Quest Journals
Journal of Research in Environmental and Earth Sciences
Volume 7 ~ Issue 9 (2021) pp: 15-21
ISSN(Online) :2348-2532
www.questjournals.org

Research Paper

Study of Bacterial Contamination in Tobruk Bay

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ABSTRACT
The purpose of this study was to assess the degree of contamination of the water of Tobruk Bay resulting from discharge of sewage water in the Bay waters. in this study the concentration of some physical properties such as temperature, conductivity, dissolved oxygen concentration and biological oxygen demand (BOD) were measured in addition to microbial contamination,the results showed the presence of bacteria in abundance and high in the concentration of biological oxygen demand (BOD) in the first sample compared to the rest of the other samples This confirms that the percentage of pollution in the first sample is the highest because of the sewage and the availability of nutrients and microorganisms where the consumption of dissolved oxygen and produce biological oxygen demand (BOD) This confirms the low salinity in the first sample due to the dilution of seawater through the wastewater.

KEYWORDS: *Tobruk Bay, Pollution, biological oxygen demand , microorganisms*

Received 21 August, 2021; Revised: 03 September, 2021; Accepted 05 September, 2021 © The author(s) 2021. Published with open access at www.questjournals.org

I. INTRODUCTION

Bacterial contamination is an important and serious problem for the environment and public health in marine coastal areas used for recreation, especially those near highly polluted areas. It is believed that human activities can accelerate nutrient inputs in water ecosystems

Once discharged into the sea, inadequate surface, municipal, domestic and industrial runoff may promote intestinal growth in the small plankton or become a source of associated microorganisms. Pathogenic bacteria can be discharged into the sea through leakage of wastewater under the sea, posing a potential risk to consumers' health of fish products. [1]

There are international laws urging studies on fecal coliforms and fecal staphylococcus in seawater to assess their quality and suitability for recreational use. [2]

Wastewater (black water) contains various hazardous components that pose a threat to human health and marine flora and fauna. Sewage contains pathogens, viruses and bacteria that may cause human diseases, including salmonella, hepatitis A and E, gastrointestinal diseases and infections. Therefore, one of the most serious threats to public health is sanitation, which is discharged in coastal areas used for recreation, such as swimming, diving and marine tourism .

The disintegration of stool residue leads to oxygen withdrawal from the surrounding water. If the amount expended is abundant, the amount of oxygen for fish and other marine organisms may be inadequate, causing marine life to die. The closed seas, the bays and the shallow seas are at greater risk, as in Tobruk Bay, and the water does not replenish well .

Nutrients in wastewater also lead to the growth of algae that reduce oxygen levels in seawater, destroy fish, coral reefs, seagrass and other marine organisms. [3]

Many communities and coastal areas are currently attempting to address water quality problems associated with undetermined sources of pollution

Sea water is contaminated with untreated sewage, municipal wastewater disposal, recreational activities, coliform bacteria, deworming, and pseudomonas. The isolated bacteria are mostly disease-causing microorganisms including Vibrio, Pseudomonas, Colon, Salmonella, and Shigella [4] .

Fecal coliforms were used as indicators of water quality in relation to the presence of human pathogens [5].

The presence of micro-organisms in water indicates that water is contaminated with fecal matter from humans or other warm-blooded animals.[6]

This type of contamination means that any type of natural plant and pathogenic microorganisms that occur in the intestines of these animals may be present [7] .

The high prevalence of intestinal viruses in sea water also indicates a chronic pollution problem and potential human risk [8].[

The microorganisms in the index are coliform bacteria that are divided into fecal coliforms and non-fecal colons. The fecal type is Escherichia coli, a natural plant found in the human intestine and other warm-blooded animals. Non-fecal species include Enterobacteraerogenes, which are commonly distributed in nature and sometimes found in the intestinal tract of warm-blooded organisms. [9]

The Bay of Tobruk has significantly reduced water quality and sewage is the discharge of liquid sewage where water has become unsuitable for recreation [10], fishing [11] and aquaculture and can cause health risks On humans .

II. MATERIALS AND METHODS

2.1- **Study Area**

The area selected for this study is the Tobruk Bay, The Tobruk city is located in the eastern part of Libya which is about 150 kilometers away from the Egyptian border.

Tobruk Bay is located east of the city between the following coordinates:

At the end of the Bay:

E235758.58 N320437.63

Starting of the Bay

E240049.51 N320531.56

The length of the Bay is about 5 km and its width at the entrance of the Bay is 2 km and at the end is 0.6 km. The depths are between 5 to 16 meters in different parts of the Bay .

