VALIDATING SPECIES DIVERSITY OF BENTHIC ORGANISMS TO TRACE METAL POLLUTION IN KUWAIT BAY, OFF THE ARABIAN GULF

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Abstract. Benthic organisms diversity were observed in the sequence of Annelida > Mollusca > Crustacea > "Diversa" group. Levels of trace metals in benthic organisms were in the range 0.12–96.86 µg/g during winter and 0.98–54.13 µg/g in summer. Species diversity index (*H*'), evenness index (*J*) and index of dominance (*λ*) were in the range 0.951–1.368 bits/unit, 0.475–0.684, and 0.19–0.33 respectively, for benthic organisms sampled in Kuwait Bay sites. Evenness index (–) was found to increase with increasing *H*'. Seasonally, an inverse correlation was observed between species richness (*R*1 and *R*2). Comparative studies revealed low diversity indices correspondingly to the increase in trace metal level in benthic species collected from four sites except Doha wherein high abundance of certain benthic species and high trace metal levels due to manmade perturbations were observed altering the diversity indices. Furthermore, these indices will validate benthic organisms as an indicator to trace metal pollution in Kuwait marine ecosystem.
Keywords. *benthic organisms; trace metals; species diversity*

Introduction

Rapid industrialization and recent 'Fish Kill' aftermath of the Gulf War I and II, has been causing concern over the stressed ecosystem in Kuwait Bay. Investigations on the harmful algal blooms (HABs), metal levels in marine organisms, bacteriological and toxicological studies and organic and inorganic pollutants supported evidences to marine pollution in this Bay [3–5, 9, 18, 23, 29, 31]. Several investigators observed metal concentrations in aquatic macro benthic organisms to be a better indicator than ambient (water) metal concentrations [2, 7, 11, 12]. Studies revealed oligochaetes as an indicator to organic and industrial pollution [6, 22]. They suggested that if benthos occurs in a density of 100–999 individuals/m^2, the water is unpolluted; 1000–5000 individuals/m^2, moderately polluted; and more than 5000 individuals/m^2 shows heavy pollution. Earlier observations showed benthic organisms to be highly sensitive to environmental stress due to trace metal pollution [13, 24, 25, 30]. They also noted anthropogenic pollution influenced by sedentary benthic organisms. In contrast to these findings, few investigators [15, 19, 26] observed low pollution levels coinciding with some oligochaetes and polychaetes densities in some geographical sites. However, in an overall view, they found the macro benthos to be an indicator of pollution. Meanwhile, studies on the concept of species diversity in community ecology intensified by ecologists over the past few decades. Observations revealed various diversity indices which responded differently to different environmental factors of biotic communities [21]. A combined index, which characterizes species abundance and evenness simultaneously, limits the dependent factors of sample size. Further, [14] observed species diversity indices are justifiable when subjected to comparative analysis. In Kuwait, no published data relating to the

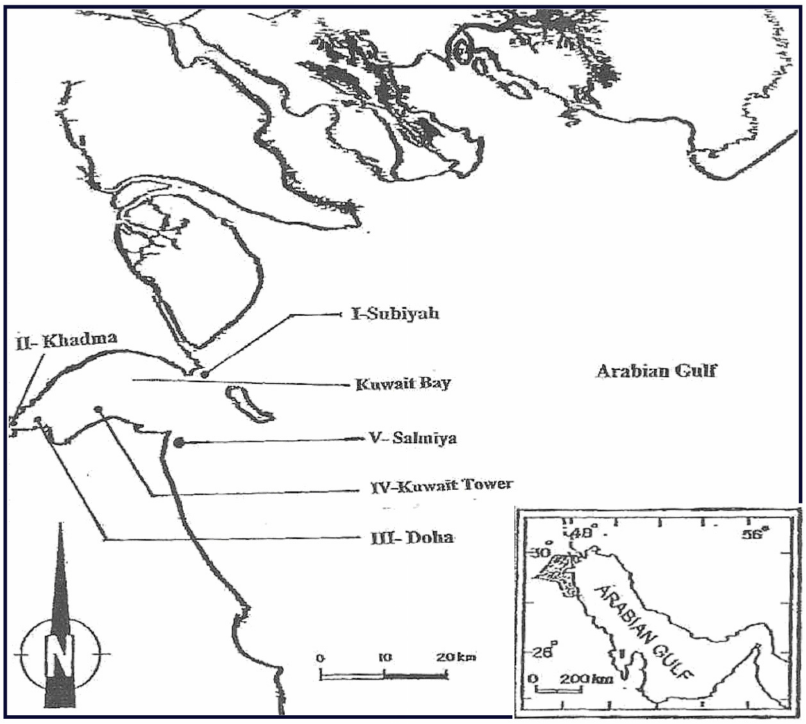

Figure 1. *Map indicating the sampling sites*

species diversity of benthic organisms exist. Therefore, investigations were carried out to: (1) determine the abundance of benthic organisms in five sites of Kuwait Bay; (2) compare predominant trace metal levels in the major and minor benthic organisms (annelids, crustaceans, molluscs and grouped "Diversa" respectively) in Kuwait Bay sites; and (3) validate the diversity of benthic species as indicators to trace metal pollution with seasonal changes (summer and winter) in the Kuwait Bay.

