

## The Influence of *Holothuria atra* (Echinodermata: Holothuroidea) on Organic Matter Assimilation, Ammonification and Nitrification Rate of Sediment, Hurghada, Red Sea, Egypt.

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### Abstract

This study was undertaken to investigate the effect of sea-cucumber (*Holothuria atra*) population density on sea bottom sediment; redox potential, Total organic matter (TOM) assimilation, ammonification and nitrification activity. In protected aquaculture ponds at National Institute of Oceanography and Fisheries (NIOF), Four treatments of high population density (108 individuals/HPDP of 30m<sup>2</sup>), medium population density (54 individuals/MPDP of 30m<sup>2</sup>), low population density (18 individuals/LPDP of 30m<sup>2</sup>) and reference (RP; Empty of *H. atra*). Intestinal (Foregut and hindgut) of *H. atra* and ambient sediment samples were used. The data showed that presence of *Holothuria atra* increase the Redox Potential of sediment and kept its characteristics in the oxidized form which enhanced the biological activities. The TOM decreased by 16.04% (22.32% to 6.28%); 5.34% (10.54% to 5.2%); 3.02% (8.02% to 5.0%) and 3.61% (8.35% to 4.74%) in HPDP, MPDP, LPDP and reference pond respectively. The maximum monthly mean of ammonia was 6.93 μg g<sup>-1</sup> in HPDP and the lowest mean was 0.09 μg g<sup>-1</sup> recorded in LPDP. Ammonia and nitrate concentrations in the ponds of *H. atra* were slightly reduced compared with the RP while nitrite concentration followed different pattern. The highest count of ammonifying, ammonium oxidizing and nitrite oxidizing bacteria were 1100 MPN/g, 460 MPN/g and 64 MPN/g recorded in foregut contents. Meanwhile the lowest count was 31.5 MPN/g, 20 MPN/g and 6.75 in RP, hindgut contents and LPDP respectively. The highest Ammonification and nitrification activity were 36.69 μg/g/h and 1.19 μg/g/h recorded in HPDP meanwhile, the lowest values were 7.38 μg/g/h and 0.07 μg/g/h in RP. Ammonification rate and nitrification rate in foregut were higher than in hindgut contents and the ambient sediment. Data analysis showed ammonia-N; nitrate-N and nitrite-N concentration varied non significantly with *H. atra* population density (p>0.05) while a highly significant effect recorded with oxidation reduction potential (Eh) and Nitrification activity (p<0.01) and significant effect on TOM matter and Ammonification rate (p<0.05). This study emphasizes the ecological role of the most dominant holothurians species, *H. atra*, especially in relation to organic matter assimilation, through its associated bacteria that enhance ammonification and nitrification processes in sediment.

**Keywords:** *Holothuria atra*, Total organic matter, Ammonification rate, Nitrification rate.

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### 1- Introduction

The holothurians, or sea cucumbers, include a class, Holothuroidea, of some 900 members within the invertebrate phylum Echinodermata (Barnes, 1987). About 80 species of sea cucumber belonging to 22 genera are recorded in the Red Sea (Vine, 1986). Holothurians and other sediment-feeders are key components of a benthic recycling system that may have some similarity to the planktonic microbial loop (Uthicke, 2001a). Holothurians are also believed to play a significant role in nutrient cycling and sediment modification in shallow-water habitats (Amon and Hemdl, 1991; Hanafy, 2011). Holothurians are common inhabitants of sheltered marine shallow-water substrates; 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 (Laubier and Sibuet, 1979; Menzies *et al.*, 1973; Graham and Ballaglene, 2004) where they are assumed to mix up the deep sea mud on a large range (Heezen and Hollister, 1971). Sediment ingested by deposit feeding holothurians is of low nutrient value. The low richness of organic matter forces the deposit feeders to ingest large amounts of sediment to obtain their required energy (Santos *et al.*, 1994).

Holothurians fecal pellets contribute to the detritus pool in coastal ecosystems. Pellets are deposited normally in an unsystematic manner, forming fecal mounds of variable size and organic matter content, often richer than the neighboring sediment (Sloan and von Bodungen, 1980). Organic matter from decaying fecal pellets can be re-suspended by waves, currents and biota and will influence the relative amounts of organic carbon (Hopkinson, 1985). Re-suspension seems to favor repositioning of particulate material rather than dissolved nutrients (Wainwright, 1990).

The use of direct count techniques demonstrated higher numbers of bacteria in the holothurian intestinal tract than in the neighboring sediment (Deming and Colwell, 1982). On the basis of these findings, the presence of an active commensal gut flora metabolizing organic matter ingested by the holothurian was suggested (Deming and Colwell, 1982). Other reports (Phillips, 1984; Barlocher and Kendrick, 1978) have suggested that gut bacteria may have an important role in the supply of particular necessary amino acids to the holothurian, in the same manner that rumen microbes supply some essential nutrients to their hosts (Purser, 1970). On the other hand Dilution plating of samples indicated 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 (Ward-Rainey *et al.*, 1996).

*Holothuria atra* is one of the most common and widest ranging tropical, coral reef sea cucumbers in the world (Skillings *et al.*, 2011). *Holothuria atra* is the most abundant and frequent holothurian in all countries within the region (common name: black lollyfish), and therefore one of the most studied species despite its low commercial value (Conand, 1996; Jaquemet *et al.*, 1999; Uthicke *et al.*, 2001b; Dhinakaran and Lipton, 2014; Conand and Mangion, 2002; Conand, 2004; Taddei, 2006).

