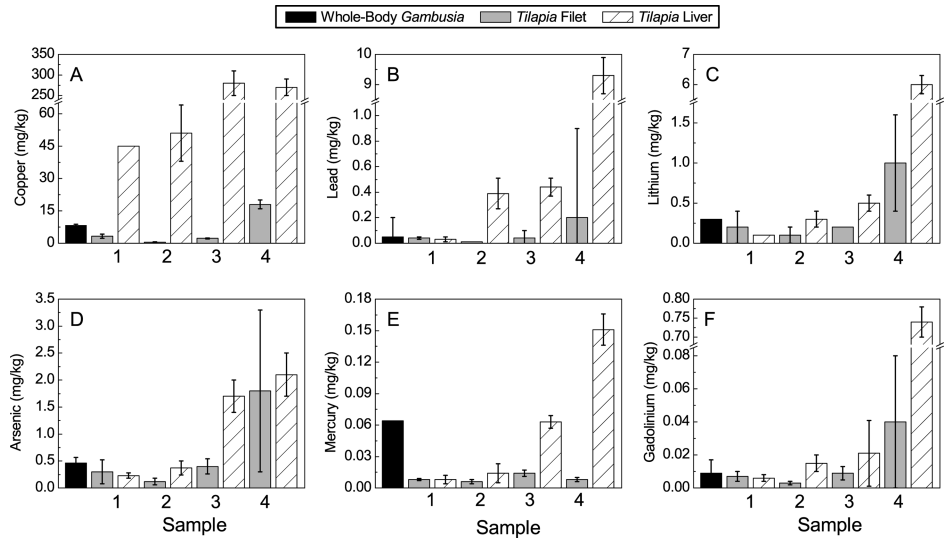

FIGURE 1. Concentrations for (A) copper, (B) lead, (C) lithium, (D) arsenic, (E) mercury, and (F) gadolinium in whole-body *Gambusia*, and in filet and liver tissue of four individual *Tilapia* specimens collected February 2000 [mg/kg, milligram per kilogram dry weight tissue concentrations; error bars indicate one standard deviation ($n = 3$)].

TABLE 3. Concentrations (Wet Weight) of Hydrophobic Organic Compounds in *Gambusia* and *Tilapia* Collected February 2000 from the Tres Rios Wetlands[a]

compound	*Gambusia* whole[b] μg/kg	*Tilapia* whole[c] μg/kg	*Tilapia* filet[d] μg/kg	*Tilapia* liver[d] μg/kg	*Tilapia* other[d,e] μg/kg
lipid (%)	3.4	1.5	0.9	3.3	1.3
cis-chlordane	E3	<5	<5	<5	<5
trans-chlordane	E4	<5	<5	<5	<5
p,p′-DDE	44	13	<5	13	6.5
dieldrin	8.3	<5	<5	<5	<5
lindane	6.4	<5	<5	<5	<5
trans-nonachlor	61	10	<5	9	E4.5
PCB	2400	<50	<50	<50	<50

[a] μg/kg, microgram per kilogram; <, less than; E, compound detected at less than quantitation limit, and concentration was estimated. [b] Composite sample prepared from ~100 individual specimens. [c] Composite sample prepared from 5 individual specimens. [d] Composite sample prepared from dissection of 5 individual specimens. [e] Other tissue consists of remaining body parts exclusive of filet and liver.

TABLE 4. Concentrations of Hydrophobic Organic Compounds and Organic Wastewater Contaminants in Semipermeable Membrane Devices (SPMD) Deployed for 28 Days During the Summer (July and August) of 1998 and the Summer (June and July) of 1999 in the Tres Rios Wetlands[a]

