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The Influence of *Holothuria atra* (Echinodermata: Holothuroidea) on Bacterial Density and Sediment Characteristics of the Red Sea, Hurghada, Egypt.

W.M. El Kazzaz[1], E.E. Shabana[2], M.R. Dar[3], A. Dewedar[1]

[1] Botany Department, Faculty of Science, Suez Canal University, Egypt.
[2] Egyptian Environmental Affairs Agency, Hurghada Branch, Red Sea, Egypt.
[3] National Institute of Oceanography and Fisheries, Hurghada, Red Sea, Egypt.
Corresponding author: E.E. Shabana. E-mail: miarmetwaly@gmail.com. Tel: +201010397020

Abstract

This study was undertaken to investigate the effect of sea-cucumber (*Holothuria atra*) population density on sediment microbial community structure. Feeding effects were investigated over six months in protected aquaculture ponds at National Institute of Oceanography and Fisheries (NIOF). Four treatments of high population density (108 individuals/30m^{2}), Medium population density (54 individuals/30m^{2}), low population density (18 individuals/30m^{2}) and Reference (pond without *H. atra*). Intestinal (Foregut and hindgut), skin of *H. atra* and ambient sediment samples were used to investigate its effect on sediment's pH, Total phosphorus (TP), grain size and culturable bacterial groups represented in; Total Viable bacteria (TVB), Total coliform (TC), *Vibrio* sp (V), Fecal coliform (FC) and *Salmonella* and *Shigella* (SS). The data showed that water temperature ranged from 20.8 ^{o}C to 33.1^{o}C during February and May 2009 respectively. The pH decreased in ponds of *H. atra* by 0.64 (7.8 to 7.16); 0.46 (7.81 to 7.35); 0.28 (7.84 to 7.56) in HPDP, MPDP and LPDP respectively, while in sediment of reference pond increased by 0.11 (7.93 up to 8.04). Dilution plating of samples indicated that numbers of culturable bacteria (TVB, V, TC and SS) in hindgut contents were higher than in foregut content samples, skin, and ambient sediment, While FC count exhibited highest count in foregut than hindgut contents, ambient sediment and skin respectively. Data analysis showed no significant effect of *H. atra* density on TP, fine sediment ($\O_{4}$ + $\O_{5}$) and *Vibrio* sp while a significant effect recorded on pH, coarse sediment ($\O_{-1+}$ $\O_{0+}$ $\O_{1}$), medium sediment ($\O_{2}$ + $\O_{3}$), TVB, FC, TC and SS. Observing reduction in Total coliforms, Fecal coliforms, *Vibrio* sp, *salmonella* and *shigella* in addition to its role as a pH controller, it can be concluded that *Holothuria atra* can be used successfully for the improvement of coastal regions with its associated fauna and flora. This study emphasizes the feeding selectivity and ecological role of the most dominant holothurians species-*H. atra* in the red sea.

Keywords: *Holothuria atra*, culturable bacteria, grain size, selective feeding

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1. Introduction

Phylum Echinoderm is a large group of marine animals worldwide, consisting of starfish, brittle star, sea urchin, feather star and sea cucumber. The holothurians, or sea cucumbers, comprise a class, Holothuroidea, of some 900 members within the invertebrate phylum Echinodermata [1]. There are about 80 species of sea cucumber belonging to 22 genera in the Red Sea [2]. Holothurians and other sediment-feeders are important components of a benthic recycling system that may have some similarity to the planktonic microbial loop [3]. Sea cucumbers obtain food by the ingestion of marine sediment, or by filtration of sea water. Deposit-feeding holothurians are well-known members of many deep benthic communities ([4, 5, 6] where they are believed to mix the deep-sea mud on a large scale [4]. Sediment ingested by deposit feeding holothuroids is of low nutrient value [7]. The low richness of organic matter forces the deposit feeders to ingest large amounts of sediment to obtain their needed energy [7].

Holothurians are also alleged to play an important role in nutrient cycling and sediment modification in shallow-water habitats [8]. Farouk *et al.* [9] isolated some bacterial strains from various tissues of the sea cucumber species, (*H. atra*). The bacterial secretions and extracts showed an interesting antibacterial activity. A culturable, mixed, bacterial population associated with the hindgut lining of abyssal holothurians has been reported by using direct count techniques the author [10] demonstrated higher numbers of bacteria in the holothurians intestinal tract than in the surrounding sediment [11]. The presence of intact bacteria directly associated with the holothurians gut lining was also demonstrated by transmission electron microscopy, on the basis of these findings, the presence of an active commensally gut flora metabolizing organic matter ingested by the holothurians was suggested [11]. Other reports [12, 13] have suggested that gut bacteria may have an important role in the supply of specific essential amino acids to the holothurians, in the same way that rumen microbes contribute some essential nutrients to their hosts [14]. Ohwada *et al*. [15] found that the gut of deep-water invertebrates has a bacterial flora abundant in *Vibrio* sp. and also suggest that some marine invertebrates may serve as reservoirs for certain potential pathogenic *Vibrio* Sp. *Vibrio* sp have been isolated from fresh- water, estuarine and marine environments [16, 17]. While some Vibrios are pathogenic to humans and lower animals, other species are not, but they play an important role in the recycling of nutrients, especially chitin [18].

The effect of feeding by the sea cucumber *Actinopyga mauritiana* on sediment characteristics in the red sea examined by [19], the study emphasizes the vital role of holothuroids in generating and/or regenerating nutrient in the marine environment. Kitano [20] reported that feeding and movements of sea cucumbers on the sea bottom recovered the bottom conditions and increased the number of diversity of species by inhibition of anaerobic conditions. It was also reported that extirpation of sea cucumbers caused hardening sea floor, eliminating habitat for other benthic and infaunal organisms [21]. The beche-de-mer fishery began in Egypt in 1998. Egypt has become one of the most important suppliers of beche-de-mer after the depletion seen other areas. By 2000, the fishery had expanded dramatically, leading to fears of overexploitation. Consequently, the Red Sea Governorate initiated a ban on sea cucumber fishing in 2001 [22]. The initial ban on fishing resulted in the development of a large illegal fishery along the coast of Egypt. In addition, pressure from the Fisheries to reopen the fishery lead to the Red Sea Governorate lifting its ban in 2002. However, preliminary data collected by the Darwin project indicates that populations of commercial sea cucumber have undergone a rapid decline, and this has now led all government agencies and departments to realize that the resource needs immediate protection. Consequently, a new prohibition was decreed in March 2003 to cover the whole coastline [22]. Ahmed and Lawrence [23] updated the situation. Following the initial study, they have re-visited some of the sites in the red sea to determine whether there was any evidence of stock recovery following the 2003 fishery ban. It appeared that four years after the ban on the fishery, there is some evidence of the return of selected commercial species to some of the sites, but no evidence of stock recovery. It was therefore the purpose of this study to explore the effect of sea-cucumber (*H. atra*) population density on sediment microbial community structure and composition.