2.2 Sampling and Analytical methods

The following five sampling locations have been selected for this study in Tobruk Bay:

Location 1: Municipal drain pipe
(Latitude:N 32°07′65″ ; Longitude: E 23°96′98″)

Location 2: The location in the bay where is suction of water for power station
(Latitude: N 32°06′42″ ; Longitude: E 23°98′45″)

Location 3: Hariga Terminal for loading of oil for export
(Latitude: N 32°06′37″ ; Longitude: E 23°99′38″)

Location 4: San George resort
(Latitude: N 32°06′89″ ; Longitude: E 24°00′53″)

Location 5: Commercial port
(Latitude: N 32°07′63″ ; Longitude: E 23°97′54″)

Samples were collected from five sites distributed in the Bay as described and shown in the map(figure 1). The water samples were collected from water surface by 50 cm from several different points to study the extent of their contamination with the bacteria where they were kept in glass bottles sterile sterilized seal , These samples were sent directly to the laboratory directly in a time not more than two hours of taking the sample and the tests were conducted.

Figure 1 :Seawater Sampling Locations inTobruk Bay

The total viable number (TVC) was determined in different samples by immunization of nutrient agar plates with adequate dilution of the water sample. The cfu / ml (colony forming unit) was used to denote the results. The colonies were formed after 48 hours of incubation. The media were used to isolate different bacteria from the stool index. Total coliforms and E. coli were identified using Mac Conkey agar and the dishes were incubated for 24 hours at 37 ° C. Fecal coliforms were identified using agar-FC agar, incubated for 24-48 hours at 44.5 ± 0.20C , Blood Agar is a general purpose enriched medium often used to grow fastidious organisms , A differential selective medium for the isolation of Salmonella and some Shigella species from clinical specimens, foods etc.

The isolations were carried out according to the standard microbiological examination methods for water and waste water (APHA- 1995).

while the identification process was carried out through biochemical examinations using the gram stain and API 20E identification Kits. [12]

2.2.1- Gram stain

The Gram stain procedure distinguishes between Gram positive and Gram negative groups by coloring these cells red or violet. Gram-positive bacteria stain violet due to the presence of a thick layer of peptidoglycan in their cell walls, which retains the crystal violet these cells are stained with. Alternatively, Gram-negative bacteria stain red, which is attributed to a thinner peptidoglycan wall, which does not retain the crystal violet during the decoloring process .

Reagent s :-

1-**Crystal violet**

2-Decolorizing agent .

3 –lugols Iodine .

4- counter stain .

2.2.1.1 Procedure :-

1- make athin smear on a clean glass slide from a specimen or bacterial sus pension

2-dry it in air or by holding it close to the flame ,then fix bypassing through flame with the smear side 2-3 times .

3-Allow fixed smear to cool,then cover the smear with crystal violet,keep for one minut .

4- wash the slid with water then cover with gram iodine and let it stand for one minut

5- wash the slide waith water .

6- Discolors with acetone /alcohol ,rocking the slide gently for 10-15 seconds till the violet colors come off the slide .

7- wash with water immediately .

8- cover the slide with counter stain ,let the counter staind for 30 seconds

9- wash with water ,blot dry and examine the oil immersion lens of a microscope .

-Interpretation of the result :-
Gram positive bacteria are seen blue to violt.
Gram negative bacteria are seen red to pink .

API 20 E Mothed -2.2.2

is a standardized identification system for Enterobacteriaceae and other non-fastidious, Gramnegative rods which uses 20 miniaturized biochemical tests and a database. The complete list of those organisms that it is possible to identify with this system is given in the Identification Table at the end of this package insert.

2.2.2.1- Principle the API 20 E

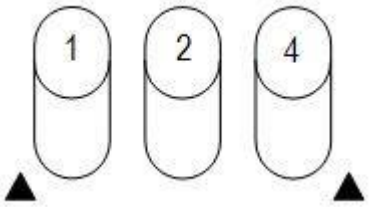
strip consists of 20 micro tubes containing dehydrated substrates. These tests are inoculated with a bacterial suspension that reconstitutes the media. During incubation, metabolism produces color changes table (1) that are either spontaneous or revealed by the addition of reagents. The reactions are read according to the Reading Table and the identification is obtained by referring to the Analytical Profile Index or using the identification software ses table (2).