The nutrition of *H. atra* has been studied, as the species is a major component of the soft-bottom reef communities and mudflats (Taddei, 2006; Ashaet *et al.*, 2015); Conand and Mangion, 2002; Conand, 2008, Purcell *et al.*, 2009). The nutrition of the two dominant species, *Holothuria atra* and *H. leucospilota*, has first been studied at two sites, one eutrophicated and one oligotrophic (Mangion *et al.*, 2004). Gut content analysis showed that the organic matter ingested from the sediment was used with 10 percent efficiency for both species (Mangion *et al.*, 2004). The carbon: nitrogen ratio decreased along the gut showing organic matter degradation. This shows the ability of these holothurians to break down the organic matter from the sediment and to make it easily available for other organisms (Mangion *et al.*, 2004).

During the last decade, fishing of sea cucumber has appeared as major added threats to Egyptian reefs of the Red Sea. After the reduction of sea cucumber fisheries in other parts of the

world (kinch, 2002). By 2000, the sea cucumber fisheries in Egypt had increased greatly as a result of high demand for beche-de-mer and the high prices paid for it. Between 2002 and 2004, extensive fishing of sea cucumber caused a serious depletion in the natural stocks where commercial species disappeared completely from many reefs (Hanafy, 2011). Lawrence *et al.*, (2004), provided detailed density estimates for different areas in Egypt. *Holothuria atra* was by far the most abundant followed by *Actinopygamauritaniana*. A second survey conducted four years following the official fishing ban, has shown that there was some evidence of the return of some commercial species, but no evidence of stock recovery (Ahmed and Lawrence, 2007). This work is designed to investigate the impact of sea cucumber removal, represented by *Holothuria atra*, on physico-chemical characteristics, specific bacterial content and the rate of ammonification and nitrification processes of in the animal gut contents and the sea floor sediment.

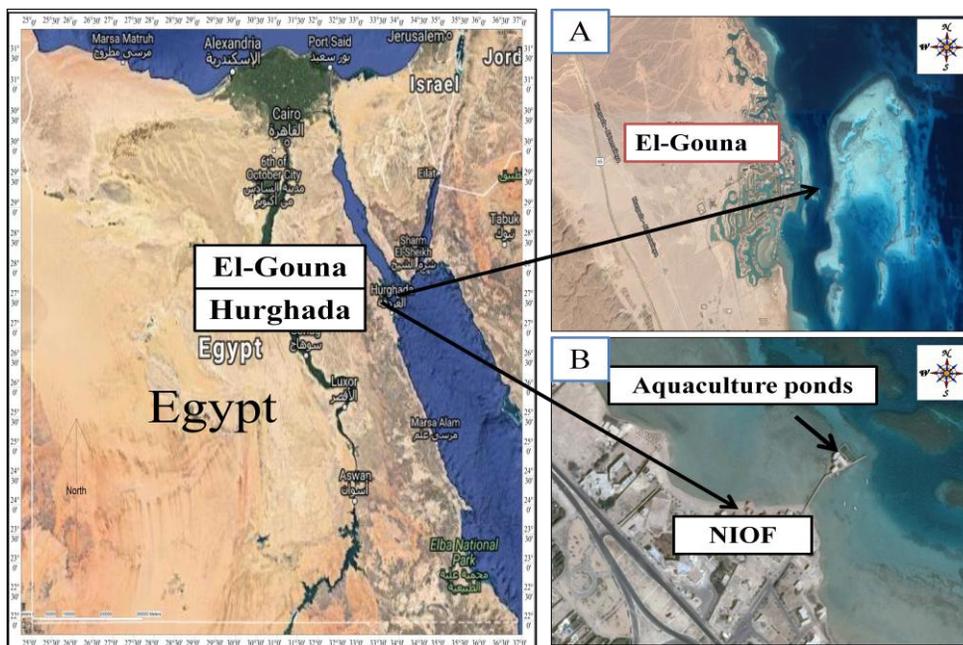
## 2-Materials and methods

### 2.1 Experimental site and design

A preliminary survey had been made to select appropriate site, characterized with high density of *H. atra*, near to Hurgada city and easily accessible at any time without obstacles. The other selected site was to establish aquaculture ponds to study the effect of *H. atra* on sediment features. As a result of survey we found 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 figure (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 shown in figure (2). The pond was 10m x 12m, divided into four sections by using green nets supported by metal stands and embedded in the pond sediment to 20 cm depth to avoid the animal escaping from one section to the neighboring one. Each of the sub-ponds had the dimensions 5m x 6m, the water get 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.

### 3.2 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, Oxidation Reduction potential (Eh) was measured by using a digital pH meter (Hanne III 9024C). Determination of weight of sediment organic matter (and thereafter percent organic matter-POM) of the digestive tract contents and the ambient sediments were determined by thesequential loss on ignition (Dean, 1974). Ammonia was measured by using the method of Steam Distillation with magnesium oxide according to the procedure described by (Bremner and Keeney, 1965). Oxidized nitrogen (nitrate + nitrite) was determined according to the method of Steam Distillation with MgO and Devarda's alloy after removal of ammonia-nitrogen by steam distillation method (Brenmer and Keeney, 1965). Nitrite was measured colorimetry based on the Griess-Ilosvy Method which reported by Barnes and Folkard(1951) and Bremner(1965).



