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Present-Day State of Coral Reefs of Nha Trang Bay (Southern Vietnam) and Possible Reasons for the Disturbance of Habitats of Scleractinian Corals

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Abstract—Our investigations were conducted from 1990 to 2002. Sampling of bottom sediments and biological objects, as well as photo and video shooting, were performed during scuba diving. The state of the environment and coral reef communities was assessed using the chemical–analytical, fluorometric, and luminometric methods, as well as the Ames test and the transect technique. The research results suggest that the spectrum and distribution pattern of persistent congeners of PCDD/Fs (dioxins) in bottom sediments are similar to those of the defoliant "Agent Orange" and that the bottom sediments are toxic and display photo inhibition and a mutagenic effect. The bottom of the bay is heavily silted throughout its depth. Many large dead colonies of corals without mechanical damage were observed everywhere. The total coverage by live corals in all sites investigated does not exceed 30%. Although, without a doubt, many factors contributed much to the disturbance of the bay ecosystems, the actual trigger for the degradation of the coral reefs seems to be the input of dioxin-containing chemicals used as defoliants during the American–Vietnamese war (Vietnam War).

Key words: Coral reefs, scleractinians, ecodiagnosis, ecotoxicology, dioxins, defoliants, "Agent Orange."

In recent decades, there has been a tendency toward the deterioration of the state of near-shore coral reefs in various regions of the world [15, 22, 24–26], and for Vietnam in particular, it has become a challenge. Vietnam has extensive sea borders, and the biological resources of the sea are of great importance in the economy of the country. The productivity of the coastal waters is largely determined by the state of the corals reefs.

It has been reported that Nha Trang Bay (South China Sea) has favorable biotopes and one of the richest scleractinian faunas in Vietnam [4, 5, 19, 23]. A series of monographs "Scleractinian Corals of Vietnam" provided a detailed account of the species composition and distribution of scleractinians in Nha Trang Bay up to the year 1986 [6–9]. The purpose of the present research is to examine the present-day state of near-shore coral reefs in Nha Trang Bay and to make a comprehensive integral evaluation (ecological diagnostic) of the quality of the benthic environments and to clarify the possible reasons for the degradation of the coral reefs of the bay.

MATERIALS AND METHODS

In planning this research and in our analysis of the results, we used, along with other publications and our own data, the available reports of expeditions of the Institute of Marine Biology (IMB, then of the Far Eastern Branch of the USSR Academy of Sciences) for the years 1981, 1982, and 1986, as well as the reports of the Russian–Vietnamese Tropical Center (Russian Academy of Sciences) and the Institute of Oceanography (National Center for Scientific Research of Vietnam) in Nha Trang.

The investigations were carried out from 1990 to 2002 at 60 stations, which were located practically throughout the bay (Fig. 1).

In planning the research, we singled out four transects within the bay:

1. Transect A—the northern bay. In this part of the bay, there is a large coral bank (stations 41, 42, 43, 44), the islands of Dum (station 45) and Tyala (station 49), and Binhkanh Bay (stations 46 and 47) with numerous shrimp farms and the Ngada River emptying into the bay.

