J. ENVIRONMENTAL SYSTEMS, Vol. 29(1) 55-70, 2002-2003

ENVIRONMENTAL ASSESSMENT OF FISHES GROWN IN TREATED DOMESTIC SEWAGE

SURESH V. INAKOLLU
Regional Research Laboratory, Bhopal, India

ASWANI WANGANEO
Bhopal University, India

ABSTRACT

Published records on the use of effluent water from domestic sewage in fish production date back to the early parts of the twentieth century. The present series of investigations was aimed at assessing fish contamination from sewage maturation ponds in tropical regions of India. Our field studies have shown that fish prefer regions with high nutrient content. Toxicological investigations accordingly should be carried out at very high concentrations of wastewater contamination. The incidence of bacterial contamination was high in all wastewater-grown fishes and virtually independent of wastewater concentration. The toxicity of heavy metals to organisms varies widely. Comparison of concentration ranges of heavy metals in surface waters with acute toxicity data from these metals shows that concentrations that have been determined to be lethal in laboratory tests may occur in nature with no noticeable effect. The present study, carried out on bioaccumulations of bacteria and heavy metals in three different fishes, revealed that the domestic wastewater is in no way harmful for rearing fish.

INTRODUCTION

The main source of problems from serious ground and surface water pollution has been improper disposal of wastewater, especially from domestic and industrial activities [1]. A principal challenge posed in assessments of environmental impacts is to isolate the effect of interest from spatial and temporal variability [2].

A range of ecological responses in fish has been attributed to sewage pollution including: increased mortality, increased or decreased abundance or diversity, and changes to size, reproduction, contaminant levels, parasites, infections or behavior [3-9]. Many authors have investigated the impacts of sewage on fish abundance and/or condition [10-13].

It is well known that recycling of waste through aquaculture has great potential. Major restraints on wastewater fish culture may include pathogenic bacteria and toxic materials apart from parasitic disease. Although it is widely accepted that almost all such pathogens do not cause infection of the fish [14], they may survive and accumulate in the animals [15, 16]. Buras et al. [17] showed that high concentrations of human enteric viruses and bacteria could be recovered from several fish organs. Above a certain threshold concentration in the ambient water, these microorganisms could be detected in the edible muscle as well [18]. Several studies have shown that when fish are grown under unfavorable environmental conditions, their defense mechanisms are affected and succumb to bacterial infection [19, 20].

Bacteriological and heavy metal evaluation from pathological and toxicological points of view is of paramount importance and becomes essential if the fishes are to be commercialized for human consumption. Fish readily absorb dissolved metals and may serve as indicators of the extent of pollution. However, wide variations exist between species insofar as the metabolic pathways are concerned, and no single fish species can truly be said to act as a reliable indicator of pollution [21, 22]. This causes difficulty in assessing the significance of heavy metal levels with respect to accumulation and exposure times in fish. In addition, considerable intraspecific variations in heavy metal levels in fish have been found. Such variations occur with tissue type [23], tissue location [24], and size [25]. The purpose of this study was to investigate the effect of these parameters in three local species of fish, in an effort to provide information on the distribution of heavy metals within the individual species. This information, in turn is essential in planning a monitoring study for any long-term data collection of bacterial and heavy metal accumulation in these species of fish.

Site Description

The Bharat Heavy Electricals Limited (BHEL), Bhopal, India has an Environmental Management Division to protect and promote public health and improve the surrounding environment. A full-fledged treatment plant is operating, to process the entire sewage generated from the residential colonies of the plant. The treatment system consists of aerobic treatment followed by anaerobic digestion of biosolids. The designed capacity of the sewage treatment plant is two million gallons per day. After hydraulic retention period of the wastewater, the wastewater is collected in a pond for growing fish and vegetables.

Wastewater

Wastewater is mostly of domestic origin with no noticeable industrial contribution. Its flow is about 4 to 5 m^3/s. The raw wastewater was pretreated for the removal of coarse material, grease, and sand, and then pumped into the treatment plant.

Treatment Plant

Wastewater treatment is achieved in a train of six sections in series with overall retention time 10.5 days. Table 1 shows the characteristics and hydraulic retention times for each section, including a series of compartments (aerobic, facultative, and fishpond).

MATERIALS AND METHODS

Biological and chemical examination of the samples of the treated effluent and produced fish were analyzed by standard methods. The species of fish investigated in this study were *Cyprinus carpio, Clarias batrachus,* and *Heteropneustes fossilis.* These three species are of major economic importance to the local fisheries industry. Samples were obtained from a local fisherman. Each fishpond, is approximately 75m × 75m × 1.5m with water depth of one meter. No supplementary feed was supplied to sewage-fed fishes. The entire fish biomass came from the organic constituents and plankton grown in the wastewater. The average weight and length of fingerlings at the time of stocking was 5 grams and 5 cm, respectively. Observation on growth of fish was made periodically by catching about 10-20 fish at random by cast netting in order to measure the weight, and releasing them into water after measurement. Effluent samples were analyzed according to standard methods for examination of water and wastewater [26].