**Figure 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.

### 3.3 GUT CONTENTS AND AMBIENT SEDIMENT SAMPLING ANALYSIS FOR BACTERIAL COUNT AND ACTIVITY.

#### 3.3.1 SPECIMEN COLLECTION

Three actively feeding *H. atra* were collected from different ponds and a replacement with the same numbers were done to keep the density. The holothurians' were placed on a sterilized surface. The dissection was carried out within 2 h 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. Ambient sediment samples also collected.

#### 3.3.2 ENUMERATION OF AMMONIFYING AND NITRIFYING BACTERIA

According to Belser and Mays (1982) and Alexander (1982) Most Probable Number MPN technique used for Enumeration of Ammonifying and Nitrifying bacteria in sediment ponds, foregut contents and hind gut contents of *Holothuria atra*, by MPN.

#### 3.3.3 Determination of Ammonification and nitrification Activity.

Potential ammonification rates were measured by following the conversion of L-arginine to ammonium (Alef and Kleiner, 1986; Nancy et al., 1995). Nitrification activity was measured according to the procedure reported by (Schmidt and Belser, 1982; Groffman, 1987).

## 4- RESULTS

The highest *Eh* value was 223 mV recorded in January at the MPDP while the minimum value 168 mV was recorded in May at RP. In the HPDP the *Eh* was increased from 207mV in the

beginning of the experiment to 221mV at the end of the experiment. Slight changes recorded in Eh value in both MPDP and LPDP. In general, the bottom sediments of the controlled experiment ponds preserve their characteristics in the oxidized form which enhanced the biological activities (Table 1; Figure2).The presence of sea cucumbers *H. atra* had a highly significant effect ( $p<0.001$ ) on the Eh values in the sediment over the 6 months period of the experiment.

The TOM decreased by 16.04% (22.32% to 6.28%); 5.34% (10.54% to 5.2%); 3.02 % (8.02% to 5.0%) and 3.61% (8.35% to 4.74%) in HPDP, MPDP, LPDP and RP, respectively. The highest TOM (23.32%) was recorded in the HPDP in December while the minimum value was 4.74 % at the RP in April (Figure 3). *H. atra* causes significant decreasing in TOM contents in sediment from 22.32% to 6.28%, from 10.54% to 5.2%, from 8.02% to 5.0% and from 8.35% to 4.74% in HPDP, MPDP, LPDP and RP respectively. TOM% in the gut content was fluctuated from 6.93% to 26.57%. The averages of TOM in gut contents recorded higher percentages than the ambient sediments in December, April and May (Table 1; Figure4).

Table 1: Overall means of Eh, (mV), TOM (%), ammonia-N( $\mu\text{g/g}$ ), nitrate N( $\mu\text{g/g}$ ) and nitrite-N( $\mu\text{g/g}$ ); in the sediment of different ponds populated with *H. atra* over experimental period of 6 months

Station	Eh (mV)	TOM (%)	Ammonia-N ( $\mu\text{g/g}$ )	Nitrate-N ( $\mu\text{g/g}$ )	Nitrite-N ( $\mu\text{g/g}$ )
HPDP	217.17	12.24	3.06	4.38	0.10
MPDP	215.83	7.49	2.82	4.09	0.10
LPDP	203.67	6.87	2.67	3.59	0.10
R.pond	182.67	6.35	3.11	3.71	0.14

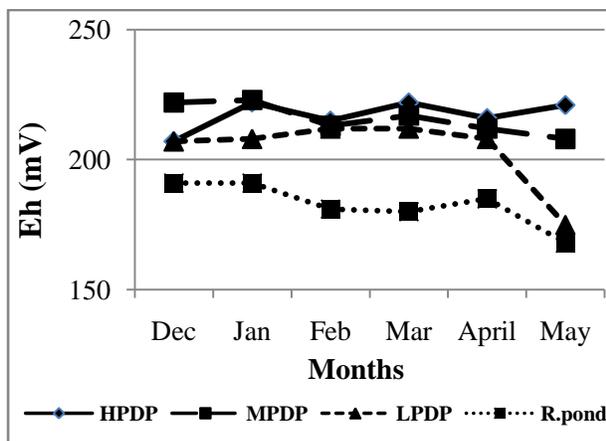


Figure 2: Average of Eh (mV) measured monthly in different population density ponds of *H. atra* (i.e. HPDP =108, MPDP=54, LPDP=18 and RP= zero individuals /pond) over 6 months.