2. Transect B—the entrance channel of the Kay River. Here, between the coral bank and Che Island

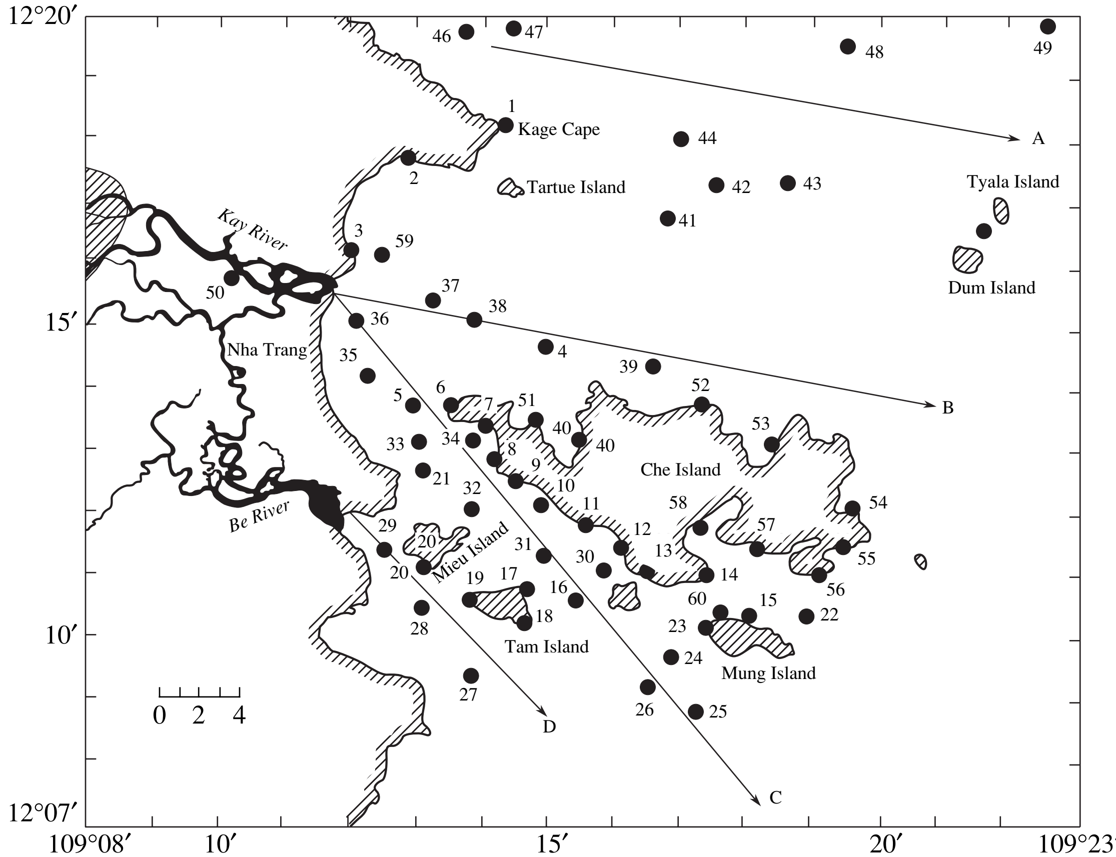

Fig. 1. The location of stations in Nha Trang Bay, 1990–2002. The shaded patch in the upper reaches of the Kay River (left upper part of the map) is the lower limit of the area where dioxin-containing defoliants were used during the American–Vietnamese war (Vietnam War). Transects A, B, C, and D are shown by arrows (explanation in the text).

(stations 4, 37, 38, 39) at a depth of 15–20 m, a non-silted sand plateau was observed in the late 1980s and the early 1990s.

3. Transect C—the area between Che Island and the islands of Mieu and Tam. By virtue of the hydrological features of the bay, a large part of the suspended material from the Kay River is deposited here.

4. Transect D—the southern bay. It receives suspended material from the Be River.

Analyses were performed in the Laboratory of Ecotoxicology, the Institute of Ecology and Evolution Problems, Russian Academy of Sciences. In the bottom sediment samples collected at stations 5, 16, 20, 21, 24, 27, 29, 30, 33, 36, 37, 39, 41, 44, 49, and 50, the total content of dibenzo-p-dioxins (PCDD) and dibenzofurans (PCDF) (in all, 17 congeners) was determined by high resolution chromato-mass spectrometry and expressed using the international equivalents of toxicity (1-TEQ or dioxin equivalent) relative to the most toxic congener 2,3,7,8-TCDD [3]. Specific isomer analysis of PCDD and PCDF was carried out on a GC-MS “Finnigan” MAT-95XL, Hewlett Packard HP 6890 Plus, at a resolution of 10000 [20]. Dioxins are long-lived superecotoxicants [3, 18] and mask well less persistent compounds (among them toxic), the components of which they were formerly.