compound	1998 inlet conc (μg/kg)	1998 outlet conc (μg/kg)	1998 removal (%)	1999 inlet conc (μg/kg)	1999 outlet conc (μg/kg)	1999 removal %
Hydrophobic Organic Compounds						
cis-chlordane	32	8.8	+73	4.6	E0.3	+93
trans-chlordane	38	10	+74	4.8	<1	- -
p,p′-DDE	13	3.6	+72	6.0	6.7	−12
dieldrin	92	26	+72	13	3.1	+76
endosulfan II	168	7.4	+96	<1	<1	- -
heptachlor epoxide	13	3.2	+75	<1	<1	- -
hexachlorobenzene	18	1.8	+90	<1	2.5	- -
lindane	140	38	+73	13	5.8	+55
cis-nonachlor	2.8	<1	- -	<1	<1	- -
trans-nonachlor	13	3.4	+74	120	16	+87
oxychlordane	4.6	<1	- -	<1	<1	- -
pentachloroanisole	36	2.4	+93	28	8.1	+71
Organic Wastewater Contaminants						
1,4-dichloro-benzene	E0.2	E0.3	- -	na	na	na
4-nonylphenol	E15	E20	- -	na	na	na
4-nonylphenoldi-ethoxylate	E0.4	<1	- -	na	na	na
normal-octyl-phenol	E0.5	E0.6	- -	na	na	na
tert-octylphenol	E0.3	E0.9	- -	na	na	na
tert-octylphenol-diethoxylate	E0.8	E3.2	- -	na	na	na
triclosan	E4.6	E4.2	- -	na	na	na

[a] Conc, concentration; μg/kg, microgram per kilogram of SPMD; removal, percent (%) change in concentration between wetland inlet and outlet, a positive value indicates decreasing concentrations and a negative value indicates increasing concentrations; <, less than; - -, removal not calculated because values were below detection limit; E, estimated value; na, not analyzed.

elements, but generally can be grouped into the following: (1) elements with less than 10% change, (2) elements with greater than 10% decrease in concentration across the wetlands, and (3) elements with greater than 10% increase across the wetlands. The increase in REE concentrations across the wetlands indicates the influence of sediment water interactions during wetland residence. Gadolinium has been used as an indicator of anthropogenic contamination, and can be enriched in WWTP effluents due to its use as a contrast agent in magnetic-resonance-imaging medical diagnostic procedures (*40*, *41*). When the dissolved REE concentrations are normalized to natural abundance using the North American Shale Composite (*42*), a well-defined positive gadolinium anomaly is present (Figure 3A) and persists across the wetlands, consistent with results from other WWTP effluent impacted waters (*38*, *40*, *41*). Gadolinium concentrations were near the method detection limit for whole-body *Gambusia* and *Tilapia* filet tissue, but when the *Tilapia* liver REE data are normalized to sediment abundance, the gadolinium anomaly is absent (Figure 3B). The absence of the anomaly in biological tissue indicates that the REE composition of the fish is more strongly influenced by sediment exposure (through ingestion) than by uptake from the aqueous phase.

Using the approach of evaluating accumulation in the various tissue compartments provides insight into potential bioaccumulation in animals that feed on the wetland fish. The low concentrations of HOC and trace-element contaminants in *Tilapia* filet indicates reduced risk if only the muscle tissue is consumed (human consumption) relative to the whole organism (wildlife consumption), which is of interest if aquaculture and fishing are water-resource management objectives (*43*). Whole-body *Tilapia* and *Gambusia* show the presence of *p,p′*-DDE and *trans*-nonachlor, with higher concentrations in *Gambusia* (Figure 4). The whole-body *Gambusia* lipid content was more than twice that of