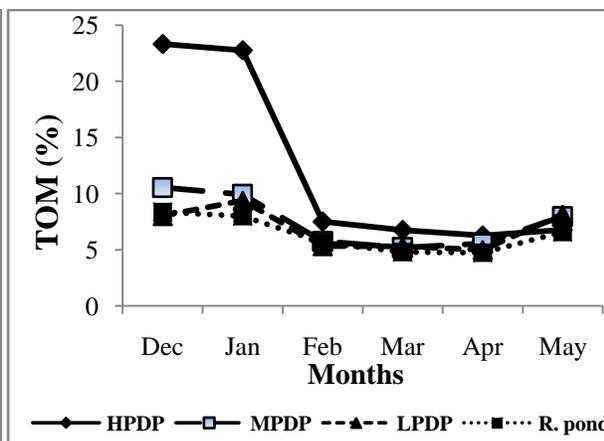
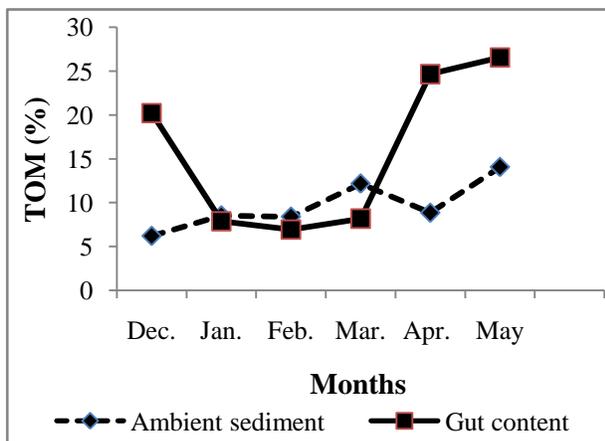
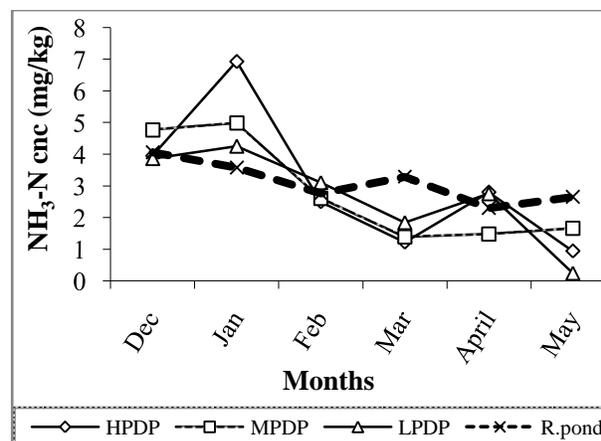


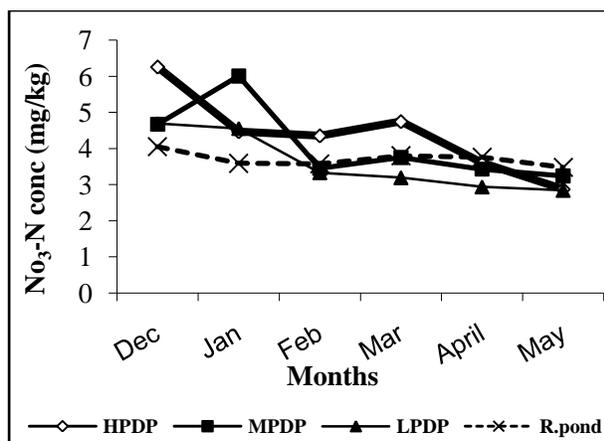
Figure 3: Average of TOM (%) measured monthly in different population density ponds of *H. atra* (i.e. HPDP =108, MPDP=54, LPDP=18 and RP= zero individuals /pond) over 6 months.



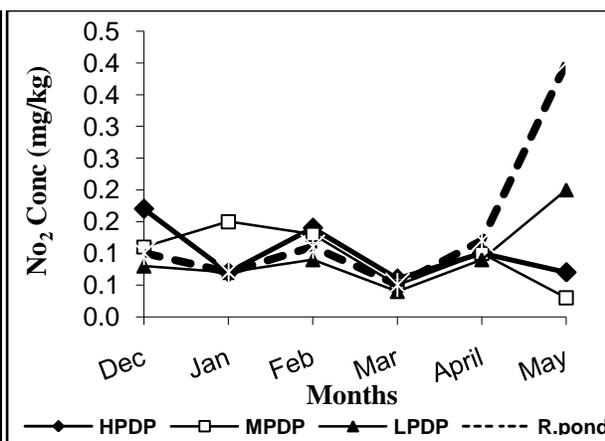
**Figure 4:** Average monthly variation of TOM (%) in gut contents of *H. atra* and ambient sediment at El Gouna Resort, Hurghada, Egypt.



**Figure 5:** Average of Ammonia-N 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.



**Figure 6:** Average of Nitrate-N 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.



**Figure 7:** Average of Nitrite-N 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.

The maximum monthly mean of ammonia  $6.93\mu\text{g g}^{-1}$  in HPDP in January and the lowest mean  $0.09\mu\text{g g}^{-1}$  is recorded in LPDP in March. Ammonia concentration in the ponds of *H. atra* was significantly low compared with the RP (Table 1; Figure 5). The highest concentration of nitrates reached  $6.25\mu\text{g g}^{-1}$  in the MPDP in January while the lowest monthly was  $2.84\mu\text{g g}^{-1}$  in the LPDP in March. The recorded concentrations of nitrates in the experimental ponds were lower than concentration in RP through the period of experiment (Table 1; Figure 6).

The recorded nitrite concentrations in the experimental ponds were inconsistent (Table 1; Figure 7), the highest mean of nitrite concentration ( $0.04\mu\text{g g}^{-1}$ ) was recorded in RP in April meanwhile the lowest mean ( $0.03\mu\text{g g}^{-1}$ ) was observed in MPDP in April.