Table 1. Volume, Area, and Hydraulic Retention Time of Each Element of the Wastewater Treatment Plant

Stage	Volume (m^3)	Area (m^2)	Retention time (days)
Primary clarifier	2000	800	2
Activated sludge	1000	800	1.5
Trickle filter	1500	700	2
Facultative pond	2000	400	2
Fish pond	1000	400	50-80
Total	7500	3100	57.5-87.5

Microbiological Analysis

Bacteriological assays included aerobic plate count (APC), enterobacteria, and fecal coliforms. In addition, the fish surface and muscles were examined for *E. coli.* A prescribed area of 30 cm^2 of surface samples of fish was taken and the samples were mixed with 20 ml of 0.1 percent peptone water. One ml of the original suspension was transferred to a sterile test tube containing 9 ml of 0.1 percent peptone water to obtain a dilution of 1/10. Serial dilutions were then prepared in peptone water to achieve a dilution of $1/10^7$. For preparation of muscle homogenate, 10 gr of muscle sample were taken from the dorsal muscle of the fish. Muscle samples homogenate was then prepared according to techniques recommended by ICMSF [27].

The surface spread plate technique was used in assaying APC; Enterobacteriaceae counts employed a plate count agar and violet-red bile glucose agar as plating media. The coliform counts were determined by multiple tube method recommended by ICMSF and APHA. Isolation of *E. coli* was carried out according to ICMSF. For *E. coli* assays, fish organ homogenates and water samples were diluted in PBS (phosphate buffered saline) and assayed by membrane filtration, using m-TEC medium [28] supplemented with 50 ppb streptomycin and nalidixic acid.

Heavy Metals Analysis

Levels of Fe, Zn, Cu, Ni, Pb, Cd, and Cr were investigated in the muscle and skin tissues, the edible portions of the fish. Each specimen was scaled, gutted, and filleted. All the three species of fish were used to study the effect of tissue location, using three individuals of identical length from each species. Each fish was cut into head, middle, and tail sections before separating the muscle from the skin. To a chopped and weighed muscle sample (30 grams), 40 ml of distilled water was added and the mixture was homogenized at high speed with a micro emulsifier. The weighed skin sections (10 grams) were blended with 5 ml of distilled water. The addition of water was necessary to achieve thorough homogenization of these samples because of the small quantities available. Aliquots of tissue blended with distilled water and equivalent to 10 grams of pre-blended fish muscle tissues were weighed accurately and then oven dried at 85 °C for 10 hours. The digestion of the samples for trace metal analysis was done following the procedure as given by Muir et al. [29]. Samples and calibration standards were prepared with the same concentrations of digestion acids and analyzed on GBC 902 atomic absorption spectrophotometer with background correction. Metal levels were determined as microgram per gram wet weight of tissue. The detection limit for each of the elements Fe, Ni, Pb, and Zn was 0.005 and the limit for cadmium and chromium were 0.0005. Metal levels in the skin and the muscle of the three species of fish investigated when compared statistically using pair data *t*-tests. The metal levels in

muscle and skin at three different locations of the species also were analyzed using two-way analysis of variance (ANOVA). Two group *t*-tests were used to compare statistical levels of each of the detectable metals (in both muscle and skin) in samples from the two different body size classes. All statistical comparisons were done at the 5 percent and 1 percent confidence levels.

RESULTS AND DISCUSSION

Effluent Quality

The treatment performances are satisfactory as shown by effluent data presented in Table 2. Based on the chemical quality, the effluent has minimum organic load throughout the year; evaluation studies of the treatment plant have been reported previously [30]. The most important criterion for a wastewater-fed fishpond is total nitrogen loading. Too much or too little nitrogen results in high variations of algal biomass in the pond, and consequently small fish yields and sometimes fish kills due to severe dissolved oxygen depletion [31]. The optimal nitrogen loading is approximately 4 kg N/ha/d [6] as estimated by the equation given by Reed [32]:

$$C_e = C_i \exp\{-[0.064(1.039)^{T-20}]\ [\theta + 60.6(pH-6)$$

Where C_e and C_i = N concentrations in effluent and influent respectively
T = Temperature in degrees Celsius
θ = Retention time in days

Table 2. Chemical Quality of Influent and Effluent (Mean Values of 15)