2. Materials and methods

2.1. Experimental site and design

Because of the vulnerability of the coast line to overfishing, the preparation dehumanizes sea cucumber decreased significantly. A preliminary survey had been made to select a suitable site to collect enough *H. atra* specimens characterized with high density, near to Hurghada city and easily accessible at any time without obstacles. Another site selected to establish aquaculture ponds to study effect of *H. atra* effect on sediment characteristic. As a result of survey we find that the best location to collect *H. atra*, identified within the scope of the El Gouna resort (N: 27°24'3.69, E: 33°41'15.68) to confront one of private villas as shown in Fig (1); it had not been subjected to overfishing until the completion of the study (El Gouna site-A).The aquaculture pond (N: 27°17'6.21, E: 33°46'23.02) of (NIOF) was selected and modified as shown in Fig. (2). The pond was 10m x12m, divided into four sections by using green nets supported by metal stands and embedded in the pond sediment to 20 cm depth to prevent the animal escaping from one section to the neighboring one. Each of the sub-ponds had the dimensions 5m x 6m, the water gets into and get out of the ponds following the high and low tide of the sea through fine crevices in the pond walls. About 200 animals collected and distributed into the pre-prepared sub-ponds. In the first section (HPDP: 108 animals) of *H. atra* was allocated while in the second 54 animals allocated to represent the MPDP and in the third 18 animals was allocated to represent LPDP, while the fourth section of the pond was left as a reference or control pond.

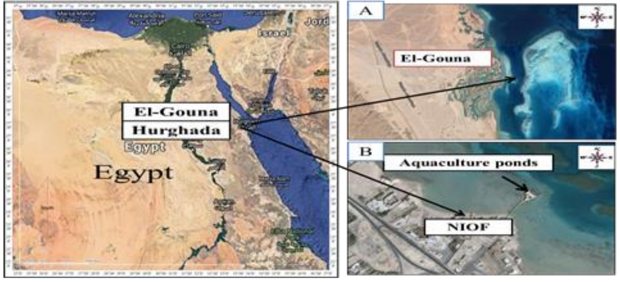

Fig (1): location map of Elgouna Resort (A: Sea cucumber collection site) and NIOF aquaculture pond (B: protected experiment), the site position in relation shoreline. Hurghada, Egypt.

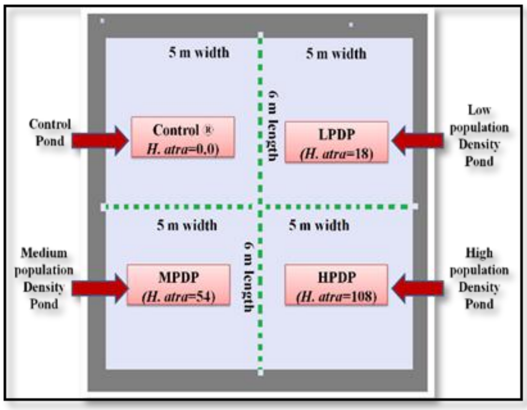

Fig (2)**:** Schematic diagram for aquaculture ponds hosted with different population densities of *H. atra*. NIOF-Hurghada-Egypt.

2.2. Sediment sampling and analysis for physico-chemical parameters of sediment.

Sediment samples were collected monthly by using a transparent cylindrical corer of 4.5 cm diameter and 50 cm length. The samples were collected in a 500g sterilized plastic bags. Three cores with 10 cm heights ambient sediment were collected randomly from each of the population density ponds and reference pond and placed into labeled plastic bags. Physico-chemical parameters were analyzed for each sample. At the time of sampling, water temperature measured in the shade using a manual thermometer 110 ºC graduated to 0.5 ºC. Hydrogen ion concentration measured by using a digital pH meter (Hanna III 9024C). Spectro-photometric method developed by [24] was used for the determination of Total phosphorus (PO_4^{-3} $mgkg^{-1}$) in sediment. Total phosphorus is extracted from finely ground sediment with conc. sulfuric acid, hydrogen peroxide, and hydrofluoric acid, according to [25]. Sieving and grain size analysis were applied to evaluate different fractions of animal gut contents and ambient sediment, by using a one-phi interval sieve set. According to [26], sediment samples were dried and sieved in order to study the degree of divergence or coincidence between them and the gut contents of the animals. Seven fractions were obtained: gravel ($Ø_{-1}$), very coarse sand ($Ø_0$), coarse sand ($Ø_1$), medium sand ($Ø_2$), fine sand ($Ø_3$), very fine sand ($Ø_4$) and mud ($Ø_5$). Each fraction was weighed and expressed as a percentage of total weight. The seven fractions grouped into three groups; Coarse sediments ($Ø_{-1+}$ $Ø_{0+}$ $Ø_{1)}$ Medium sediments ($Ø_2$ + $Ø_3$), and fine sediments ($Ø_4$ + $Ø_5$). This grouping is more efficient for determining sediment variations in the different population density ponds and gut contents [27].

2.3. Gut contents and ambient sediment sampling for micro-biological investigation.

2.3.1. Specimen collection

Three actively feeding *H. atra* were collected monthly from different ponds and a replacement with the same numbers were done to keep the density. Ambient sediment samples were collected.

2.3.2. Dissection

The holothurians were placed on a sterilized surface. The dissection was carried out within 2 hours of collection, under aseptic conditions. The animal's body wall was cut away to expose the intestine, which was removed to a sterile container. An incision was made at each end of the gut, and samples of gut content were removed from the fore and hindgut areas.