Table 2: Average count of Ammonifying Bacteria; Ammonium Oxidizing Bacteria; Nitrite oxidizing bacteria; ammonification rate and Nitrification rate in sediment of different population density ponds and the gut contents of *H. atra*

Stations	Ammonifying Bacteria (MPN g <sup>-1</sup> )	NH <sub>3</sub> -N Oxidizing Bacteria (MPN g <sup>-1</sup> )	NO-N Oxidizing Bacteria (MPN g <sup>-1</sup> )	Ammonification rate (μg NH <sub>3</sub> -N g <sup>-1</sup> soil h <sup>-1</sup> )	Nitrification rate (μg NO <sub>3</sub> -N g <sup>-1</sup> h <sup>-1</sup> )
HPDP	313.25	88.85	45.75	20.94	0.69
MPDP	176.75	32.25	51.00	18.91	0.51
LPDP	265.00	130.75	10.88	15.44	0.42
RP	85.25	50.40	12.25	11.67	0.14
Foregut	780.00	350.00	46.00	22.16	2.83
Hindgut	305.00	155.00	19.50	17.94	1.87

The highest count of ammonifying bacteria was 1100 MPN/g in foregut contents in May, while the lowest Ammonifying bacterial count 31.5 MPN/g was recorded in RP in April (Table 2; Figure 8). Meanwhile the highest count of ammonium oxidizing bacterial count (*Nitrosomonassp.*, *Nitrosospira sp.* and *Nitrosolobussp.*) 460 MPN/g was observed in the foregut contents in May, while the lowest was 20 MPN/g in hindgut contents during April (Table 2; Figure 9). Nitrite oxidizing bacterial (*Nitrobacter winogradskyi*) counted the highest value 64 MPN/g in foregut contents during April and the lowest count 6.75 MPN/g was in LPDP in May (Table 2; Figure 10).

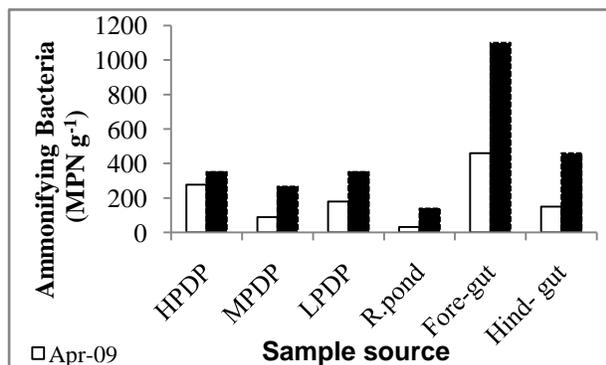


Figure 8: Variations in count of Ammonifying bacteria as MPN g<sup>-1</sup> in sediment of different population density ponds, gut contents and skin of *H. atra*.

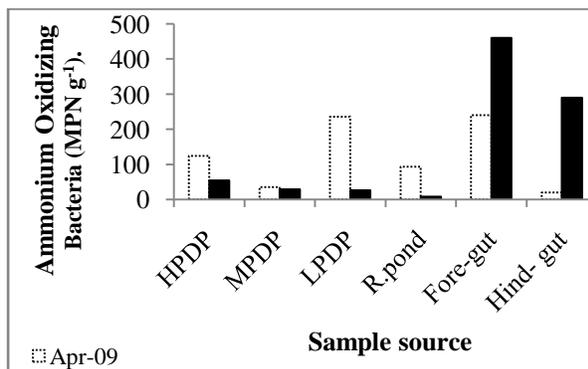
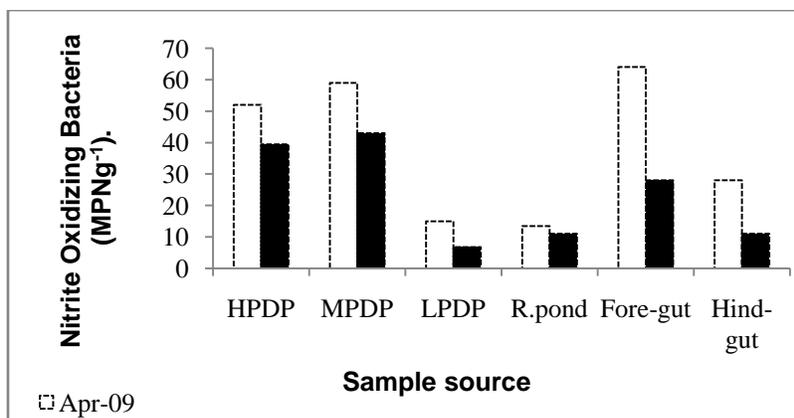


Figure 9: Variations in count of ammonium oxidizing bacteria as MPN g<sup>-1</sup> in sediment of different population density ponds, gut contents and skin of *H. atra*.



**Figure 10:** Variations in count of Nitrite oxidizing bacteria as MPN g<sup>-1</sup> in sediment of different population density ponds, gut contents and skin of *H. atra*.

**Table 3:** Changes in average ammonification rate as  $\mu\text{g/g/h}$  in sediment of different population density ponds and the gut contents of *H. atra*.