As is customary in chemico-analytical research, for averaging of small-scale nonuniformity in the distribution of the constituents of bottom sediments, samples at each station were taken at 4–5 points lying about 1 m from each other. Bottom sediments were removed to a depth of 10–15 cm (on hard sands) or 50–70 cm (on soft silts). Samples collected at the same station were pooled, while under water, into an integrated sample and placed in a 1.5-liter tightly sealed plastic vessel. Subsamples of the integrated samples were used in the toxicological research.

The following biological methods of environmental diagnostic [14] were used: fluorimetric, bioluminometric, and genetic methods and the transect technique.

Coral coverage and the state of bottom communities were assessed using the transect technique. Scleractinian corals were selected as the major object of study because, as was noted above, they are edificatory species indicative of the state of coral communities. Transects were made at stations 1, 3, 6, 11, 12, 15, 18, 19, 23, 25, 40, 42, 46, 47, and 49. The transect (graduated rope 100 m long) was perpendicular to the shoreline starting with a zero depth. Along the transect, at 10 m intervals, photo and video shooting of objects was made within a 1×2 m aluminium frame with internal 25×25 cm quadrates.

Sediment sampling and photo and video shooting were made during scuba diving. The exact position of station was fixed from the ship using a Garmin GPSIII plus; where possible, the location of stations was tied to on-land reference points. The depth and transparency of the water were registered at each point of sampling.

The toxicity of the bottom sediments in the bay was studied using samples collected on four transects at stations 5, 16, 20, 24, 27–29, 30, 32, 33, 36, 37, 39, 41, 44, 47, 49, and 59, which more or less fully represent the diversity of the biotopic and hydrological characteristics of the bay. In addition, a sample of sediments from the former riverbed of the Kay River, which is completely inundated during the floods (station 50), was analyzed.

The effect of bottom sediments on photosynthetic activity was examined using cultures of the marine algae *Thalassiosira weissflogii* (Grunow) Fryxell et Hasle (Bacillariophyta), *Tetraselmis viridis* (Rouch) Morris (Prasinophyta), *Nannochloris* sp. (Chlorophyta), and *Isochrysis galbana* Parke (Prymnesiophyta). These algae—routine objects of marine toxicology—belong to the main large taxonomic groups of native inhabitants of marine plankton and periphyton. Scleractinian corals harbor photosynthgetic organisms (zooxanthellae), and fluorimetric studies were made to reveal the adverse effects of the environment on the zooxanthellae, which inevitably tell on the coral host [16]. The working concentration of the bottom sediment suspension in toxicological experiments was 20 mg/ml. Cultures (10 ml) without bottom sediment suspension were the control. Photosynthetic activity (PA) was estimated by the relative proportion of pulse-amplitude-modulation fluorescence $q = F_v/F_m = (F_m - F_o)/F_m$ when measuring the chlorophyll fluorescence rate in open (F_o) and closed (F_m) reaction centers [10]. F_o and F_m in algal suspension were recorded with a portable two-beam impulse fluorimeter designed by the Chair of Biophysics of the Biological Faculty of Moscow State University [1]. The impulse duration of the testing excitation light of fluorescence was 4 μs; the average power density of the exciting light in the measurement of F_o and F_m was 0.4 and 3000 Wt/m^2, respectively. The fluorescence rate was measured before adding the bottom sediment suspension and 24, 48, and 72 h after the addition; the same was done to the control cultures at the same time intervals. Prior to measurements, the algal cultures with the bottom sediment suspensions added were thoroughly stirred. A series of methodical experiments showed that at a sediment concentration of 20 mg/ml, the suspension of particles does not interfere with the estimation of the fluorescence parameters of the algae. From the q values, the inhibition coefficient of PA was estimated:

$$K_{\mathrm{PA}} = (1 - (q_{\mathrm{exp}}/q_{\mathrm{contr}})) \times 100\%,$$

where q_{exp} is the relative contribution of pulse-amplitude-modulation fluorescence in algae in the experimental cultures exposed to the bottom sediment suspension; and q_{contr}, in control cultures. The methodical tests showed that the standard deviation value of K_{PA} changes in some cultures in the range from 0 to 9%; hence, K_{PA} values exceeding 2δ (18%) were considered significant.