Parameter (mg/l)	Influent	Effluent
pH	6.94	7.50
Conductivity (mmhos/cm)	1.45	0.786
TSS	252	165
TDS	750	380
Chloride	264	334
Calcium	75	92
Magnesium	40	51
Sodium	83	58
Potassium	51	33
Total-N	41	28
Ammonical-N	22	15
Total-P	8	3.5

Taking C_i as 40 mg/l with a design temperature of 25 degrees Celsius and the pH as 8 gives a value of C_e of 20 mg/l. The retention time was calculated to check the excess algal growth and consequent problems. The overall performance of the fishpond was satisfactory in terms of nitrogen loading and retention time (Table 1).

Microbiological Assay

Results of bacteriological monitoring of the pond water during fish rearing period are shown in Table 3. Due to the high concentrations of microorganisms, attachment of potential pathogens to the skin of the fish and subsequently to internal organs are possible. This is in agreement with the findings of Sedik et al. [33] and Fattal et al. [18], who reported that the flora of fish associated with the environment (and hence of fish caught in polluted water) may be carriers of food poisoning microorganisms.

The fecal coliform (FC) numbers of fishpond water was calculated by the equation of Marais [34]:

$$N_p = \frac{N_i}{(1+\theta_a)(1+k\theta_f)+1+k\theta_p)}$$

Where N_p and N_i = FC per 100 ml in effluent and influent
K = First order rate constant in d^{-1} ($= 2.6\ (1.19)^{T-20}$)
θ_a, θ_f, θ_p = Retention time of the treatment area of the plant

Bacteria on the surface of three different fishes are presented in Table 4. The low bacterial counts in zero days indicate that the fingerlings used in the study originated from uncontaminated waters. After introduction of fingerlings in the pond, the bacterial surface load significantly increased and over a period of time

Table 3. Bacterial Counts of Treated Wastewater during Fish Rearing Period

Days	APC/100 ml	Coliform/100 ml
0[a]	3×10^7	3×10^5
50	1×10^6	2×10^3
100	4×10^4	50
150	4×10^3	30
200	1×10^3	35
250	2×10^2	40

[a]Samples before stocking in the pond.

Table 4. Bacterial Counts on Surface of Fish Grown in Wastewater

	Clarias batrachus		Cyprinus carpio		Heteropneustes fossilis	
Days	APC/cm^2	Coliform/cm^2	APC/cm^2	Coliform/cm^2	APC/cm^2	Coliform/cm^2
0[a]	70	85	100	67	124	25
50	8×10^5	6×10^5	8×10^5	6×10^4	6×10^6	5×10^3
100	8×10^4	40	7×10^4	30	7×10^3	19
150	3×10^4	20	1×10^4	10	2×10^4	14
200	7×10^3	10	9×10^3	7	7×10^3	6
250	1×10^2	7	3×10^2	7	2×10^3	23

[a]Samples before stocking in the pond.

was reduced tremendously. Table 5 shows the log mean concentration of *E. coli.* The highest concentration was found in the digestive tract followed by the liver. The lowest concentration was found in muscle. There were no significant discrepancies in between the levels of *E. coli* in the water and fish tissues (ANOVA, $P < 0.05$).

The relatively high concentrations of bacteria recovered from the skin correlate with the findings of Crause-Eisnor et al. [35]. It would seem that the bacteria are not associated with the skin tissue but rather are attached to mucus or trapped beneath the scales. A high correlation has been observed between bacterial concentrations in the skin and digestive tract. These studies are also similar to the findings of Hejkal et al. [36]. Feachem et al. [37] detected low levels of *E. coli* within the muscle even when fish are exposed to very high concentrations of bacteria levels greater than those present in water. These findings contradict the previous studies reported by Buras et al. [16, 17] that fish muscle is always contaminated by bacteria once the microbial biomass in the water exceeds a limit of about 5×10^4/ml. The results of the study indicate that the fish organs, and particularly the digestive tract, can harbor high levels of microorganisms originating in the wastewater. Such levels of microbiological contamination may pose a potential public health risk if fish are harvested from sewage-fed fishponds, although the microbiological examination of fish reared in treated sewage at this farm indicate no evidence of public health concern.