	HPDP	MPDP	LPDP	R.pond	Fore-gut	Hind- gut
Dec.	11.78	13.82	19.25	16.41	-	-
Jan.	22.16	17.17	17.25	12.03	-	-
Feb.	36.69	30.21	23.11	13.15	-	-
Mar.	22.45	24.14	11.65	10.20	-	-
April	16.98	14.98	10.76	10.85	23.83	18.43
May	15.57	13.14	10.63	7.38	20.48	17.44
Average	20.94	18.91	15.44	11.67	22.16	17.94

**Table 4:** Changes in average Nitrification rate as  $\mu\text{g/g/h}$  in sediment of different population density ponds and the gut contents of *H. atra*.

	HPDP	MPDP	LPDP	R.pond	Fore-gut	Hind- gut
Dec	0.37	0.47	0.17	0.15	-	-
Jan	0.50	0.79	0.35	0.07	-	-
Feb	1.19	0.77	0.67	0.20	-	-
Mar	0.74	0.30	0.27	0.10	-	-
April	0.84	0.37	0.77	0.17	2.73	1.27
May	0.52	0.37	0.30	0.15	2.93	2.46
Average	0.69	0.51	0.42	0.14	2.83	1.87

The overall means in ammonification activity values (Table 2) exhibited that the sediment of the investigated ponds with different densities of *H. atra*, RP and gut contents were varied significantly where the overall mean values of ammonification activity were 20.94  $\mu\text{g/g/h}$ ; 18.91  $\mu\text{g/g/h}$ ; 15.44  $\mu\text{g/g/h}$ ; 11.67  $\mu\text{g/g/h}$ ; 22.16  $\mu\text{g/g/h}$ ; 17.94  $\mu\text{g/g/h}$  recorded for HPDP; MPDP; LPDP; RP; Fore-gut and Hind- gut respectively in addition to the highest ammonification activity

for the samples collected in April and May was 23.83  $\mu\text{g/g/h}$  recorded in fore-gut contents in April. Over the period of six months of study, the highest value of ammonification activity recorded was 36.69  $\mu\text{g/g/h}$  observed in HPDP in February and the lowest 7.38  $\mu\text{g/g/h}$  was observed in the RP in May. Ammonification rate in foregut content was reached 23.83  $\mu\text{g/g/h}$  in April and May declined to 20.48  $\mu\text{g/g/h}$  in May ((Table 3).

In the dry sediments, the highest activity of nitrification was 1.19  $\mu\text{g/g/h}$  recorded in the HPDP in February while, the lowest activity was 0.07  $\mu\text{g/g/h}$  was observed in RP in January. The nitrification in foregut content showed high activities in April and May, the highest activity was in the foregut 2.93  $\mu\text{g/g/h}$  in May and the lowest was 1.27  $\mu\text{g/g/h}$  in addition to the overall means were 0.69  $\mu\text{g/g/h}$ ; 0.51  $\mu\text{g/g/h}$ ; 0.42  $\mu\text{g/g/h}$ ; 0.14  $\mu\text{g/g/h}$ ; 2.83  $\mu\text{g/g/h}$ ; 1.87  $\mu\text{g/g/h}$  recorded for HPDP; MPDP; LPDP; RP; Fore-gut and Hind-gut respectively (Table 2 and 4).

Data analysis showed ammonia-N; nitrate-N and nitrite-N concentration varied non significantly with *H. atra* population density ( $p > 0.05$ ) while a highly significant effect recorded with Eh and Nitrification activity ( $p < 0.002$ ) and significant effect on TOM and ammonification rate ( $p < 0.05$ ). This study emphasizes the ecological role of the most dominant holothurians species, *H. atra*, especially in relation to organic matter assimilation, through its associated bacteria that enhance ammonification and nitrification processes in sediment.

## 5- Discussion.

Oxidation-reduction potential hereafter referred to as redox potential, can be used as an indicator of the degree of oxidation of sediments. Submerged sediments display a range of redox potentials from +700 mV, which indicates highly oxidized sediment, to -300 mV, which indicates highly reduced sediment (DeLaune *et al.*, 1976). Intense Reduction in submerged sediments tends to buffer sediments close to pH 7.0 (Ponnamperuma, 1972). During the current study oxidation reduction potential (Eh) increased in sediment of HPDP from 207 mV in the beginning of the experiment to 221 mV at the end of experiment. Slight changes recorded in Eh value in both medium and low population density ponds. While (Eh) in reference pond decreased from 191 mV to 168 mV. In general, the present study found that *Holothuria atra* density increase the redox potential of sediment and kept its characteristics in the oxidized form which enhanced the biological activities, in addition to bioturbation (Mixing) of sediment by *Holothuria atra* and sediment reworking are important in establishing the sediment redox conditions and provide insight into the reactivity of sedimentary organic matter. Gordon *et al.*, (1980) maintained that Sediment pH and oxidation-reduction potential were Important factors in governing the population of hydrocarbon degrading microorganisms in the sediment and subsequent mineralization rates. Highest mineralization rates occurred at pH 8.0, and the lowest occurred at pH 5.0.