Materials and methods

Employing a 'Van-Veen' grab with a mouth opening of 0.1 m^{-2}, benthic organisms were collected from five Kuwait Bay sites. Further, from each site, five transect samples were collected to minimize sampling errors (*Fig. 1*).

The samples were separated from the sediment using a 0.5 mm sieve [13]. Following the classification [1, 8, 20] benthic samples were rinsed, weighed, dried at 50 °C in an oven (Gallencamp II) and homogenized. Benthic mollusc and crustacean species were removed from their shells during preparation but other organisms were analyzed without modification. Unidentified encrusting algae were scraped from mollusc shells. Dried benthic samples weighing 2 g were pre-treated in 3 ml nitric acid (Aristar grade, v/v) and 1 ml HCl (Aristar grade, v/v) in a Fischer brand sterile centrifuge disposable tube (50 ml capacity). After 48 hours, the samples were diluted to 50 ml in double distilled de-ionized water, digested in an automatic microwave digester (Spectro Prep-CEM) and the metal levels (µg/g) measured by atomic absorption spectrophotometer (AAS, Perkin Elmer 5100). The accuracy of the method was verified using standard material, DORM-1 for benthic samples from the National Research Council of Canada and average recoveries (95%) of all the trace metals achieved.
Species richness was calculated using the known indices [16, 17] as given below:

$$R1 = S - \frac{1}{\ln n}, \quad \text{(Eq. 1)}$$

$$R2 = S - \frac{S}{\sqrt{n}}, \quad \text{(Eq. 2)}$$

wherein, *R*1 and *R*2 represent the species richness indices. The utility of *R*2 holds well, when a functional relationship exists between *S* and *n*. *S* is the total number of species and *n* is the total number of individuals. If the assumption fails, the richness index will change with sample size in an unknown manner. Thus, *R*1 and *R*2 combination was worked out to draw better conclusions by another index [28]:

$$\lambda' = \sum_{i=1} n_i \frac{(n_i - 1)}{n(n-1)}. \quad \text{(Eq. 3)}$$

For a finite community, Simpson's index (λ) gives the probability of two individuals drawn randomly from a population belonging to the same species. However, in the present study, infinite population was found and it was impossible to count all members and hence, the Simpson's unbiased estimator (λ') was used. n_i is the number of individual of the *i*th species.

Diversity index [27] is:

$$H' = \sum_{i=1}^{s} [(n_i/n)\ln(n_i/n)]. \quad \text{(Eq. 4)}$$

This index (*H*') is a measure of the average degree of 'uncertainty' in predicting to what species an individual chosen at random from a collection of *S* species and *N* individuals will belong to. Thus, *H*' = 0 if there is one species in the sample and *H*' is maximum only when all *S* species are represented by the same number of individuals, that is a perfectly even distribution of abundances.

Evenness index [21]:

$$J = H' / H'_{max}. \quad \text{(Eq. 5)}$$

This index represents *H*' relative to the maximum value that *H*' can obtain when all the species in the samples are even with one individual per species. In the present study,

$H'_{max} = \log_2 S$

was used. (Eq. 6)

Results and discussion

The abundance of benthic samples incorporated the mean counts/m^2 from the five transects collected from each site, off the Kuwait Bay (*Tables 1* and *2*).

Observations showed that the annelids increased in density during summer than in winter in Kuwait Bay, which supports the earlier study [2]. The overall counts were lower in Mollusca, Crustacea and "Diversa" groups when compared to Annelida, during summer and the reverse during winter. This could be attributed to (1) the high trace metal levels and other pollutants resulting in mortality of the shelled organisms and "Diversa" groups during summer, and (2) the high tolerance to environmental stress by the annelids in the Bay and supports the earlier findings [18, 30].

Abundance of benthic annelids, molluscs, crustaceans and „Diversa” group at different sites were noted in the sequences of Doha > Kuwait-Tower > Salmiya > Subiyah > Khadma during summer (*Table 3*) and Doha > Kuwait-Tower > Khadma > Salmiya > Subiyah during winter (*Table 4*).

The Kuwait Bay sites revealed the total abundance of more than 1000 pollution indicator benthic species / m^2 irrespective of seasonal variation. Therefore, the classification of ‘moderately polluted areas’ with individuals >1000 species / m^2 in Kuwait Bay sites showed evidences to studies [6, 22].