Fish Growth

Wastewater fish culture experiments conducted in developed as well as developing countries have confirmed that domestic wastewater has tremendous

Table 5. Log Mean of *E. coli* Concentrations in Water and Fish Tissues

		Clarias batrachus (cfu/cm^2)			Cyprinus carpio (cfu/cm^2)			Heteropneustes fossilis (cfu/cm^2)		
Days	Water cfu/ml	DT cfu/gr	Liver cfu/gr	Muscle cfu/gr	DT cfu/gr	Liver cfu/gr	Muscle cfu/gr	DT cfu/gr	Liver cfu/gr	Muscle cfu/gr
0[a]	8.90	3.03	3.18	0.19	3.63	1.96	0.70	3.50	0.35	0.19
50	9.15	4.88	1.52	0.12	3.69	2.08	0.12	1.60	3.28	0.23
100	3.89	2.25	1.30	0.10	3.49	1.53	0.12	4.76	1.96	0.55
150	9.35	4.4	0.79	0.70	3.82	0.33	0.18	3.79	2.08	0.12
200	9.76	2.56	2.79	0.19	3.79	1.30	0.91	3.79	1.59	0.12
250	6.50	4.80	0.74	0.94	3.44	0.67	0.91	3.40	1.60	0.12

[a]Samples before stocking in the pond.
DT = digestive tract

potential in fish growth and yield due to the natural amount of food present in domestic wastewater [38-40]. The growth rates of three different fishes in the present study represented in Figure 1 show results similar to prior work [41, 42]. The higher growth is due to rich nutrient food engulfed and higher metabolic pressure present in these organisms [43, 44].

Heavy Metals Assessment

Apart from indicator organisms, other contaminants such as heavy metals may pose potential health hazards in domestic wastewater. They cannot be destroyed through biological degradation, unlike most of the organic pollutants. In view of its importance, an analysis was made to gauge the contamination and bioaccumulation of heavy metals. The levels of heavy metals in the skin were slightly higher than in the muscle in all three species of fish investigated (Tables 6, 7, and 8). The variations in these metals between muscle and skin may be due to the higher degree of pigmentation in the skin tissue than in the muscle tissue. This point is supported by studies that suggest that these metals are accumulated in the skin, hair, and feathers of man, rat, bird, and fish in proportion to the degree of pigmentation [45, 46]. The ANOVA treatment of the data indicated that heavy metal levels in head, middle, and tail muscle sections were not significant. However, variations in levels of some of the metals among the head, middle, and tail sections of the skin tissue were shown to be real.

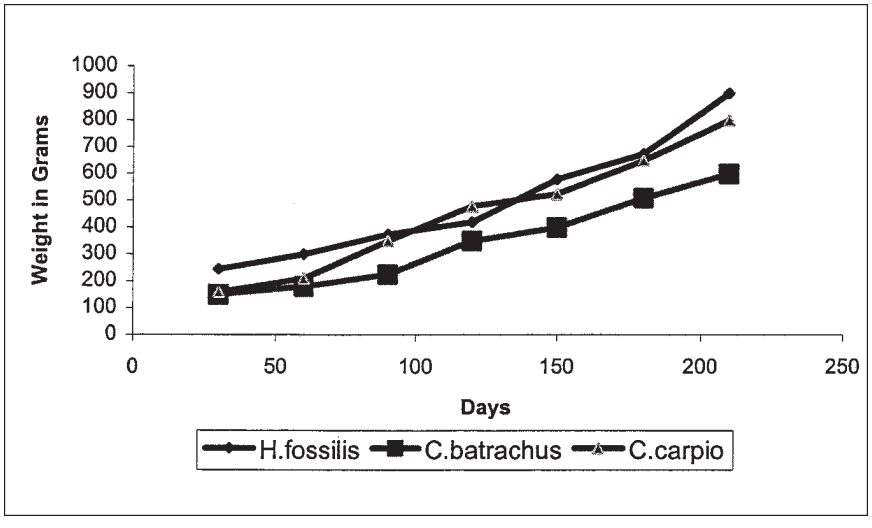

Figure 1. Observed growth of fishes grown in wastewater.

Table 6. Heavy Metal Distribution in Different Sections of the Fish *Clarias batrachus* (μg/gm Wet Weight)

	Fish		Head section		Mid section		Tail section	
Metal	Length (cm)	Weight (grams)	Tissue	Skin	Tissue	Skin	Tissue	Skin
Cd	15.5	325	0.05	0.90	0.07	0.70	0.08	0.50
Cu	18.5	376	0.08	1.03	0.65	1.22	0.20	0.70
Fe	21.6	435	2.89	4.87	2.58	7.90	2.78	3.67
Ni	23.8	450	0.02	0.91	0.01	0.23	0.04	0.08
Pb	17.5	375	0.09	0.96	0.002	0.087	0.07	0.10
Zn	16.5	325	0.68	1.89	1.23	2.46	0.89	2.78

Table 7. Heavy Metal Distribution in Different Sections of the Fish *Cyprinus carpio* (μg/gm Wet Weight)