The effects of bottom sediments on the vital activity of aerobic nonphotosynthesizing heterotrophic bacteria, as well as the degree of integral toxicity of the bottom sediments were evaluated using the “Biotox-6” and “Biotox-10” devices. This investigation was undertaken because heterotrophic bacteria are important in maintaining the near-shore ecosystem stability in the South China Sea [15] and because an integral evaluation of the toxicity of the bottom sediments for humans is needed. The rate and degree of suppression of the bioluminescence of biosensors was measured. The biosensors were test cultures of heterotrophic chemoluminescent bacteria. The response of the bacteria to supplemented substances is analogous to that in other living organisms, and the value of 50% fluorescence quenching in bacteria completely correlates with a 50% lethal dose for humans. The intensity of bioluminescence of special biosensors (“Ecolum” biosensor in our investigations) containing chemoluminescent bacteria changes in relation to the toxic effect and is represented by the index of toxicity [2, 11]

$$T = 100(I_o - I)/I_o,$$

where I_o and I are respectively the intensity of fluorescence of the control and the experiment at a fixed time of exposure of the test solution with the test object. According to the “Biotox” user’s manual [12], a value of the toxicity index from 0 to 20 indicates that the test substances are virtually nontoxic; with index values of 20 to 50, the test substance is toxic; and with values exceeding 50, toxicity is high. Negative values of the toxicity index are indicative of the presence in samples of substances stimulating metabolic processes in bacteria. In tests with chemoluminescent bacteria, the procedure for the preparation of bottom sediment suspensions was analogous to that used in the fluorimetric study. Samples of bottom sediments were dried and ground in porcelain dishes, then 5 g of the sediment was diluted in 25 ml of filtered pasteurized seawater (distilled water for river samples) to attain a working concentration of 20 mg/ml. For each sample, the toxicity of the suspensions was measured in 12 replicas.

Increased mutagenicity of the environment is one of the factors that not only induces negative responses in some organisms (including humans), but also disturbs the stability of the population structure and ecosystems as a whole. Mutagenic effects of bottom sediments were evaluated using generally accepted methods. Acetone–hexane extracts were made from dried (to con-

Table 1. Mutagenic index of extracts from the bottom sediments in the presence of (+MA) and without (–MA) the metabolic activation system of the strain TA-98 sensitive to mutagens inducing the shift of the reading frame of the genetic code and the strain TA-100 sensitive to mutagens causing mutations of the base substitution type

Reagent			Dose per petri dish	*Salmonella typhimurim* strains			
				TA-98		TA-100	
				+MA	–MA	+MA	–MA
Control	DMSO		0.1 ml	1.0	1.0	1.0	1.0
	2- aminoanthracene		0.5 g	**44.8**	0.9	**8.5**	1.0
Extracts of bottom sediments from stations	River	50	0.1 ml	1.1	**2.3**	0.9	1.5
	Transect C	36	0.1 ml	0.9	0.9	**2.6**	**2.0**
		5	0.1 ml	**1.8**	1.6	0.7	0.9
		30	0.1 ml	**5.4**	1.6	1.0	1.3
	Transect D	29	0.1 ml	**3.0**	1.1	0.9	1.2
	Transect A	44	0.1 ml	**1.8**	**1.9**	0.8	1.2

Note: Bold-faced numerals indicate a significant mutagenic effect.

stant mass at 50°C) bottom sediment samples. The extracts were evaporated, dissolved in dimethylsulfoxide (DMSO), and tested according the Ames method in three replicas. A positive control test with promutagen 2-aminoanthracene was run simultaneously. *Salmonella typhimurim* TA-98 and TA-100 strains were used. The strain TA- 98 is sensitive to mutagens inducing the shift of the reading frame of the genetic code, while TA-100 is susceptible to mutagens causing mutations of the base substitution type. Promutagens (mutagens present in the environment in the inactive state) were revealed according to the standard method [21] using the metabolic activation of bottom sediment extracts. The results of tests (Table 1) are represented as the mutagenic index (MI) reflecting the ratio of the number of colonies his^+ *Salmonella* revertants in the experiment to the control (DMSO). With MI from 1.7 to 10, the mutagenic effect was considered positive; from 10 to 100, moderate; and more than 100, high [17].