The present study five trace metals such as Cu, Zn, Fe, Ni and Pb were chosen, and observed: (1) within the detectable limits in AAS, (2) to cause significant impact on marine organisms and (3) in high levels in Kuwait Bay waters. In general, trace metal levels were in the sequence of Mollusca (0.1–96.86 µg/g) > Crustacea (0.98–28.12 µg/g) > Annelida (0.08–4.71 µg/g) > and “Diversa” group (0.15–4.06 µg/g) irrespective of the

***Table 1**. Composition and relative abundance (% frequency) of benthic organisms during summer in Kuwait Bay*

species	site				
	Subiyah	**Khadma**	**Doha**	**K. Tower**	**Salmiya**
benthic Annelida					
Eulalia viridis	9	8	10	9	8
Polydontes melanoleis	3	—	4	2	6
Syllis gracillis	—	2	3	3	2
Ceratonereis erythroensis	9	6	15	11	7
Nephys tulerensis	5	3	5	4	3
Glycera convolute	2	3	3	2	2
Eunice indica	8	9	6	9	7
Marphysa sanguinea	1	4	1	1	2
Janua kayi	3	5	1	7	4
Megalomma quadrioculatum	—	3	1	—	4
total	40	43	49	48	45
benthic Mollusca					
Tellidora pellyana	6	5	5	5	5
Solen vagina	5	4	6	4	4
Donax scalpellum	5	6	7	7	5
Dentalium octangulatum	3	6	5	5	5
Cerithium scabridum	3	8	8	9	8
total	22	29	31	30	27
benthic Crustacea					
Platyischnopus longimanus	1	1	2	1	1
Balanus tintinnabulum	6	13	13	10	8
Diogenes avarus	1	1	1	4	1
Alpheus djeddensis	1	1	2	2	1
Pagarus perspicax	1	1	1	1	1
total	10	17	19	18	12
“Diversa” group					
Oligochaeta sp.	23	8	1	3	12
Nematode sp.	2	2	—	1	1
Nemertina sp.	1	—	—	—	1
Spongia sp.	1	1	—	—	1
Turbellaria sp.	1	—	—	—	1
total	28	11	1	4	16

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two seasons which supports an earlier investigation [4] in Kuwait Bay. Trace metal levels were observed in the sequence of Zn > Fe > Cu > Ni > Pb in annelids and„Diversa" but Fe > Zn > Cu > Ni > Pb in molluscs and crustaceans, irrespective of seasons. Among the five metals, Zn was predominant in the bodies of annelids. The above-mentioned metals could be transferred or assimilated in their bodies from the sediment when compared with the other metals [11]. Meanwhile, observation showed a sequential change in Fe over Zn levels in molluscs and crustaceans, nevertheless the other metals observed in the sequence to that of the annelids. Doha consistently had the high trace metals level (0.93–96.86 µg/g) in all the samples than the other Kuwait Bay sites. This attributes to (1) stagnant water in the Bay causing accumulation of metals in these organisms, and (2) the discharge of domestic and wastewater into the Bay which supported earlier observation [5]. The

***Table 2**. Composition and relative abundance (% frequency) of benthic organisms during winter in Kuwait Bay*

species	site				
	Subiyah	**Khadma**	**Doha**	**K. Tower**	**Salmiya**
benthic Annelida					
Eulalia viridis	1	—	2	3	2
Polydontes melanoleis	1	—	—	1	—
Syllis gracillis	—	—	1	1	1
Ceratonereis erythroensis	3	4	6	5	4
Nephys tulerensis	—	1	—	1	—
Glycera convolute	1	1	—	—	1
Eunice indica	1	—	—	—	1
Marphysa sanguinea	—	2	3	2	—
Janua kayi	1	1	2	1	2
Megalomma quadrioculatum	1	1	2	1	2
total	9	10	16	15	13
benthic Mollusca					
Tellidora pellyana	1	1	4	1	1
Solen vagina	2	4	4	3	2
Donax scalpellum	1	3	3	1	1
Dentalium octangulatum	2	5	4	5	1
Cerithium scabridum	8	4	5	8	10
total	14	17	20	18	15
benthic Crustacea					
Platyischnopus longimanus	6	5	7	8	5
Balanus tintinnabulum	7	11	12	9	7
Diogenes avarus	6	8	7	6	6
Alpheus djeddensis	3	2	3	4	4
Pagarus perspicax	1	2	3	3	3
total	23	28	32	30	25
"Diversa" group					
Oligochaeta sp.	6	7	13	12	8
Nematode sp.	11	12	8	5	8
Nemertina sp.	12	9	6	6	12
Spongia sp.	13	9	2	8	9
Turbellaria sp.	12	8	3	6	10
total	54	45	32	37	47