	Fish		Head section		Mid section		Tail section	
Metal	Length (cm)	Weight (grams)	Tissue	Skin	Tissue	Skin	Tissue	Skin
Cd	25.6	400	0.09	0.12	0.005	0.17	0.07	0.16
Cu	32.5	435	0.82	0.98	0.82	1.35	0.60	0.89
Fe	30.7	430	5.85	6.25	6.92	8.00	2.46	3.56
Ni	28.2	400	0.08	0.08	0.08	0.10	0.09	0.13
Pb	28.5	375	0.005	0.10	0.07	0.15	0.005	0.10
Zn	30.5	400	0.69	1.95	0.96	1.21	0.68	0.92

The statistical analysis of the levels of heavy metals and fish body length and weight by the two group *t*-test are given in Tables 9 and 10. The effect of size on the muscle levels of these metals is mixed for the different species. At the intraspecific level, if metal accumulation is dependent principally on the duration of exposure, it seems fair to assume that it will be enhanced with age. However, consideration must be given also to the excretion rates, as these may eventually match or exceed uptake rates. This factor may eventually result in a decrease in the heavy metal concentration with age [47].

Table 8. Heavy Metal Distribution in Different Sections of the Fish *Heteropneustes fossilis* (μg/gm Wet Weight)

	Fish		Head section		Mid section		Tail section	
Metal	Length (cm)	Weight (grams)	Tissue	Skin	Tissue	Skin	Tissue	Skin
Cd	20.5	300	0.09	0.12	0.08	0.11	0.03	0.25
Cu	22	300	0.06	0.13	0.09	0.13	0.08	0.25
Fe	18.8	280	6.91	7.81	7.81	4.62	5.91	2.34
Ni	25.5	310	0.05	0.10	0.008	0.17	0.06	0.15
Pb	20	280	0.002	0.012	0.005	0.12	0.01	0.10
Zn	20	275	0.60	0.91	0.45	0.68	0.62	1.56

Table 9. Results of Statistical Analysis of Data for Effect of Fish Length

	Cyprinus carpio (t_s)		*Clarias batrachus* (t_s)		*Heteropneustes fossilis* (t_s)	
Metal	Muscle	Skin	Muscle	Skin	Muscle	Skin
Cd	0.421[a]	1.669[a]	0.118[a]	3.334[b]	0.318[a]	2.363[a]
Cu	0.677[a]	3.013[a]	4.009[c]	0.101[a]	1.800[a]	4.301[b]
Fe	1.867[a]	0.169[a]	5.288[c]	0.604[a]	2.405[a]	0.469[a]
Ni	0.913[a]	2.987[b]	0.614[a]	0.293[a]	0.660[a]	4.104[b]
Pb	0.257[a]	2.819[b]	0.142[a]	0.145[a]	2.160[a]	0.025[a]
Zn	0.127[a]	0.770[a]	5.335[c]	1.547[a]	1.973[a]	1.201[a]

[a]Not significant. [b]Significant at $0.05 \geq p > 0.01$. [c]Significant at $0.05 \geq p > 0.001$.

Relationships of various metals in wastewater pond with fish samples were also studied (Table 11). It appears that accumulations of certain metals are positively correlated, while others are not correlated, with wastewater, as bioaccumulation depends upon the concentration of specific forms of the metals in bioavailable form, rather than the total metal concentration. The biologically available fraction of a metal is that part of the total trace metal available for uptake by biota [48]. Metal uptake by aquatic organisms is generally described as involving an initial reaction of the free metal ion with a membrane-embedded-transport system, either a channel or ion carrier, that takes the metal across the external membranes of the exchange surfaces [49]. Complexation with water-soluble organic ligands

Table 10. Results of Statistical Analysis of Data for Effect of Fish Weight

	Cyprinus carpio (t_s)		*Clarias batrachus* (t_s)		*Heteropneustes fossilis* (t_s)	
Metal	Muscle	Skin	Muscle	Skin	Muscle	Skin
Cd	1.816[a]	1.786[a]	0.768[a]	1.142[a]	3.546[a]	0.765[a]
Cu	2.376[a]	0.675[a]	0.155[a]	0.246[b]	0.987[a]	0.876[a]
Fe	0.678[a]	0.456[a]	0.303[a]	0.987[a]	0.564[a]	0.543[a]
Ni	0.345[b]	0.190[a]	1.656[a]	0.675[a]	0.765[a]	0.564[a]
Pb	4.009[b]	0.134[a]	0.294[a]	0.456[a]	0.675[a]	0.564[a]
Zn	0.134[a]	0.670[a]	0.134[a]	0.765[a]	0.987[a]	0.987[a]

[a]Not significant. [b]Significant at $0.01 \geq p > 0.001$.