2.4. Isolation and identification of bacteria

According to [28] for each sample five dilutions containing 95 ml of marine salt solution "MSS diluent" [29] was prepared, capped and autoclaved at 121ºC for 15 min. A portion of 10g of each moist sample (foregut contents-hindgut contents and ponds sediment) was added to MSS. Pieces measuring 10g of skin was dissected, washed gently with sterile MSS and placed in 95 ml sterile MSS. Serial dilutions were prepared up to (10^{-5}). Bacterial groups cultured on selective media that prepared using Artificial seawater instead of normal distilled water [30]: TVB on Simidu medium [31]; TC on Makonky agar [32]; Vibrio on TCBS agar [33]; FC on M. Endo agar [34]; SS on SS agar medium [35]. Dilutions that gave the optimum numbers of organisms for counting were used. Starting with the most dilute bottle, 1 ml portion of a freshly mixed dilution was transferred into each three sterile Petri dishes using a sterile pipette. The solid media inoculated in triplicate with 1.0 ml samples of the appropriate dilutions and incubated aerobically at 22°C for 24 hours. The dilution that yields from 30 to 300 colonies/ plate was selected for count record. Colonies counted with the aid of Quebec colony counter. Total Viable bacteria (TVB), *Vibrio* sp (V), Total coliform (TC), Fecal coliform (FC) and *Salmonella* and *Shigella* (SS) calculated as cfu/g. Isolation and identification of bacteria from hindgut and foregut has been done to cover the possible different colonies according to their morphological characteristics. Bacterial colonies were purified on the basis of their colonial characteristics on nutrient agar medium. The isolation program ended with 21 isolates, however, a number of these isolates lost viability after sequence laboratory cultivation. A total of 15 isolates were characterized. A series of preliminary tests was performed to build a phenotypic profile of each isolate. These studies: Gram stain, Spore stains [36]. Catalase test [37]. Kovac's oxidase test [38, 39]. Motility has been studied in hanging-drop wet preparation. The oxidation fermentation (O.F) tests [40, 36, 41]. API- 20 NE (Ref: 20050) and API-20 E System (Ref: 20100/20160) used for the identification of gram negative rods.

3. Results

The minimum recorded value of water temperature was 20.8 ºC during February while the maximum value was 33.1 °C in May (Fig. 3). The recorded mortality (%) of animals in the protected experiment were 6.8%, 0.31%, 4.4%, 0.31% and 1.5 % in January, February, March, April and May respectively (Fig. 4). The presence of sea cucumbers *H. atra* had a highly significant effect ($p<0.001$) on the pH values in the sediment over the 6 months period of the experiment. The pH decreased in ponds of *H. atra* by 0.64 (7.8 to 7.16); 0.46 (7.81 to 7.35); 0.28 (7.84 to 7.56) in HPDP, MPDP and LPDP respectively, while in sediment of reference pond increased by 0.11(7.93 up to 8.04) as shown in (Fig 5 and Table 1)

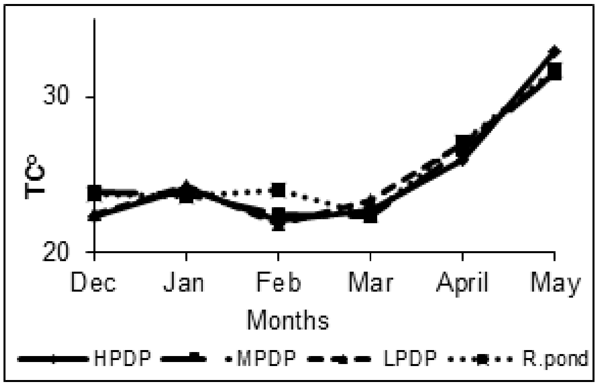

Fig (3): Average of monthly water temp. (ºC) in different population density ponds of *H. atra* (i.e. HPDP =108, MPDP=54, LPDP=18 and RP= zero individuals/pond) over 6 months

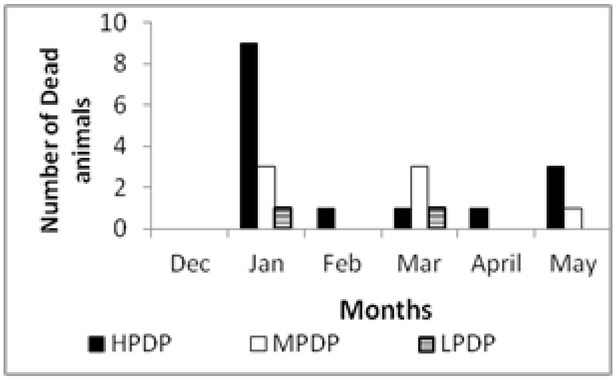

Fig (4): Mortality of *Holothuria atra* over 6 months of study.

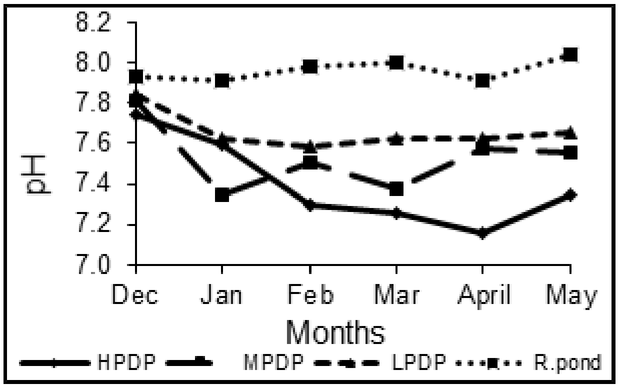

Fig (5): Average of pH measured monthly in different population density ponds of *H. atra* (i.e. HPDP =108, MPDP=54, LPDP=18 and RP= zero individuals /pond) over 6 months

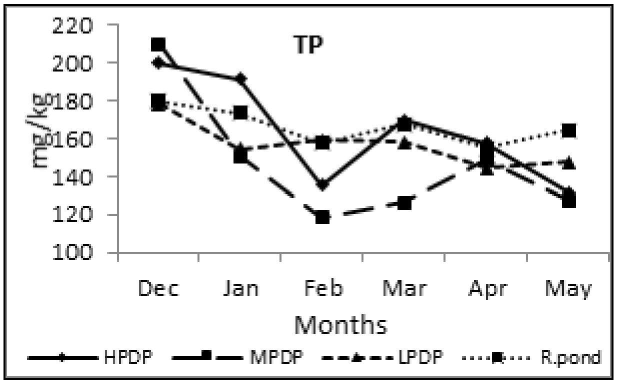

Fig (6): Average of T.P in different population density ponds of *H. atra* (i.e. HPDP =108, MPDP=54, LPDP=18 and RP= zero individuals /pond) over 6 months

The overall mean values of (T.P) in the HPDP, MPDP and LPDP were 164.4 µg/g, 147.0µg/g, 157.0 µgg^{-1}, compared with 166.4µg/g in the Reference pond (RP), although, the T.P values fluctuated with time (months) in all ponds (Table 1and Fig. 6). The presence of *H. atra* had a non-significant effect ($p>0.05$) on the total phosphorus in sediment of different density ponds while a significant effect recorded with months ($p<0.05$). The RP had the highest values of T.P.