***Table 3**. Benthic organism count (×10^2 /m^2) and diversity indices during summer in Kuwait Bay*

species	site

	Subiyah	Khadma	Doha	K. Tower	Salmiya
Annelida	4	5	15	12	8
Mollusca	2	4	13	10	3
Crustacea	1	3	10	9	2
"Diversa"	8	2	1	1	3
total	15	14	39	32	16
*R*1	1.107	1.136	0.818	0.865	1.082
*R*2	1.032	1.069	0.640	0.707	1.000
λ	0.333	0.236	0.307	0.296	0.291
H'	1.136	1.333	1.176	1.196	1.234
J	0.568	0.665	0.588	0.598	0.617

*R*1: Margalef's index; *R*2: Menhinick's index; *λ*: Simpson's index; *H*': Shannon-Weaver's index; *J*: Pielou's index

***Table 4**. Benthic organism count ($\times 10^2/m^2$) and diversity indices during winter in Kuwait Bay*

species	site				
	Subiyah	Khadma	Doha	K. Tower	Salmiya
Annelida	2	3	9	5	3
Mollusca	1	2	6	3	2
Crustacea	1	2	4	3	2
"Diversa"	3	2	1	1	1
total	7	9	20	12	8
*R*1	1.541	1.365	1.001	1.207	1.442
*R*2	1.133	1.000	0.670	0.866	1.060
λ	0.190	0.250	0.300	0.240	0.250
H'	0.951	1.368	1.192	1.265	1.320
J	0.568	0.665	0.588	0.598	0.617

*R*1: Margalef's index; *R*2: Menhinick's index; *λ*: Simpson's index; *H*': Shannon-Weaver's index; *J*: Pielou's index

overall analyses revealed trace metal levels at 0.12–96.86 µg/g in benthic organisms during winter than in summer (0.98–54.13 µg/g). The reasons may be due to the trace metals transfer from sediment to the benthic organisms and supports the earlier findings [2, 5]. Comparative studies in most of the observations (*Table 5*) revealed higher trace metal levels in benthic molluscs and annelids in Kuwait Bay than in the earlier observations from other countries [3, 10, 24, 26]. Publications were recorded on the „Diversa" group in the light of abundance [30], but no studies related to their trace metal levels.

Species diversity studies revealed (a) species richness (*R*1), (b) species richness (*R*2) to be the least but (c) Simpson index (*λ*) to be the highest in Doha when compared to the other sites during both seasons, (d) Shannon-Weaver's diversity index (*H*') between 0.951 and 1.368 bits/unit during both seasons and framed within the diversity of 2.000 bits/unit. In general, the species diversity indices were higher during winter than in summer. Quantitatively, benthic species were observed in high numbers in Doha site but, the *H*' and *J* indices were found moderate when compared to the other sites. This attributes to (1) the dominating mollusc and annelid species, and (2) those abundant species that could tolerate the metal polluted Doha site. Index (*J*) [21], revealed the highest value in Khadma with high *H*' but with low trace metal levels.

Table 5. *Comparative analyses on the mean trace metals levels in benthic organisms from different areas of the world*

trace metals (μg/g)	benthic organisms Annel.	Mollus.	Crusta.	"Div."	location	references
Cu	3.14	51.01	5.21	2.18	Kuwait Bay	present study
Zn	4.71	68.01	22.93	4.06		
Fe	4.38	96.86	28.12	3.13		
Ni	1.01	1.27	2.48	0.90		
Pb	0.98	1.21	2.05	0.87		
Cu	NS	2.43	1.61	NS	Taiwan	[10]
Cu	4.13	2.16	NS	NS	Cheaspeake Bay, U.S.A.	[24]
Cu	2.40	NS	NS	NS	Spain	[26]
Cu	2.40	NS	NS	NS	UK estuaries	[3]
Zn	6.80	NS	NS	NS		
Fe	4.92	NS	NS	NS		
Ni	1.30	NS	NS	NS		
Pb	0.30	NS	NS	NS		
Cu	3.10	55.10	7.10	NS		
Zn	25.00	82.50	35.12	NS		
Fe	NS	148.12	45.80	NS		
Ni	NS	NS	4.30	NS		
Pb	2.50	1.20	2.30	NS		

Annel. = Annelida; Mollus. = Mollusca; Crusta. = Crustacea; "Div." = "Diversa"; NS = not studied

Conclusions

The present findings revealed the significance of trace metal levels in benthic organisms to the stressed ecosystem of Kuwait Bay. Observations showed relatively high (*H*') species diversity index [27] in sites with low trace metal levels that justifies high or low abundance of benthic organisms, validating these benthic organisms as an indicator to metal pollution and enable environmentalists to take precautionary measures.

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