Table 11. Correlation Coefficient Values (R) between Metal Concentrations in Wastewater and Accumulation in Fish Samples

	Wastewater—*Cyprinus carpio*		Wastewater—*Clarias batrachus*		Wastewater—*Heteropneustes fossilis*	
Metal	Muscle	Skin	Muscle	Skin	Muscle	Skin
Cd	–0.11*	–0.25*	0.19	0.61*	–0.25	0.68*
Cu	0.02	–0.11*	–0.24	0.27	–0.25	0.68*
Fe	0.61*	0.06	0.45*	0.11*	0.68*	0.45*
Ni	0.23*	–0.25*	0.19	0.23*	0.21*	0.45*
Pb	0.06	0.22*	–0.81*	0.06	0.45*	0.19
Zn	0.28*	0.46*	0.45*	0.28	0.86	0.27

*Significance at 1% level.

generally decreases the uptake of heavy metals by aquatic organisms by reducing the activity of metal ion in the solution. Consequently, variations in metal uptake are usually best explained on the basis of changes in free metal ion, rather than by changes in total metal concentration.

Beveridge et al. [50] reported that, if there is too great an abundance of essential heavy metals, the metal content in the organism could be regulated by homeostatic control mechanisms. However, if the heavy metal concentration at the source is too high, the homeostatic mechanisms are inhibited and accumulation proceeds, as uptake exceeds the loss rate.

In general, body length is not necessarily an indication of the age of the fish and consequently, larger body length does not indicate a longer duration of exposure. The observations of bioaccumulation studies can be based on a combination of uptake/excretion rates, increasing or decreasing physiological needs, metabolic turnover, and/or varying exposure times. The high metal concentration in skin is explained by the fact the skin is one of the sites to which metabolites and waste products are transported in fish for storage or elimination, and therefore both increased metabolic turn over and age will tend to enhance rather than reduce metal levels there [46].

The results indicate clear variations in the detected metal levels with respect to tissue type, location, and the species investigated. The results give support to the view that no single fish species can truly be said to act as a reliable indication of pollution. This is because different species of fish will exhibit varying levels of metals as determined by a combination of factors that operate within the individual species. Thus, it is evident from the present study that the levels of heavy metals found in the fish were not excessive, and indeed are several times below the acceptable threshold levels and are safe for human consumption.

ACKNOWLEDGMENTS

We thank Professor T. C. Rao, Director, RRL, Bhopal, and Late Prof. G. P. Bhatnagar for providing the necessary laboratory facilities.

REFERENCES

1. Z. Dubinsky and N. Stambler, Marine Pollution and Coral Reefs, *Global Change Biology, 2,* pp. 511-526, 1996.
2. R. J. Schmitt and C. W. Osenberg, *Detecting Ecological Impacts, Concepts and Applications in Coastal Habitats,* Academic Press, San Diego, California, 1996.
3. C. Tsai, *Effect of Sewage Treatment Plant Effluents on Fish: A Review of Literature,* CRC Publication No. 36, University of Maryland, Maryland, 1975.
4. M. S. Love, B. Axell, P. Morris, R. Collins, and A. Brppks, Life History and Fishery of the Californian Scorpion Fish *Scorpaena guttata,* within the Southern California Bight, *Fish Bulletin, 85,* pp. 99-116, 1987.
5. A. Wanganeo, S. Pani, M. J. Nandan, and I. V. Suresh, Fish Mortality in Lower Lake of Bhopal, *Geobios, 21*:2, pp. 145-146, 1994.
6. R. W. Grigg, Effects of Sewage Discharge, Fishing Pressure and Habitat Complexity on Coral Ecosystems and Reef Fishes in Hawaii, *Marine Ecology Progress Series, 105,* pp. 25-34, 1994.
7. R. W. Grigg, Coral Reefs in an Urban Embayment in Hawaii: A Complex Case History Controlled by Natural and Anthropogenic Stress, *Coral Reefs, 14,* pp. 253-266, 1995.
8. R. Siddall, A. W. Pike, and A. H. McVicar, Parasites of Flatfish in Relation to Sewage Sludge Dumping, *Journal of Fish Biology, 18,* pp. 193-209, 1994.
9. A. D. Lemly, Wastewater Discharge May be Most Hazardous to Fish During Winter, *Environmental Pollution, 93,* pp. 169-174, 1996.