Table (1): Overall means of the measured parameters in the sediment of different ponds populated with *H. atra* (i.e. HPDP =108, MPDP=54, LPDP=18 and RP= zero individuals /pond), gut contents (foregut and hindgut) and skin over experimental period of 6 months.

	water Temperature ºC	pH	T.P µgg^{-1}	TVB Cfu/g	TC Cfu/g	V Cfu/g	FC Cfu/g	SS Cfu/g
HPDP	25.0	7.4	164.4	543.9	157.5	722.3	4.8	1.8
MPDP	25.1	7.5	147.0	921.8	159.0	578.5	4.6	2.2
LPDP	25.1	7.7	157.0	1031.7	227.5	739.5	5.5	8.3
RP	25.4	8.0	166.4	399.2	456.0	892.7	12.3	24.3
Foregut	-	-	-	1219.2	6548.3	870.0	987.3	324.7
Hindgut	-	-	-	2344.7	14071.7	2031.7	400.0	601.8
Skin	-	-	-	2.1	4.3	4.0	0.0	1.0

Table (2): Overall means of sediment fractions (%) and group percentages of ambient sediments (ponds) and gut contents of *H. atra*; Gravel ($Ø_{-1}$), Very Coarse Sand ($Ø_0$), Coarse Sand ($Ø_1$), Medium Sand ($Ø_2$), Fine Sand ($Ø_3$), Very Fine Sand ($Ø_4$) and Mud ($Ø_5$), Coarse Sediments ($Ø_{-1+}$ $Ø_{0+}$ $Ø_1$), Medium Sediments ($Ø_2$ + $Ø_3$), and Fine Sediments ($Ø_4$ + $Ø_5$) over six months.

	$Ø_{-1}$	$Ø_0$	$Ø_1$	$Ø_2$	$Ø_3$	$Ø_4$	$Ø_5$	$Ø_{-1}$+$Ø_0$+$Ø_1$	$Ø_2$+$Ø_3$	$Ø_4$+$Ø_5$
HPDP	9.56	14.54	12.93	17.53	30.07	12.21	3.17	37.02	47.60	15.38
MPDP	7.61	9.38	10.66	18.47	34.66	15.41	3.81	27.65	53.13	19.23
LPDP	3.51	6.03	8.28	18.47	42.29	18.09	3.33	17.82	60.76	21.42
RP	4.12	7.22	9.21	19.47	43.58	14.30	2.09	20.55	63.05	16.39
Ambient sediments	6.9	10.0	10.6	18.2	35.7	15.2	3.4	27.5	53.8	18.7
Gut contents	16.0	22.9	22.4	18.7	12.2	6.8	1.0	61.3	30.9	7.8

As represented in (Table 2 and Fig. 7, 8, 9 and 10) the sediment at the investigated ponds with different densities of *H. atra*, RP and gut contents comprise biogenic gravel, sand and mud. The overall mean values of coarse sediment in the HPDP, MPDP and LPDP and RP of the experiment period of six months were 37.02%, 27.65%, 17.82% and 20.55% respectively compared with 61.3% in the animal gut contents. The overall mean values of Medium sediment in the HPDP, MPDP and LPDP and RP were 47.60%, 53.13%, 60.76% and 53.8% respectively compared with 30.9% in the animal gut contents. The overall mean values of fine sediment in the HPDP, MPDP and LPDP and RP were 15.38%, 19.23%, 21.42% and 16.39% respectively compared with 7.8% in the *H. atra* gut contents. The presence of sea cucumbers *H. atra* had a highly significant effect on the sediment grain size values represented in Gravel ($p<0.002$), Very Coarse Sand ($p<0.002$), Coarse Sand ($p<0.002$), Fine Sand ($p<0.02$), Coarse Sediments ($p<0.002$), Medium Sediments ($p<0.002$) while the effect of *H. atra* density varied non-significantly with Medium Sand $p>0.05$), Very Fine Sand ($p>0.05$), Mud ($p>0.05$), and Fine Sediments ($p>0.05$) over experimental period of 6 months. Data of the gut contents exhibited the feeding selectivity of *H. atra* where the overall means of coarse, medium and fine sediment values of gut contents were 61.3%, 30.9% and 7.8% compared with the overall mean values 27.5%, 53.8% and 18.7 respectively of ambient sediment of populated ponds.

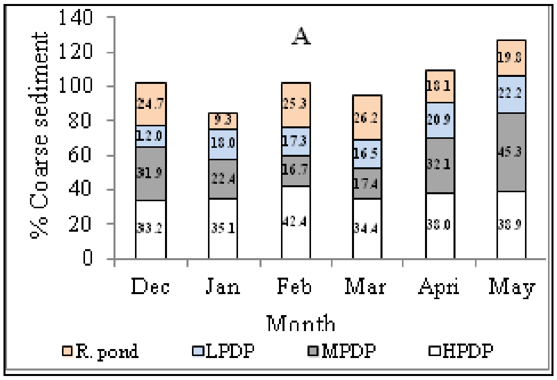

Fig (7): Average monthly variations of coarse sediment (%) in the populated ponds and Reference pond over 6 months

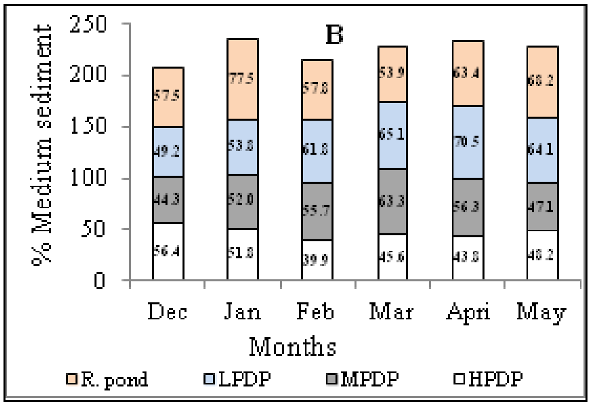

Fig (8): Average monthly variations of medium sediment (%) in the populated ponds and Reference pond over 6 months.