RESULTS

Long-term studies of the bottom biotopes using the transect method revealed a more or less pronounced tendency toward the degradation of coral reefs, siltation, and the suppression of scleractinians at all depths and at all stations in the bay. We compared the results of charting coral reefs made by T.A. Britaev and M.V. Pereladov (unpublished report of the Tropical Center for 1990) and the results from our survey in the early 1990s at stations 8, 9, 11, 12, 13, 14, 51, 40, and others adjacent to Che Island with the data obtained by the expeditions of the IMB in 1983 and 1986. Over the following 6–7 years, once rich scleractinian settlements had degraded markedly but still remained. By 2001, in many areas neighboring Che Island scleractinian settlements had disappeared almost completely; dead corals overgrown with periphyton, coral debris covered with silt, or even continuous silt fields were observed on the bottom. Particularly marked siltation occurred in the western and southern bay in the area between Che Island and the mainland (transects C and D, stations 5, 16, 21, 25–33, 35–37, and 59; see Fig. 1). In extensive areas of the bay, the silt thickness was up to several tens of centimeters. In bottom areas where the silt layer exceeded 1–3 cm, live scleractitians were completely lacking. Young scleractinian colonies (seldom more than 10 cm in diameter) or small end growths on old dead colonies of branched corals occurred in weakly silted areas of the bottom (Fig. 2b). Relatively large colonies (up to several tens of centimeters in diameter) were usually encountered on boulders, raised fragments of dead coral reefs, or elevations of the bottom (Fig. 2a).

Earlier ([4, 6], unpublished reports of expeditions of the IMB and the Tropical Center of the RAS until the year 1990), Nha Trang Bay was characterized by a high coverage (up to 100%) of the substratum with live corals in coral habitats. Toward the end of the study period, large dead coral colonies without mechanical damage were found everywhere (Fig. 2c). Mainly small (young) specimens of other hermatypic coral species were found. The total live coral coverage was not above 30% at all points surveyed. The maximum coverage was found for the near-shore reefs of Cape Kage (Station 1), Mung Island (stations 15 and 60), and Tam Island (station 19), where at depths down to 10 m a considerable quantity of live corals was observed on the bottom elevations (ridges) against the background of the silty bottom between the ridges (Fig. 2a).

The fluorimetric studies showed that in most cases the photosynthetic activity was inhibited after a 1-day exposure of algae with bottom sediment suspension and sensitivity varied among the algal species (Table 2). Pooling the data on the suppression of photosynthetic