Results of the present study showed that OM decreased by 16.04% (22.32% to 6.28%); 5.34% (10.54% to 5.2%); 3.02 % (8.02% to 5.0%) and 3.61% (8.35% to 4.74%) in HPDP, MPDP, LPDP and reference pond respectively. The activities of *Holothuria atra* enhanced bacterial abundance, which facilitated mineralization processes and provides a mechanistic explanation for the observed reduction in sediment OM content. The low population density pond exhibited the same pattern of the reference pond. The presence of intact bacteria directly associated with the holothurians gut lining was also demonstrated by transmission electron microscopy (Deming and Colwell, 1982). Moriarty, (1982) measured organic carbon and nitrogen and bacterial biomass in the sediments and gut contents of *Holothuria atra* and *Stichopus chloronotus* on the Great Barrier Reef. The author found that significantly higher values of organic carbon and nitrogen and

muramic acid were found in foregut contents of the holothurians, indicating selective feeding on organically rich components of the sediment. Carbon values were 16-34% higher in the foregut than in the sediment. Nitrogen values 35-111% higher and muramic acid values 33-300% higher

On the basis of these findings, the presence of an active commensal gut flora metabolizing organic matter ingested by the holothurian was suggested (Deming and Colwell, 1982). In another study, it was reported that sea cucumber prevented the formation of anaerobic conditions (Uthicke, 2001c; Kitano *et al.*, 2003) by consuming high amount of organic matter inside the sediment. Moreover, it was observed that sea cucumber caused a reduction of ammonium level by consuming organic matter of sediment, by this way it was also able to prevent excess production of algae by stabilizing chlorophyll concentration (Moriarty, 1982).

Since there were fluctuations in ammonia, nitrate and nitrite concentrations in the present study, increase of ammonia followed by nitrate refer that there was ammonification in the sediment hosted by *Holothuriaatra*. Decrease of nitrite concentration may refer to nitrification process. Although, controversial results were reported for instance; Uthicke and Klumpp (1998) reported that ammonium nitrogen increased in the oligotrophic areas with sea cucumber. Uthicke (2001a) reported that there were no significant contribution on the total nitrogen in the areas of *Holothuriaatra* and *Stichopus chloronolus*. Therefore *Holothuriaatra* may accelerate nitrification by increasing ammonia as a result of active ammonification and could decrease ammonium concentration in sediment of the population density ponds during the present study. Webbet *al.*, (1977) found that ammonia release rates for three species of holothurians, *H. atra*, *H. difficilis* and *Actinopygamauritiana* were weight specific and the release of ammonia by *Holothuriaatra* was equivalent to 9% of the total ammonia exported from the reef proper.

It has been demonstrated that the holothurians and its immediate environment harbour a wide range of microorganisms. However, the presence in *Holothuriaatra* of a commensally gut flora, independent of that ingested in the sediment, has not been convincingly demonstrated (Ward-Rainey *et al.*, 1996). The obtained results in the current study are consistent with findings of Hatmanti and Purwati (2011) who demonstrated that, there are many species of bacteria in the substrates and inside intestine of sea cucumber. There are no specificity in performance, species and number of bacteria which found in anterior or posterior of intestine, as well in their substrates. 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 (Hatmanti and Purwati, (2011).

MacTavish *et al.* (2012) demonstrated that bacteria proliferated in the presence of sea cucumbers and this promoted organic matter decomposition. The average maximum value of ammonification activity and nitrification activity were 36.69  $\mu\text{g NH}_4\text{-N g}^{-1} \text{h}^{-1}$  and 1.19  $\mu\text{g NO}_2\text{-N g}^{-1} \text{dry soil h}^{-1}$  respectively recorded for HPDP during while, the minimum values were 7.38  $\mu\text{g NH}_4\text{-N g}^{-1} \text{h}^{-1}$  and 0.07  $\mu\text{g NO}_2\text{-N g}^{-1} \text{h}^{-1}$  recorded for reference pond. On the other hand ammonification rate and nitrification rate in foregut content is higher during the investigation period in April to May. The average of ammonification activity and nitrification activity in foregut were ranged from 20.48 to 23.83  $\mu\text{g NH}_4\text{-N g}^{-1} \text{h}^{-1}$  and 2.73 to 2.93  $\mu\text{g NO}_2\text{-N g}^{-1} \text{h}^{-1}$  respectively.

These findings supported by the obtained results of ammonifying bacteria, ammonium oxidizing bacteria and nitrite oxidizing bacterial count in foregut content were the highest during the period of study when compared the same parameters to that recorded in sediment of different population density ponds, reference pond hindgut and skin in addition to ANOVA two way showed Ammonification rate and nitrification rate were varied significantly ( $P < 0.01$ . in different

population density ponds of *Holothuriaatra*. MacTavish *et al.* (2012) demonstrated that bacteria proliferated in the presence of sea cucumbers and this promoted organic matter decomposition. by facilitating bacterial abundance and suppressing microphytobenthos, deposit-feeding sea cucumbers shift the microbial balance in organically enriched marine sediments and redistribute dissolved nutrients from the sediments into the pelagic environment. Moriarty (1982) indicated that bacteria and nitrogenous components of the organic matter were selectively eaten according to his findings that maintained that, Values for organic carbon and nitrogen and muramic acid were generally lower in the hindgut than in the foregut, due to digestion and assimilation. Assimilation efficiencies averaged 30% for organic carbon, 40% for organic nitrogen and 30-40% for muramic acid (bacteria). Detritus (non-living matter) probably constituted 60-80% of the organic matter in the sediment and thus the food of the holothurians.