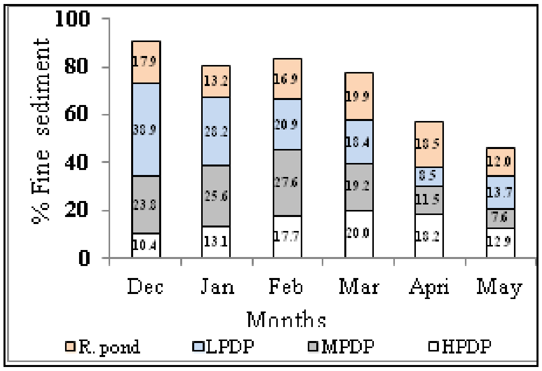

Fig (9): Average monthly variations of Fine sediment (%) in the populated ponds and Reference pond over 6 months

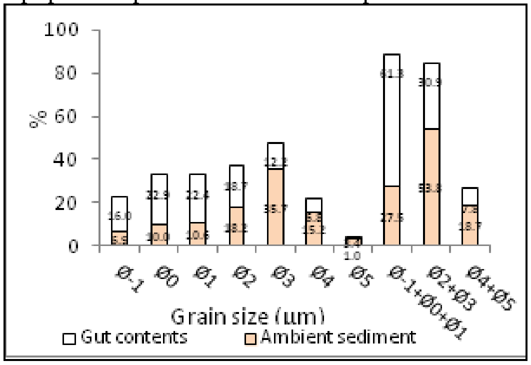

Fig (10): Overall means of sediment fractions (%) and group percentages of ambient sediments (ponds) and gut contents of *H. atra*

H. atra showed a significant effect ($p<0.05$) on bacterial count of Fecal coliform, Total coliform, *Salmonella* and *shigella* and Aerobic heterotrophic bacteria in the sediment while non-significant correlation on *Vibrio* sp count along the six months of experiment. The maximum average count of TVB in sediment of the controlled experiment was 1494 cfu/g recorded in RP during March while the minimum was 95.5 cfu/g in December at MPDP (Fig. 11). TVB count in hindgut contents was higher than TVB count in foregut content samples, skin, and sediment samples of different population density of *H. atra.* The maximum average TVB count (Fig. 12) in gut contents was detected in hindgut contents 3650 cfu/g in February, while the lowest was 1 cfu/g recorded in skin during April. The overall mean of TVB in HPDP, MPDP and LPDP was 543.9 cfu/g, 921.8 cfu/g and 1031Cfu/g respectively compared with 399.2 Cfu/g, 1219.2 Cfu/g, 2344.7 Cfu/g and 2.1 Cfu/g in RP, foregut, hindgut and skin (Table 1).

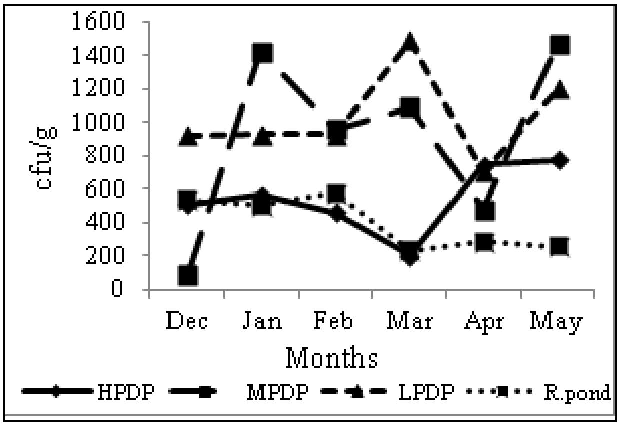

Fig (11): Monthly variation of TVB bacteria in the populated ponds and Reference pond.

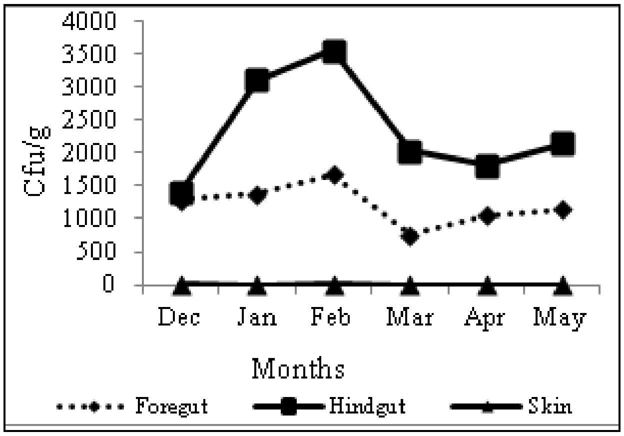

Fig (12): Monthly variation of TVB bacteria in gut contents and skin of *H. atra*

The maximum average count of TC in sediment of the experiment was 663 cfu/g recorded in RP during April while the minimum was 60 cfu/g in March at LPDP (Fig. 13). TC count in hindgut contents was greater than that in foregut, skin, and sediment samples of different population density of *H. atra*, during the period of study. The maximum average Total coliform count in gut content samples was detected in hindgut sediment samples 25500 cfu/g in May, while the minimum was 2.0 cfu/g recorded in skin during April (Fig. 14). The overall mean of TC in HPDP, MPDP and LPDP was 157.5cfu/g, 159.0cfu/g and 227.5 Cfu/g respectively compared with 456.0 Cfu/g, 6548.3 Cfu/g, 14071.7 Cfu/g and 4.3 Cfu/g in RP, foregut, hindgut and skin (Table 1).

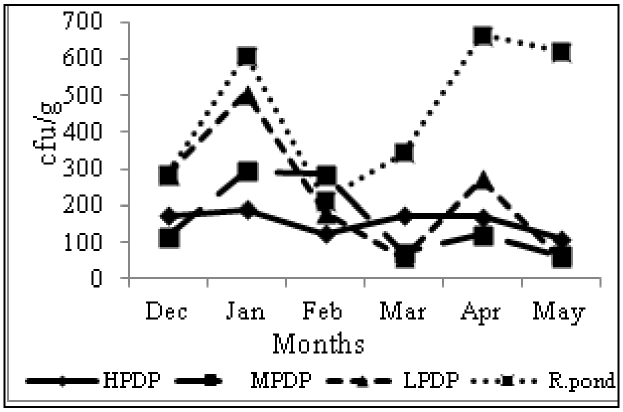

Fig (13): Monthly variations of TC bacteria in the populated ponds and Reference pond.