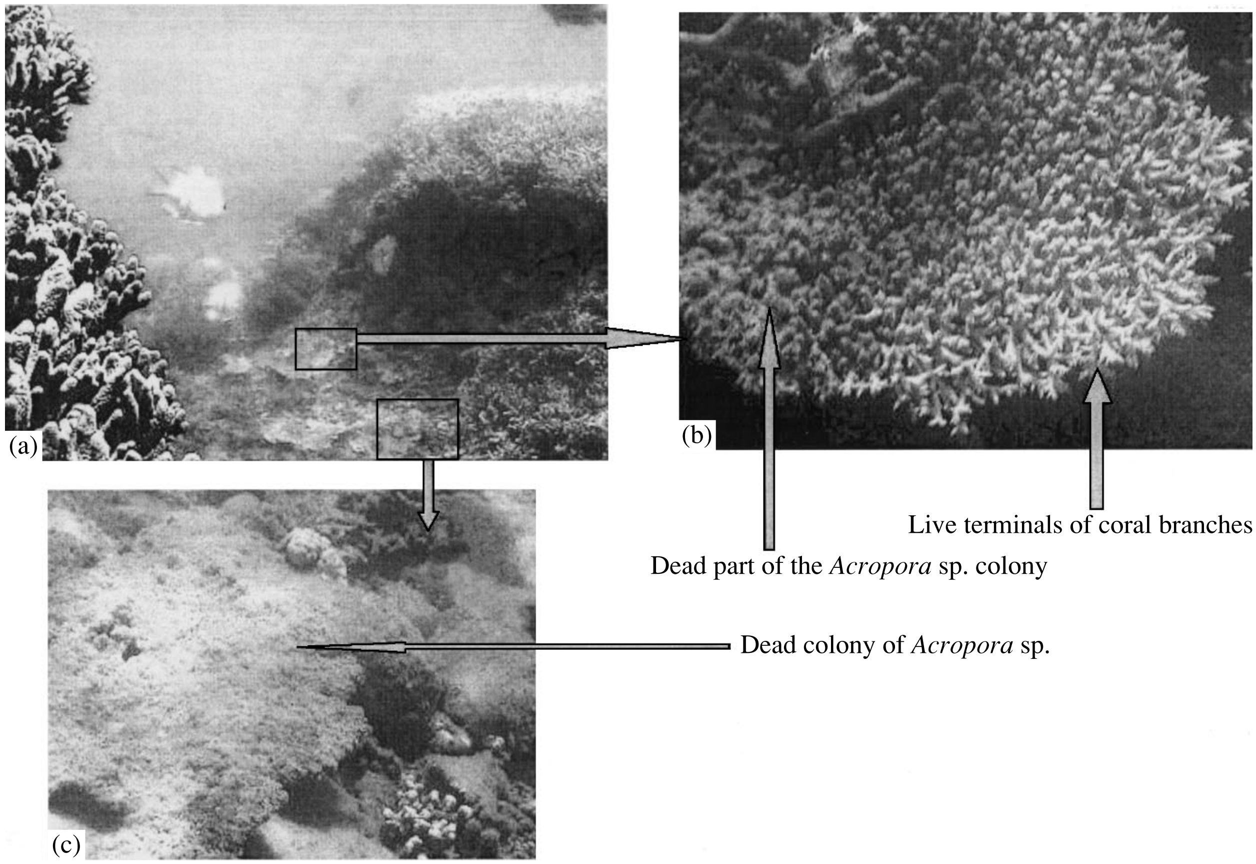

Fig. 2. Station 15 (Mung Island). Depth is about 4 m and transparency about 12 m (see text for explanation).

activity 24 h after supplementing the bottom sediment suspension for four test cultures, we calculated the average values of $^{24}K_{PA(avg)}$ (Table 2), which, to some degree, represent the total response of the multispecies natural community of photoproducers. Most of the tested samples exerted a significant inhibitory effect on photosynthetic activity (Table 2). Inhibition of photosynthetic function was highest ($^{24}K_{PA(avg)}$ = 60%) with the bottom sediments sampled at station 5. In most cases (10 stations), the values of $^{24}K_{PA(avg)}$ varied in the range of 23–37%. In *Nannochloris* sp. exposed to the bottom sediment suspension with the highest content of dioxins (4–20 ng/kg) (stations 16, 33, and 59), K_{PA} increased on the 2nd–3rd day, while at a dioxin concentration of less than 4 ng/kg, K_{PA} decreased with time (Table 2). The exception was a decrease in K_{PA} after the first day of exposure with sediments from the former bed of the Kay River at a dioxin concentration of 7.9 ng/kg (station 50). It is possible that the decrease was due to the different composition of toxic substances in the sediments of the river and the sea bay.