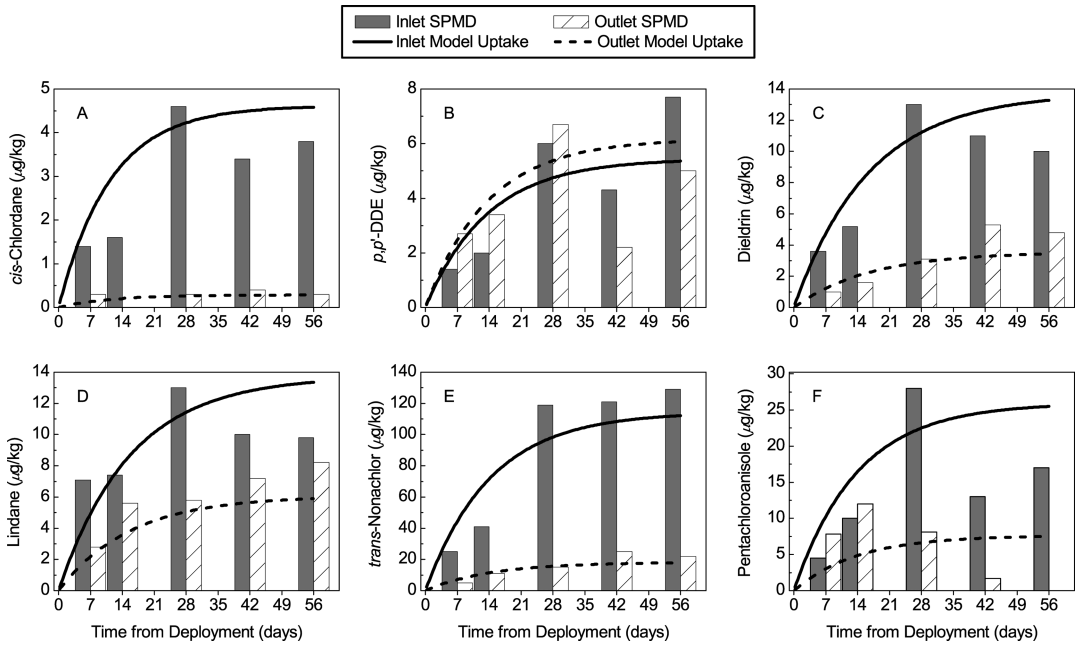

FIGURE 2. Concentrations of (A) *cis*-chlordane, (B) *p,p′*-DDE, (C) dieldrin, (D) lindane, (E) *trans*-nonachlor, and (F) pentachloroanisole in semipermeable membrane devices (SPMD) deployed June to August 1999. Modeled uptake curves for the inlets and outlets were calculated using eqs 3 and 4 and C_w, K_{SPMD}, and R_s data given in Table 5 [μg/kg, microgram per kilogram of SPMD].

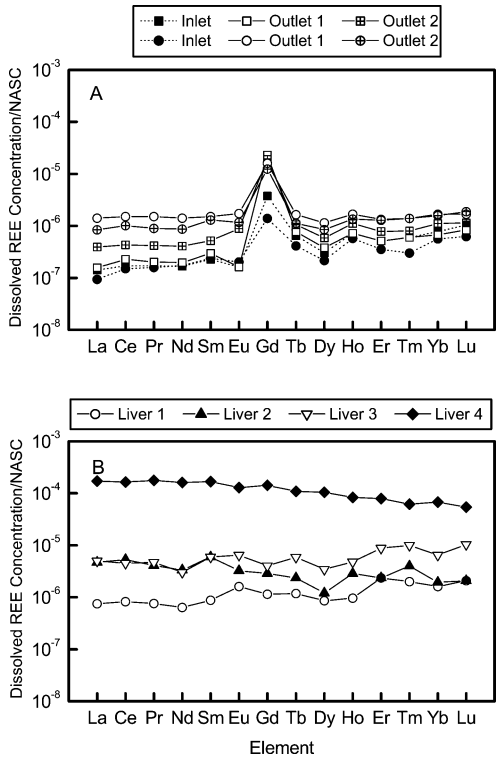

FIGURE 3. Rare earth element (REE) concentrations normalized to the North American Shale Composite (NASC) background composition (*42*) for (A) filtered water samples collected from the Tres Rios wetland inlet and outlets on 2 days in February 2000, and (B) individual *Tilapia* liver tissue samples collected from the Tres Rios wetlands, February 2000 [The REEs are lanthanum (La), cerium (Ce), praseodymium (Pr), neodymium (Nd), samarium (Sm), europium (Eu), gadolinium (Gd), terbium (Tb), dysprosium (Dy), holmium (Ho), erbium (Er), thulium (Tm), ytterbium (Yb), and lutetium (Lu)].

whole-body *Tilapia* (Table 3), and lipid-normalized *p,p′*-DDE concentrations were 1300 and 870 μg/kg and lipid-normalized *trans*-nonachlor concentrations were 1800 and 670 μg/kg for *Gambusia* and *Tilapia* respectively. The *Gambusia* specimens were collected from a different wetland unit than the *Tilapia* and SPMD, and accumulated several HOC that were detected in the SPMD but not *Tilapia*, as well as some only detected in *Gambusia*. Differences such as observed for PCBs (present in *Gambusia* but not *Tilapia* or SPMD) may be due to differences in landscape characteristics because the wetlands are supplied by the same WWTP effluent. The two fish species have different life histories and associated food-webs. *Tilapia* are omnivorous and consume phytoplankton, periphyton, zooplankton, invertebrates, and *Gambusia*, whereas *Gambusia* prey primarily on zooplankton (*43*).

The SPMD accumulated *p,p′*-DDE and *trans*-nonachlor at the inlet and outlet, consistent with the *Tilapia* and *Gambusia* results (Figure 4). Both fish species and the SPMD also accumulated dieldrin. Lindane, *cis*-chlordane, and *trans*-chlordane accumulated in the SPMD and *Gambusia* but not *Tilapia*. In a field study involving SPMD, caged channel catfish (*Ictalurus punctatus*), and feral fish in the Upper Mississippi River (*44*), a similar suite of HOC was detected but concentrations were lower than those for the effluent dominated wetlands.