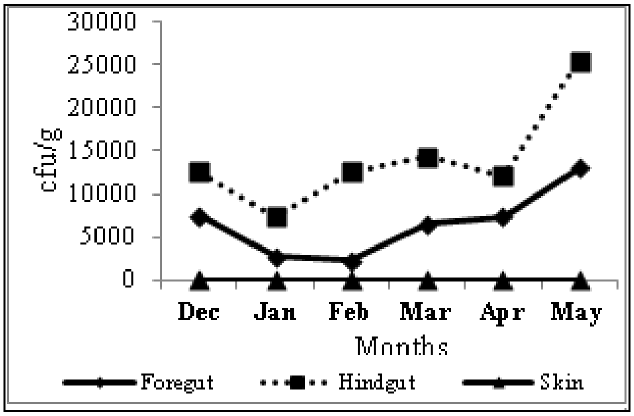

Fig (14): Monthly variation of TC bacteria in gut contents and skin of *H. atra*

The overall mean of *Vibrio* sp in HPDP, MPDP and LPDP was 722.3cfu/g, 578.5 cfu/g and 739.5 cfu/g respectively compared with 892.7cfu/g, 870.0 cfu/g, 2031.7 cfu/g and 4.0 cfu/g in RP, foregut, hindgut and skin (Table 1). The maximum average count of *Vibrio* sp in sediment of the *H. atra* experiment was 2110.0 cfu/g recorded in LPDP during December while the minimum average count was 339.0 cfu/g in May (Fig. 15). *Vibrio* sp count in hindgut contents was higher than in foregut content samples, skin, and sediment samples of different population density of *H. atra*, during the period of study. The maximum average *Vibrio* sp count in gut content samples was detected in hindgut contents 3680.0 cfu/g in March, while the minimum was 2.0 cfu/g recorded in skin during May (Fig. 16).

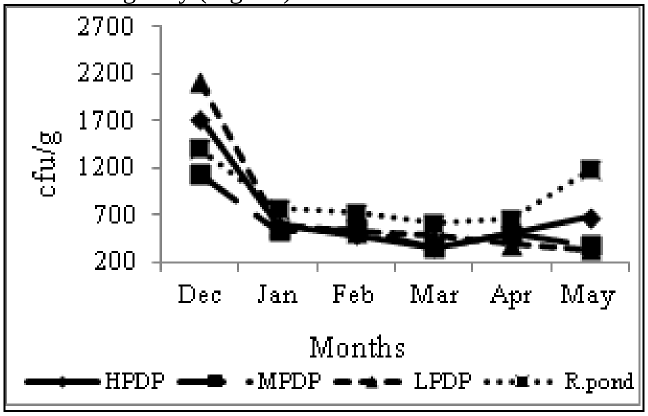

Fig (15): Monthly variations of *Vibrio sp* count in the populated and Reference pond

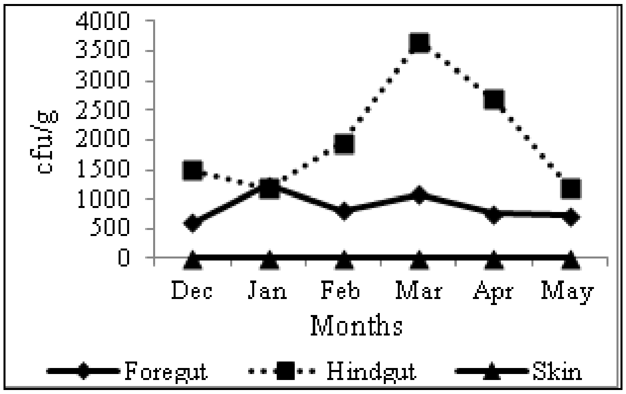

Fig (16): Monthly variation of *Vibrio sp* count in gut contents and skin of *H. atra*

The maximum average count of FC in sediment of the controlled experiment was 20.0 cfu/g in RP and LPDP in December and January while the minimum was decreased to undetectable value in different population density ponds (Fig. 17). Fecal Coliform count in foregut contents was higher than Fecal Coliform bacteria count in hindgut content samples, skin, and sediment samples of different population density ponds and The overall mean of FC in HPDP, MPDP and LPDP was 4.8 cfu/g, 4.6 cfu/g and 5.5 cfu/g respectively compared with 12.3 cfu/g, 987.3 cfu/g, 400.0 cfu/g and 0.0 cfu/g in RP, foregut, hindgut and skin (Table 1). The maximum average Fecal Coliform bacteria (FC) count in gut content samples and skin was detected in foregut contents 2750 cfu/g in January, while the minimum was 150 cfu/g recorded in hindgut during May, while there were no counts of FC detected in skin samples (Fig. 18).

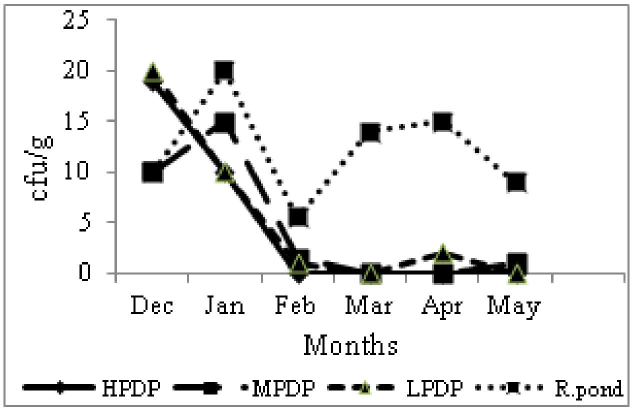

Fig (17): Monthly variations of fecal coliform in populated and Reference pond of *H. atra*

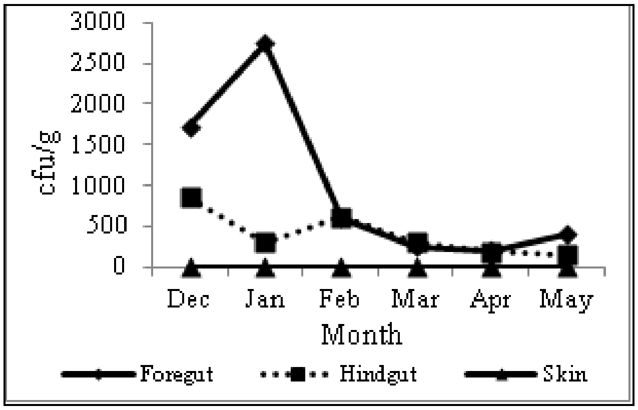

Fig (18): Monthly variation of fecal coliform count in gut contents and skin of *H. atra*

The maximum average *Salmonella* and *Shigella* count (SS) in was detected in hindgut contents 900.0 cfu/g in May, while the minimum was 130.0 cfu/g recorded in foregut during January and undetected in skin samples (Fig. 19). The maximum average count of SS in sediment of the controlled experiment was 48.0 cfu/g recorded in RP during December while the minimum average count recorded was decreased to undetectable value in different population density ponds (Fig. 20). SS count in hindgut contents was greater than in foregut content samples, skin, and sediment samples of different population density of *H. atra*. The overall mean of FC in HPDP, MPDP and LPDP was 1.8 cfu/g, 2.2 cfu/g and 8.3 cfu/g respectively compared with 24.3 cfu/g, 324.7 cfu/g, 601.8 cfu/g and 1.0 cfu/g in RP, foregut, hindgut and skin (Table 1).