10. M. S. Adams, Biological Indicators of Stress in Fish, *American Fisheries Symposium, 8,* Bethesda, Maryland, 1990.
11. C. A. Gray, N. M. Otway, F. A. Laurenson, A. G. Miskiewicz, and R. L. Pethebridge, Distribution and Abundance of Marine Fish Larvae in Relation to Effluent Plumes from Sewage Outfalls and Depth of Water, *Marine Biology, 113,* pp. 549-559, 1992.
12. O. Svanberg, Monitoring of Biological Effects, *Resources Conservation and Recycling, 16,* pp. 351-360, 1996.
13. A. K. Smith and I. M. Suthers, Effect of Sewage Effluent Discharge on the Abundance, Condition and Mortality of Hulafish, *Trachinops taeniatus* (Plesiopidae), *Environmental Pollution, 106,* pp. 97-106, 1999.
14. P. Edwards, *Use of Human Wastes in Aquaculture: Public Health Aspects—A State of the Art Review,* Division of Food and Agriculture Engineering, Asian Institute of Technology, 1987.
15. D. A. Baker, R. O. Smitherman, and T. A. McCaskey, Longevity of *Salmonella typhimurium* in *Tilapia aurea* and Water from Pools Fertilized with Bovine Waste, *Applied Environmental Microbiology, 45,* pp. 1548-1554, 1983.
16. N. Buras, L. Duek, and S. Niv, Reactions of Fish to Microorganisms in Wastewater, *Applied Environmental Microbiology, 50,* pp. 989-995, 1985.
17. N. Buras, L. Duek, S. Niv, B. Hepher, and E. Sandbank, Microbiological Aspects of Fish Grown in Wastewater, *Water Research, 21*:1-10, 1987.
18. B. Fattal, A. Dotan, and Y. Tschorsh, Rates of Experimental Microbiological Contamination of Fish Exposed to Polluted Water, *Water Research, 25*:12, pp. 1621-1627, 1992.
19. G. Peters, H. Delventhal, and H. Klinger, Stress Diagnosis for Fish in Intensive Culture Systems, in *Proceedings of World Symposium on Aquaculture in Heated Effluents and Recirculation Systems, Stavenger, Norway, 2,* pp. 239-248, K. Tiews (ed.), Heinamann, Berlin, 1981.
20. I. Bejerano and S. Sarig, Stress Induced Infection of *Sarotherodon aureus* under Laboratory Conditions, in *Intensive Aquaculture, 6,* pp. 69-79, H. Rosenthal and J. O. H. Oren (eds.), European Mariculture Society Special Publication, 1981.
21. R. W. Bradley and J. R. Morris, Heavy Metals in Fish from a Series of Metal Contaminated Lakes Near Sudbury, Ontario, *Water, Air, Soil Pollution, 27,* pp. 341-354, 1986.
22. R. Dallinger, F. Prosi, H. Segner, and H. Back, Contaminated Food and Uptake of Heavy Metals by Fish: A Review and Proposal for Further Research, *Oecologia, 73,* pp. 91-98, 1987.
23. K. Itano, S. Kawai, N. Miyazaki, T. Tatsukawa, and T. Fujiyama, Body Burdens and Distribution of Mercury and Selenium in Striped Dophin *Stenella coeruleoalba, Agricultural Biology and Chemistry, 48,* pp. 1117-1122, 1984.
24. K. Honda, M. Matsuda, and R. Tatsukawa, Distribution of Heavy Metals and Its Characteristics in Albacore (*Thunus alalunda*) and Bonito (*Katsuwonus pelamis*), *Nippon Nogei Kagaku Kaishi, 53,* pp. 177-182, 1979.
25. W. S. Vinikour, R. M. Goldstein, and R. V. Anderson, *Bulletin of Environmental Contamination and Toxicology, 24,* pp. 727-734, 1980.
26. APHA, *Standard Methods for the Examination of Waters and Wastewaters,* American Public Health Association, New York, 1989.