The modeled SPMD uptake curves (Figure 2) were calculated using eqs 3 and 4 and C_w, K_{SPMD}, and R_s values in Table 5, and show good agreement with field uptake for *cis*-chlordane, dieldrin, lindane, and *trans*-nonachlor. There was spatial variation in SPMD uptake, as illustrated by greater *p,p′*-DDE concentrations in the outlet than the inlet during 1999, possibly resulting from *p,p′*-DDE associated with the agricultural soils underlying the wetlands. Field data for pentachloroanisole deviates from the SPMD model uptake curves due to clearance, showing the dynamic nature of HOC exposure in the wetlands. Because the C_w values of the HOC detected in fish tissue were below the aqueous analytical method detection limits, concentrations were estimated from eq 5 using SPMD concentrations at 28 days (Table 4) and K_{SPMD} values (Table 5). Although the SPMD did not reach equilibrium during the 56-day deployment, by 28 days uptake had reached the steady-state phase of the curve (Figure 2). The calculated C_w values were used to estimate BCF values for *Gambusia* and *Tilapia*, which ranged from 530 to 150 000 and increased with increasing K_{ow}. The whole-body *Gambusia*

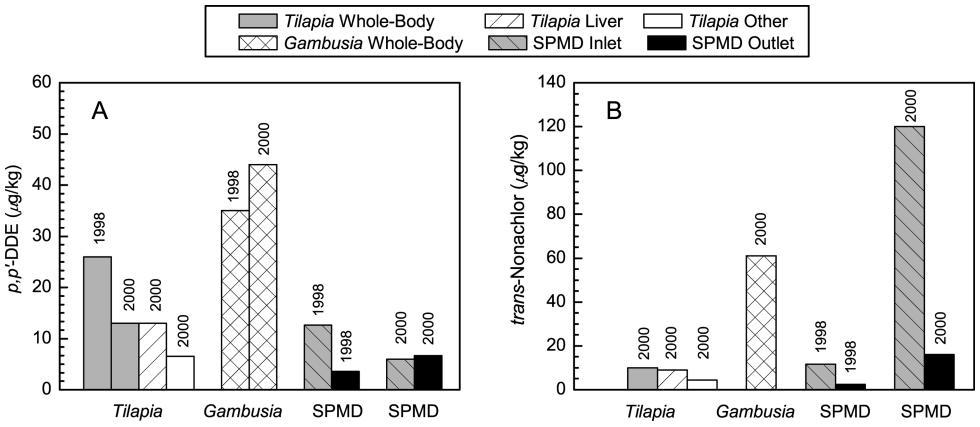

FIGURE 4. Concentrations of (A) *p,p′*-DDE, and (B) *trans*-nonachlor in whole-body *Gambusia*, whole-body *Tilapia*, *Tilapia* liver, and *Tilapia* other tissue collected July 1998 and February 2000, and in semipermeable membrane devices (SPMD) deployed July and August 1998 and June and July 1999 [μg/kg, microgram per kilogram].

TABLE 5. Summary of Hydrophobic Organic Compound Aqueous Concentrations (C_w) and Bioconcentration Factors (BCF) at the Tres Rios Wetlands Calculated Using Concentrations from Semipermeable Membrane Device (SPMD) Deployed for 28 Days During Summer 1999 (Table 4)[a]

compound	log K_{ow}[b]	R_s L/d[b]	log K_{SPMD}[b]	C_w[c] inlet ng/L	C_w[c] outlet ng/L	BCF[e] *Gambusia* whole L/kg	BCF[e] *Tilapia* whole L/kg	BCF[e] *Tilapia* liver L/kg	BCF[e] *Tilapia* other[f] L/kg
cis-chlordane	4.1	5.4	4.2	0.32	0.02	9400	- -	- -	- -
trans-chlordane	4.1	6.8	4.3	0.26	nd	15000	- -	- -	- -
p,p′-DDE	6.0	7.3	4.3	0.30	0.34	150000	43000	43000	22000
dieldrin	3.5	3.8	4.0	1.2	0.31	6900	- -	- -	- -
heptachlor epoxide	2.6	2.0	3.8	2.2[d]	0.5[d]	- -	- -	- -	- -
hexachlorobenzene	6.2	8.2	4.4	0.7[d]	0.1[d]	- -	- -	- -	- -
lindane	3.9	0.4	3.1	12	5.3	530	- -	- -	- -
cis-nonachlor	na	5.1	4.2	0.2[d]	nd	- -	- -	- -	- -
trans-nonachlor	5.6	7.0	4.3	6.4	0.80	9500	1600	1400	700
oxychlordane	na	7.6	4.3	0.2[d]	nd	- -	- -	- -	- -
pentachloroanisole	5.1	5.7	4.2	1.8	0.53	- -	- -	- -	- -