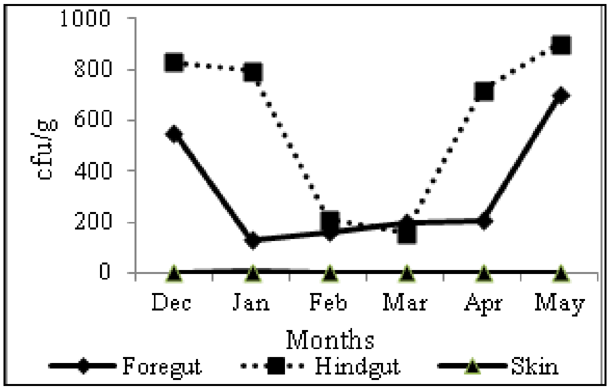

Fig (19): Monthly variation of *Salmonella* and *Shigella* in gut contents and skin of *H. atra*

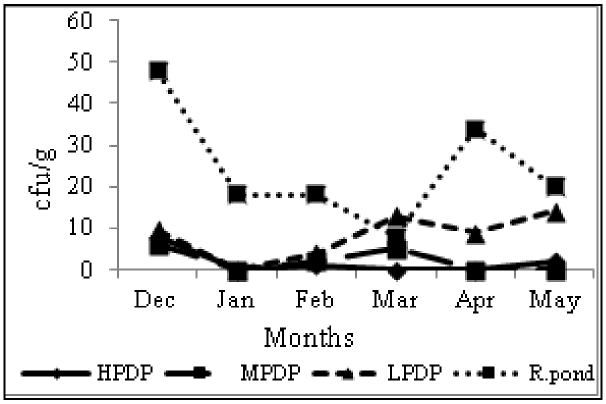

Fig (20): Variations of *Salmonella* and *Shigella* in populated and Reference pond of *H. atra.*

Table (3): bacterial strains isolated from foregut and hindgut contents of *Holothuria atra*,

NO	ONPG	ADH	LDC	ODC	CIT	H_2S	URE	TDA	IND	VP	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA	OX	Bacterial strain
Foregut contents of Holothuria atra																						
1	+	-	+	+	-	-	-	-	+	+	+	+	-	-	-	-	-	-	+	-	+	Vibrio vulnificus
2	+	-	+	-	+	-	-	-	+	+	+	+	+	+	+	-	+	-	+	-	+	Aeromonas Hydrophila
3	+		+	+	-	-	-	-	+	-	-	+	+	-	-	-	-	-	-	-	+	Vibrio vulnificus
4	+	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	+	+	-	+	Vibrio vulnificus
5	+	-	+	+	+	-	-	-	+	-	+	+	+	-	-	-	-	-	+	-	+	Vibrio vulnificus
Hindgut contents of Holothuria atra																						
1	+	-	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	+	Photobacterium damselae
2	+	+	-	-	-	-	+	-	-	-	-	+	+	+	-	-	-	+	-	-	+	Vibrio alginolyticus
3	-	-	+	+	-	-	+	-	+	-	-	+	+	-	-	-	-	-	-	+	+	Vibrio parahaemolyticus
4	-	-	-	+	-	-	+	-	+	+	+	+	+	-	+	+	+	+	+	-	+	Pseudomonas fluorescens
5	-	-	+	+	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	+	+	Vibrio parahaemolyticus
6	-	-	+	+	-	-	-	-	+	-	-	+	+	-	-	-	+	-	-	+	+	Vibrio parahaemolyticus
7	-	-	-	+	-	-	-	-	+	-	-	+	+	-	+	+	+	+	+	-	+	Pseudomonas aeruginosa
8	-	-	+	+	-	-	-	-	+	-	-	+	-	-	-	-	-	-	+	+	+	Vibrio parahaemolyticus

As shown in table (3), Foregut contents of *Holothuria atra* were hosted with two bacterial species, *Vibrio vulnificus* and *Aeromonas hydrophila*. The most dominant species in foregut were *Vibrio vulnificus*, while in the hindgut contents the bacterial species recorded were 5 related to 3 genera. The most dominant species in the foregut contents samples were *Vibrio parahaemolyticus* followed by *Photobacterium damselae*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens* and *Vibrio alginolyticus*.

4. Discussion.

During the current study the minimum value of water temperature was 20.8 oC in the February while the maximum was 33.1 oC in May, the current data confirmed by [42, 43] maintained that, water temperature showed fluctuation between 20.2 oC and 28.1oC for Red Sea. The same results were noted by [44, 45, 46]. The recorded mortality (%) of animals in the controlled experiment were 6.8%, 0.31%, 4.4%, 0.31% and 1.5 % in January, February, March, April and May respectively, the recorded mortality observed between large sized animals than small animals, mortality may be related to fluctuations of high and low tide rather than temperature, which exposed animals in crevices and that attached to walls of ponds to dehydration. H. atra has two distinct morphotypes, the large morphotype, sexual form found in deeper areas where water tends to be cooler; and the small morphotype, the asexual form found in shallow reef flats and warmer water [47]. The small morphotype population was observed to have a wider thermal performance curve with a maximal respiratory performance at a higher temperature (37.95 ºC) than that of Cook's Bay (33.49 ºC), suggesting that the small morphotype may have adapted to live in warmer conditions. The results from this study provide optimistic evidence of potential long-term resilience of *H. atra* sea cucumbers to climate change in the future.

Results showed that pH value of sediment samples clearly decreased in the populated ponds with *H. atra* while pH value in RP was higher at the end of experiment than at the beginning. The effect of sea cucumber on pH value may be due to the acidic execrations of the animal in the surrounding environment. This finding confirmed by [48] who found that the pH values of the gut lumen ranged from 7.0 to 7.6 when digestive tracts were filled with sediment compared with 6.1–6.7 in animals with empty digestive tracts. In addition to one-way ANOVA showed that there were highly significant differences in pH, ($p<0.02$) with different population density ponds. In another study, foregut pH determinations showed that the pH levels in the foreguts of five holothurians had slightly acid foreguts when empty. Accordingly, Holothurians have acid pH levels in the gut when it is empty [49, 50] mentioned that most holothurians have slightly acid fore-guts, and that the stomach and the intestine also have low pH levels.