Observing reduction in concentrations of ammonium, nitrate, nitrite, organic matter and accelerating ammonification and nitrification rate with preventing anaerobic conditions in the sediment by *Holothuriaatra*. it can be concluded that *Holothuriaatra* can be used successfully for improvement of coastal regions with its associated fauna and flora especially coral reef. In another words, *Holothuriaatra* may both remove biological pollution and improve water quality in such areas. It is crucial 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 (Moriarty, 1982).

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## الملخص باللغة العربية

تأثير خيار البحر من نوع هولوثوريا اترا على تمثيل المواد العضوية- معدل انتاج الامونيا ومعدل النيترة في الرواسب القاعية. الغردقة - البحر الاحمر- مصر.

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أجريت الدراسة لبحث تأثير الكثافة العددية لخيار البحر على خصائص التربة القاعية ومن العوامل التي تم دراستها، جهد الأكسدة والاختزال - استيعاب المواد العضوية ومعدل انتاج الامونيا ومعدل النيترة. في أحواض تربية الاحياء البحرية المحمية بالمعهد القومي لعلوم البحار والمصائد تم وضع كثافات عددية من خيار البحر، حيث قطن الحوض الاول 108 فرد ليمثل الكثافة العددية العالية والحوض الثاني 54 فرد ليمثل الكثافة العددية المتوسطة والحوض الثالث عدد 18 فرد ليمثل الكثافة المنخفضة بينما الحوض الرابع لا يحوى خيار بحر ليمثل الحوض المرجعي. أظهرت النتائج ان وجود خيار البحر من نوع هولوثوريا اترا أدى الى زيادة امكانية الأكسدة والاختزال في الرواسب وحافظ على خصائص التربة القاعية في شكل أكسدة وهو ما يعزز من الانشطة البيولوجية. إنخفاض تركيز المواد العضوية بنسبة 16.04% في الحوض ذات الكثافة العالية وبنسبة 5.34% بالحوض ذات الكثافة المتوسطة وبنسبة 3.02 في الحوض ذات الكثافة المنخفضة، بينما في الحوض المرجعي انخفضت بنسبة 3.61%. تراجعت تركيزات الأمونيا في أحواض خيار البحر مقارنة بالحوض المرجعي بينما تبع تركيز النيتريت نمطا مغايراً. سُجلت البكتيريا المنتجة للأمونيا بقيمة عددية قصوى في الأمعاء الأمامية لحيوان خيار البحر في الحوض ذات الكثافة العليا في شهر مايو ب -  $1100 \text{ MPN g}^{-1}$ ، بينما كانت القيمة الصغرى لها  $31.5 \text{ MPN g}^{-1}$  وسُجلت البكتيريا المنتجة للأمونيا بقيمة عددية قصوى في الأمعاء الأمامية للحيوان في الحوض المرجعي في شهر مايو ب  $1100 \text{ MPN g}^{-1}$ ، بينما كانت القيمة الصغرى لها  $31.5 \text{ MPN g}^{-1}$  وسُجل أعلى تعداد للبكتيريا المؤكسدة للأمونيا في محتوى الأمعاء الأمامية لخيار البحر بقيمة  $460 \text{ MPN g}^{-1}$ ، بينما احتوت الأمعاء الخلفية لخيار البحر على القيمة الصغرى التي كانت  $20 \text{ MPN g}^{-1}$  وسُجل أعلى تعداد للبكتيريا المؤكسدة للنيتريت في محتوى الأمعاء الأمامية بقيمة  $64 \text{ MPN g}^{-1}$ ، بينما سُجلت القيمة الصغرى في الحوض المحتوي على أقل كثافة عددية من حيوان خيار البحر وكانت  $6.75 \text{ MPN g}^{-1}$ . كانت أعلى قيمة لمتوسط نشاط انتاج الأمونيا وهي  $36.69 \text{ } \mu\text{g NH}_3\text{-N g}^{-1} \text{ h}^{-1}$  وتم تسجيلها في الحوض ذات الكثافة العليا من حيوان خيار البحر بينما كانت أدنى قيمة وهي  $7.38 \text{ } \mu\text{g NH}_3\text{-N g}^{-1} \text{ h}^{-1}$  وقد سُجلت في الحوض المرجعي. بلغت أعلى قيمة لمتوسط نشاط انتاج الأمونيا وهي  $23.83 \text{ } \mu\text{g NH}_3\text{-N g}^{-1} \text{ h}^{-1}$  وتم تسجيلها في الأمعاء الأمامية لحيوان خيار البحر بينما كانت أدنى قيمة  $20.48 \text{ } \mu\text{g NH}_3\text{-N g}^{-1} \text{ h}^{-1}$ . معدل النيترة في محتوى الأمعاء الأمامية لخيار البحر هو الأعلى أثناء فترة الدراسة، حيث بلغت القيمة القصوى لمتوسط معدل النيترة  $2.93 \text{ } \mu\text{g NO}_2\text{-N g}^{-1} \text{ h}^{-1}$  بينما سجل أدنى معدل للنيترة ما قيمته  $1.27 \text{ } \mu\text{g NO}_2\text{-N g}^{-1} \text{ h}^{-1}$ . من النتائج تبين أن حيوان خيار البحر من نوع هولوثوريا اترا يمثل احد العناصر البيولوجية الهامة في البيئة البحرية وخصوصا فيما يتعلق بدورة المغذيات.