The data on the effect of bottom sediment suspension on bioluminescence agreed well with the data on photosynthetic activity (Table 2). A pronounced toxic effect on bacteria was found for sediments from almost all of Nha Trang Bay, excluding stations 20, 27, 28, 29 (channel of the Be River), station 49 at Tyala Island (which is the farthest from the mouth of the Kay River), and station 47 in Binhkanh Bay. Despite the strong anthropogenic impact (except station 49), bottom sediments from these points produced a pronounced positive effect, increasing bioluminescence. Bottom sediments from the cutoff meander of the Kay River (station 50), despite the high dioxin content (I-TEQ = 7.9), induced no pronounced inhibition of bioluminescence. Bioluminometric data, along with the results of other tests, suggest the toxicity of bottom sediments from transects C, B, and partly A, as well as the mosaic distribution pattern and different present-day composition of toxicants in bottom sediments of the bay.

All the extracts of bottom sediments tested displayed a weak mutagenic effect (Table 1). The extracts of sea bottom sediments showed a mutagenic effect only following metabolic activation and induced a shift of the reading frame of the genetic code. On the other hand, extracts of the bottom sediments from station 36 located in the immediate proximity of the Kay River mouth and sediments from station 44 (coral bank) showed a direct mutagenic effect, which was retained after metabolic activation. Bottom sediment extracts from station 36, in contrast to all others, caused mutations of the base substitution type. River sediment

Table 2. Inhibition of the photosynthetic activity of algae (% of the control) on 24, 48, and 72 h exposure and of chemoluminescence of heterotrophic bacteria (toxicity index) in the presence of the bottom sediment suspension

Transect	Station	Depth, m	1-TEQ, ng/kg	Coefficient of inhibition of algae photosynthesis (K_{PA}) on 24, 48, and 72 h exposure													Toxicity index (I_t) for 30 min exposure
				Tetraselmis viridis			*Nannochloris* sp.			*Isochrysis galbana*			*Thalassiosira weissflogii*			$^{24}K_{PA}$ (avg)	
				24	48	72	24	48	72	24	48	72	24	48	72		
River	50	1.5	**7.9**	37	21	15	22	16	14	41	42	29	38	30	20	**35**	15
C	36	15.4	**2.4**	28	30	20	14	17	14	71	87	80	24	27	16	**34**	**44**
	5	25.7	**3.1**	39	38	30	32	32	22	94	69	91	76	41	55	**60**	**49**
	33	22.7	**4.2**	20	24	18	14	12	19	32	15	2	33	25	0	**25**	**73**
	32	19.4	–	8	25	18	11	17	13	24	0	0	18	0	0	15	**44**
	30	21.5	**1.8**	27	20	8	14	19	23	29	8	0	22	0	9	**23**	**62**
	16	20.6	**20.8**	11	17	17	9	17	16	23	0	0	18	0	0	15	**37**
	24	32.7	**0.4**	29	19	23	13	7	5	26	18	10	30	28	14	**25**	**94**
B	59	7.5	**16.8**	27	25	8	20	18	31	30	17	0	31	6	3	**27**	**80**
	37	21.8	**1.9**	14	10	0	9	9	13	22	7	14	8	0	0	13	**70**
	39	20.5	**0.8**	9	4	8	0	3	0	0	18	0	2	0	0	3	**30**
D	29	14.0	**1.0**	21	16	3	8	12	1	16	16	2	26	20	2	**18**	–97
	28	10.6	–	39	29	2	28	19	1	22	21	20	26	26	22	**29**	–47
	27	9.5	**2.1**	58	45	23	24	19	3	22	14	0	42	24	13	**37**	–108
	20	4.5	**0.7**	2	0	0	4	9	0	0	0	0	2	0	0	2	–157
A	41	21.0	**0.8**	29	25	13	15	9	4	31	18	17	15	13	10	**23**	**50**
	44	18.0	**0.5**	2	0	2	0	0	0	0	2	6	0	0	0	1	**49**
	49	18.0	**1.3**	0	0	0	0	0	0	0	8	6	0	0	0	0	–201
	47	5.0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–65

Note: The depth and total amount of dibenzo-p-dioxins (PCDD) and dibenzofurans (PCDF) represented by the international equivalents of toxicity (I-TEQ) are given for stations of bottom sediment sampling. Bold-faced values indicate a significant toxic effect; “–”—measurements were not made.

extract also induced a direct mutagenic effect, which, however, was lost following metabolic activation. We note that a reduction in the mutagenic index in some experiments, compared to the control (MI < 1), might indicate the presence of compounds toxic to *Salmonella*. Thus, the Ames test, suggesting the presence in bottom sediment samples of mutagens and toxicants, indirectly supports the above assumption on the different present-day composition of toxicants in the bottom sediments of the river and the sea bay.