In the present study the concentration of Total phosphorus gradually decreases in different population density ponds in comparison with the reference pond where, slightly decrease recorded. The release of phosphorus by *H. atra* followed the general rules set for size - metabolism relationships. Nitrogen/ phosphorus release ratios are 25:1 for 1-gram fresh weight animals and 42:1 for 60-gram animals [51]. Coral has a high ability to absorb nitrogen and phosphorus compounds from seawater and has a role as producer and consumer of detritus [52].

Particle selectivity by deposit feeders is an important concept in the ecology of benthic species. The holothurians, as deposit feeders, utilize the organic matter that coats sediment and detritus particles as food. Thus, particle size has been proposed as one resource axis along which niche separation can occur in optimal foraging strategy. Interspecific differences in particle size preference are thought to reduce the interspecific competition among benthic deposit-feeder species [53]. During the present study, investigation of the gut content of *H. atra* showed that the animal exhibited selectivity of sediment grain size where, the most dominant grain size in the gut content of *H. atra* was coarse sediment with the overall mean value 61.3% followed by medium sediment 30.9% and fine sediment which represented a small portion of *H. atra* diet as the overall mean was 7.8%. These results are consistent with the findings of [53] where they illustrated the specificity of some Holothurian species. This feeding behavior is discussed between species and sites. Analysis of sediments, gut contents and fecal pellets of *H. atra* indicates that *H. atra* is a selective feeder with an assimilation efficiency of about 40%. Dissolution of calcium carbonate by holothurians is estimated to be about 2.5 gm^2day^{-1}, equivalent to 25% of the net calcification rate of the reef proper [51].

The current study investigated the culturable bacterial flora of the sea-cucumber *H. atra*, and the bacterial population of the animal's immediate environment (marine sediment). Dilution plating of samples indicated that numbers of culturable bacteria; Aerobic Heterotrophic bacteria, *Vibrio* sp, Total coliform and *Salmonella* and *Shigella* in hindgut contents were higher than in foregut content samples, skin, and ambient sediment, While Fecal coliforms count exhibited highest count in foregut than hindgut contents, ambient sediment and skin respectively. The gut bacteria may have an important role in the supply of specific essential amino acids to the holothurians, in the same way that rumen microbes contribute some essential nutrients to their hosts [14, 12, 13]. The use of direct count techniques demonstrated higher numbers of bacteria in the holothurians intestinal tract than in the surrounding sediment [11] in addition to the author found that and total bacterial counts in sediments recently ingested by the animals were 1.5- to 3-fold higher than in surrounding sediments at the deepest station beside, the lowest counts were observed consistently in the foregut, where the digestive processes of the holothurians are believed to occur. In most animals, counts increased 3- to 10-fold in the hindgut [11]. On the other hand, in aquarium experiments and during field observations, *Holothuria* (Halodeima) *atra* and *Stichopus chloronotus* consumed an average of 67 and 59 g dry weight of sediment individual^{-1} d^{-1}, respectively. By processing the sediment, sea cucumbers may reduce bacterial and microalgal production within the sand, preventing overgrowth and eutrophication [54]. In another study [55] it was founded that numbers of culturable bacteria were greater in the hindgut than in the foregut, but in both cases less than that found in the surrounding sediment in addition to, higher numbers of viable bacteria were obtained from the foregut of a starved animal than from that of a normal feeding animal. In some crustaceans, it has been observed that after elimination of fecal pellets, the number of bacteria in the digestive tract dramatically declined [56] and it was suggested that there may be a small number of bacteria present in the digestive tract between feedings, with an increase in numbers only during and just after feeding. The proliferation of the intestinal microflora after feeding, however, may contribute significantly to the recycling of organic material in deep waters [10]. Bacteria proliferated in the presence of sea cucumbers and this promoted organic matter decomposition [57].

The recorded count of Fecal coliform during the present study was greater in foregut contents than hind gut contents, skin and marine sediment of different population density ponds. In addition to that, *Vibrio* sp count decreased in the ponds containing *H. atra* when compared with the reference pond. This may be proving and add value regarding the effect of *H. atra* on removal of some biological pollutants. Deming and Colwell [11] mentioned that the bacteria associated with Holothurians gut are believed to be carried as an actively metabolizing, commensally gut flora that transforms organic matter present in sediments ingested by the holothurians. The authors also found that, sediment containing organic matter altered by microbial activity cleared the holothurians gut every 16 h, suggesting that holothurians and their associated gut flora are important participants in nutrient cycles of the benthic ocean.

Foregut contents of *H. atra* during the present study were hosted with two bacterial species, *Vibrio vulnificus* (4) and *Aeromonas hydrophila* (1). The most dominant species in foregut were *Vibrio vulnificus*, while in the hindgut content the bacterial species identified were 5 related to 3 genera. The most dominant species in the hindgut contents samples was *Vibrio parahaemolyticus* (4) followed by *Photobacterium damselae* (1), *Pseudomonas aeruginosa* (1), *Pseudomonas fluorescens* (1) and *Vibrio alginolyticus* (1). *Pseudomonas fluorescens* was recorded only in hindgut samples. Ward *et al*., [55] in their study isolated 43 strains of bacteria from *H. atra;* foregut content (5), hindgut content (6), foregut wall (6), hind gut wall (4), foregut fluids (2) and skin (9) and sediment (11). The author demonstrates that the majority of the isolates fall within the phylogenetic confines of the genus *Vibrio* and related organisms. It has been demonstrated that the holothurians and its immediate environment harbour a wide range of microorganisms [55]. The results we have obtained in the current study are consistent with findings of [58] demonstrated that, there is a lot of bacterial species in the substrates and inside intestine of sea cucumber. There is no specificity in performance, species and number of bacteria which found in anterior or posterior of intestine, as well in their substrates [58]. Besides that, the holothurians do not choose specific bacteria for their feed. Bacteria are the consequence of substrates ingested. Some of them can be microflora in holothurians intestine [58]. In echinoderms, *Benthodytes sp*. (Holothurian) *Vibrio* sp populations ranged from 2.3 x 10^5 to 7 x 10^5 vibrios/g digestive tract content, and out of 36 isolates included four species. *Vibrio marinus* (19 of 20 isolates) and *Vibrio metschnikovii* were observed in the holothurians [59]. Observing reduction in Total coliforms, Fecal coliforms, *Vibrio sp*, *salmonella* and *shigella* in addition to its role as a pH controller, it can be concluded that *Holothuria atra* can be used successfully for the improvement of coastal regions with its associated fauna and flora. In another word, *H. atra* may both remove biological pollution in such areas. It is essential to develop as many biological/ecological studies as possible in order to test and apply effective management measures that avoid overexploitation of this, or other, sea cucumber species.

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