27. ICMSF, *The International Commission on Microbiological Specifications for Foods. Microorganisms in Foods, Their Significance and Methods of Enumeration* (2nd Edition), University of Toronto, 1978.
28. A. D. Dufour, E. R. Strickland, and V. J. Cabelli, Membrane Filter Method for Enumeration of *Escherichia coli, Applied Environmental Microbiology, 41,* pp. 1152-1158, 1981.
29. D. G. G. Muir, R. Wagemann, N. P. Griffith, R. J. Norstrom, M. Simon, and J. Lein, Organochlorine Chemical and Heavy Metal Contaminants in White Beaked Dolphin (*Lagenorynchus alborostris*) and Pilot Whales (*Globecephala malaena*) from the Coast of Newfoundland, Canada, *Archives of Environmental Contamination and Toxicology, 17,* pp. 613-629, 1988.
30. I. V. Suresh, A. Wanganeo, M. V. R. L. Murthy, S. K. Sanghi, and R. N. Yadava, Impact of Storm Water Runoff on Efficiency of Effluent Treatment Plant—A Case Study, *Journal of Environmental Science and Health, A31*:4, pp. 811-824, 1996.
31. P. Edwards, *Reuse of Human Wastes in Aquaculture: A Technical Review,* Water and Sanitation Report No. 2, The World Bank, Washington, D.C., 1992.
32. S. C. Reed, Nitrogen Removal in Wastewater Stabilization Ponds, *Journal of Water Pollution Control Federation, 57*:1, pp. 39-45, 1985.
33. M. F. Sedik, E. E. Safwat, A. M. Ibrahim, and A. I. Shaaban, Studies on Some Species of Locally Produced Fish, *Veterinary Medical Journal Giza, 37*:2, pp. 197-207, 1989.
34. G. V. R. Marais, Fecal Bacterial Kinetics in Waste Stabilization Ponds, *Journal of Environmental Engineering ASCE, 100*:EE1, pp. 119-139, 1974.
35. R. A. Crause-Eisnor, D. K. Cone, and P. H. Odense, Studies on Relations of Bacteria with Skin Surface of *Carassius auratus* and *Poecilia reticulata, Journal of Fish Biology, 27,* pp. 395-402, 1985.
36. T. W. Hejkal, C. P. Gerba, S. Henderson, and M. Freeze, Bacteriological, Virological and Chemical Evaluation of a Wastewater-Aquaculture System, *Water Research, 17,* pp. 1749-1755, 1983.
37. R. G. Feachem, D. J. Bradley, H. Garelick, and D. D. Mara, *Sanitation and Disease: Health Aspects of Excreta and Wastewater Management,* Wiley, New York, 1983.
38. A. Ghosh, L. H. Rao, and S. K. Saha, Culture Prospect of *Sarotherolon mossambicus* in Small Fertilized with Domestic Sewage, *Journal of Inland Fishing Societies of India,* pp. 171-173, 1980.
39. V. S. Govindan, Food and Feed Production of Municipal Wastewater Treatment, *Biological Wastes, 30,* pp. 169-179, 1989.
40. S. A. Ali, H. M. Raju, J. Peter, and S. A. Ayesha, Histopathological Evaluation of Liver and Kidney of *Cyprinus carpio* Cultured in Domestic Wastewater Ponds, *Indian Journal of Zoological Spectrum, 4*:1&2, pp. 23-27, 1993.
41. G. H. Allen and B. Hepher, *Advances in Aquaculture,* Proceedings of FAO Technical Conference on Aquaculture, Japan, May 26-June 2, 1976.
42. A. B. Akolkar, Ph.D. thesis, Department of Biological Sciences, Bhopal University, Bhopal, 1983.
43. G. W. Wohlfarth and R. Moav, *Verhandlungen der Internationalen verinigung fürTheoretische and Angewandte Limnologie, 17,* pp. 702-704, 1969.
44. G. L. Schroeder and B. Hepher, *Advances in Aquaculture,* T. V. R. Pillai and A. W. Dill (eds.), p. 487, 1979.

45. E. J. Underwood, *Trace Elements in Human and Animal Nutrition* (3rd Edition), Academic Press, New York, 1971.
46. K. Honda, R. Tatsukawa, and T. Fujiyama, Distribution Characteristics of Heavy Metals in the Organs and Tissues of Stripped Dophin *Stenella coeruleoalba, Agricultural Biology and Chemistry, 46,* pp. 3011-3022, 1982.
47. J. G. Singh, Y. Ivon-chang, V. A. Stoute, and L. Chatergoon, Distribution of Heavy Metals in Skin and Muscle of Five Tropical Marine Fishes, *Environmental Pollution, 69,* pp. 203-215, 1991.
48. D. R. Turner, Biological Availability of Trace Elements, in *Biogeochemical Processes at the Land-Sea Boundary,* P. Lasserre and L. M. Martin (eds.), Elsevier, Amsterdam, The Netherlands, pp. 191-200, 1986.
49. K. Simkiss and M. Taylor, Metal Fluxes across the Membranes of Aquatic Organisms, *CRC Critical Review of Aquatic Science, 1,* pp. 173-188, 1989.
50. M. C. M. Beveridge, E. Stafford, and R. Coutts, Metal Concentration in the Commercially Exploited Fishes of the Endorhiec Saline Lake in the Tin-Silver Province of Bolivia, *Aquacultural Fish Management, 16,* pp. 41-54, 1985.

Direct reprint requests to:

Dr. Aswani Wanganeo
Department of Limnology
Bhopal University
Bhopal, 462 026, India