The results of chromatographic and mass spectrometric analyses revealed the presence of persistent congeners PCDD and PCDF in bottom sediment samples from the bay. The spectrum and distribution pattern of congeners in all samples were close to those of the defoliant "Agent Orange" (predominantly 2,3,7,8-TCDD, 1,2,3,4,6,7,8-HpCCD, OCCD, and 1,2,3,7,8,9-HxCDF, OCDF). The total amounts of dioxins in the bottom sediments at sampling stations varied from 0.409 to 20.806 ng/kg in I-TEQ (Table 2), which is several orders of magnitude higher than the accepted sanitary standards.

DISCUSSION

The impairment of the state of near-shore coral reefs in Nha Trang Bay has been explained by various reasons. Among them global warming of the climate, much increased recreation pressure and coral harvest for souvenirs, injurious use of corals for lime production, fishing with the use of explosives, and pollution of the Kay and Be rivers by agricultural and municipal waste water.

Our integral assessment of the state of the bottom habitats in the bay suggests that bottom sediments contain long-lived components of herbicide chemicals of the "Agent Orange" type and that the environment is unfavorable (toxic) to many groups of organisms in the greater part of the bay. The diagnostic data allow us to come back to the earlier hypothesis [13] that despite the undeniable and apparently considerable adverse affects of the above factors on the bay's ecosystems, one of the most probable reasons and a trigger for the degradation of the reef-forming communities were toxic dioxin-containing components of herbicides, which were used as defoliants during the American–Vietnamese war (Vietnam War) and have entered the bay via the Kay River run-off.

In the basin of the Kay River and its tributaries, hundreds of thousands of highly toxic defoliants were sprayed in the course of military operations. Both soluble and little-soluble highly toxic components of dioxin-containing chemicals were transferred into the bay via floodwaters and heavy fractions of river silts and detritus. The range of long-lived ecotoxicants (PCDD and PCDF) in the modern sediments of the river and the bay bears a sharply pronounced resemblance to that of soils in areas treated with "Agent Orange" during the war [20]. This suggests that the transfer into the bay of at least part of the dioxins occurred via river run-off from the defoliant-treated areas.

It is very likely that over a relatively short period of time (2–3 years) after the massive treatments of the jungles were stopped considerable amounts of dioxin-containing defoliants, which were sprayed over the extensive watershed of the Kay River, were transferred via river run-off into the sea. Little-soluble and adsorbed components of toxicants together with bottom silts were subsequently redistributed over the bay. The toxic action of dioxin-containing defoliants on marine organisms entailed the inhibition of primarily hermatypic photosynthetic organisms and aerobic heterotrophic bacteria, which are the main components of reef ecosystems maintaining their stability. The primary massive chemical action was aggravated due to the periodic (during the rainy seasons) input of defoliant components from the river valley and to increasing anthropogenic pressure. Thus, the state of the bottom environments and the near-shore bottom communities in Nha Trang Bay has deteriorated markedly. The results of our investigations suggest that the presence of residues of dioxin-containing ecotoxicants in the marine bottom sediments is evidently the major stress factor (along with significant anthropogenic pressure) for the modern near-shore communities of Southern Vietnam. The adverse effect of dioxin-containing ecotoxicants is aggravated by the periodic stirring-up of silts during the storms and generally increased turbidity of the water as the results of the reduced coverage by live corals, which are the natural biological filters in the near-shore tropical ecosystems.

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