[a] Bioconcentration factors (BCF) were determined for *Gambusia* and *Tilapia* collected February 2000 using calculated C_w and measured tissue concentrations (Table 3). K_{ow}, octanol/water partition coefficient; R_s, SPMD sampling rate in liter per day, L/d; K_{SPMD}, SPMD/water partition coefficient; ng/L, nanogram per liter; L/kg, liter per kilogram; na, not in ref *21*; nd, not detected in SPMD thus C_w was not calculated; - -, compound not detected in fish tissue. [b] From Huckins and others (*21*). [c] Calculated from eq 5 using 1999 SPMD results (Table 4). [d] Calculated from eq 5 using 1998 SPMD results (Table 4). [e] Calculated from eq 2 using inlet C_w values. [f] Other tissue consists of remaining body parts exclusive of filet and liver.

lipid-normalized BCF value for lindane was 16 000, for *trans*-nonachlor was 280 000, and for *p,p′*-DDE was 4 300 000. Using the correlation (*15*) between BCF and K_{ow} (log BCF = 0.893 log K_{ow} + 0.607; log K_{ow} values given in Table 5) results in predicted BCF values of 12 000 for lindane, 400 000 for *trans*-nanochlor, and 920 000 for *p,p′*-DDE, which are in reasonable agreement with the measured lipid-normalized values.

The organic wastewater contaminant nonylphenol is a potentially toxic and endocrine disrupting compound (*31*, *45*). Although fish tissue was not analyzed for nonylphenol, it was detected in the water and SPMD indicating potential for bioaccumulation. On the basis of a reported BCF value of 410 (*46*) and an average measured inlet C_w of 0.65 μg/L, the estimated nonylphenol fish tissue concentration of 270 μg/kg is in agreement with values (66−110 μg/kg) reported for carp (*Cyprinus carpio*) from a wastewater-affected stream (*47*). Using a log K_{ow} value of 4.7 for nonylphenol (*48*) results in a predicted BCF of 64 000, which is considerably greater than measured values (*46*) even when normalized to lipid content, suggesting that other factors such as metabolism are reducing the BCF. The major chlorobenzene detected in the WWTP effluent was 1,4-dichlorobenzene (log K_{ow} = 3.5; *49*) whereas hexachlorobenzene (log K_{ow} = 6.2; Table 5) was detected in the SPMD. Although not detected in the fish tissue, the predicted BCF values of 5400 for 1,4-dichlorobenzene and 1 400 000 for hexachlorobenzene are similar to lipid-normalized values reported elsewhere (*49*).

Concentrations of most trace elements were greater in the liver tissue than in the filet tissue, consistent with observations by others (*50*). Measured trace-element BCF are shown in Table 2 and values for whole-body *Gambusia* ranged from a low of 5 (lithium) to a high of 58 000 (mercury). The ratio of the liver BCF to filet BCF ranged from less than 1 to 130 and indicates preferential accumulation of some trace elements in the liver. The measured BCF values for the trace elements were of the same order of magnitude but slightly less than those for the HOC (Table 5). Elements such as aluminum have very low water solubility at circumneutral pH conditions but are present at high concentrations in sediment and can be transferred to an organism through sediment ingestion. Although the source of most trace elements in aquatic environments is natural, elements such as cadmium, copper, lead, and zinc have major anthropogenic uses.

Aquatic organisms living in wastewater-treatment wetlands are exposed to a wide range of both inorganic and organic contaminants at concentrations greater than those typically found in natural wetland systems. Contemporaneous multi-media sample collection for comprehensive chemical analysis provides insight into bioaccumulation, and illustrates that aquatic organisms are exposed to a complex chemical matrix. Treatment wetlands generally remove anthropogenic contaminants present in WWTP effluent, but

some trace elements and HOC increase in the wetlands due to their association with the landscape.

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