Table.1 API-20E

Test	Code	Negative result	Positive result
β-galactosidase	ONPG	Yellow	Yellow
Arginie Dihydrolysis	ADH	Red-orange	Red-orange
Lysine Decarboxylase	LDC	Red-orange	Red-orange
Ornithine Decarboxylase	ODC	Red-orange	Red-orange
Citrate Utilization	CIT	Green-blue	Green-blue
Hydrogen Sulfide	H2S	Black sediment	Black-sediment
Urease production	URE	Red-orange	Red-orange
Tryptophan Deaminase	TDA	Dark brown	Dark brown
Indole production	IND	Red ring	Red ring
Acetone production	VP	Pink-red	Pink-red
Gel Hydrolysis	GEL	Black pigments	Black pigments
Glucose	GLU	Yellow	Yellow
Manitol	MAN	Yellow	Yellow
Insonitol	INO	Yellow	Yellow
Sorbitol	SOR	Yellow	Yellow
Rhaminose	RHA	Yellow	Yellow
Sucrose	SAC	Yellow	Yellow
Melibiose	MEL	Yellow	Yellow
Amayloid	AMY	Yellow	Yellow
Arabinose	ARA	Yellow	Yellow

2.2.2.2 API Reading Scale (color chart)

1. Mark each test as positive or negative on the lid of the tray
2. The wells are marked off into triplets by black triangles, for which scores are allocated as follows:



Triad	I			II			III			IV			V			VI			VII		
Tube	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	oxidase
Reaction	+	+	+	-	-	-	+	+	-	+	-	-	-	+	+	-	-	+	+	-	+
Point	1	2	4	0	0	0	1	2	0	1	0	0	0	2	4	0	0	4	1	0	4
Add	7			0			3			1			6			4			5		
7-digital Code	7031645																				

Table.2 API-20E

3- Add up the scores for the positive wells only in each triplet. Supplementary tests, e.g.: oxidase may also be included in the profile. The highest score possible for a triplet is 7 (the sum of 1, 2 and 4) and the lowest is 0 .

Some of the physical properties of the waters of Tobruk Bay were also measured for their importance and their effect on the bacteria present in the water .
The temperature of the sea water is very important to know the difference in temperature between different locations of the samples, where the temperature was measured directly by the (Hach device).
The conductivity of the Bay water was measured directly by using a special device from aAmerican Hach Company . The conductivity is important to know how much amount of drainage water discharged into the waters of Tobruk Bay.
The concentration of dissolved oxygen was measured directly in the water using a portable dissolved oxygen meter. Because melting dissolved oxygen decreases with increasing water temperature, high water temperatures limit the availability of dissolved oxygen (DO) for aquatic life asThe dissolved oxygen test (DO) was performed to measure oxygen concentration in seawater directly by Dissolve Oxygen (DO) (HACH, HQ40). An adequate amount of dissolved oxygen in water is essential for marine life. the samples of water were transferred from each location to BOD bottles,
When biodegradable organic matter (including organic waste) is present in waters it provides nutrient for the growth of bacteria and other microorganisms causing them to multiply and, where bacterial numbers are sufficient causing a depletion of dissolved oxygen in the water[13].
The BOD (5day) test is a measure of the amount of oxygen consumed by microorganisms in breaking down the organic matter.
The quality of oxygen used up by microorganisms at 27ºC and in darkness during 5 days in breaking down organic wastes in a water body is called its biological oxygen demand (BOD)

$$BOD5 = (DO - DO5)\ Sample$$

III. RESULTS AND DISCUSSION

3.1.1physica Parameters
After studying the results and measurements obtained as shown in Table
(1), it was observed that the temperature is almost identical to all samples with a slight rise in sample 2. Due to the abundance of hot water, discharged into the Bay water and the source of this hot water station Tobruk Steam generators for electricity production.
The results showed that the highest concentration of dissolved oxygen was obtained at the location of sample (4), where the value was 8.4 mg/l . This increase is due to its proximity to open sea water, which has the least pollution and less oxygen consumption .
The lowest concentration of dissolved oxygen was in sample 1, where the value of was 7 mg/l, due to the pollution resulting from the sewage that is being discharged in the waters of Tobruk Bay.

It was observed that sample No1 has the highest Biological Oxygen Demand (BOD*)* value at a concentration of 6 mg / L. The reason is the presence of microorganisms that consume dissolved oxygen in the process of organic activity (digestion of organic matter). The sample No 4 is slightly higher than the rest of the other samples, with a concentration of 1 mg / L due to the proximity of the sample No1 and sample No 4 of the wastewater discharge site shown in Table 1

Samples	**physical parameters Seawater**			
	Temp. C	**Cond .(ms/cm)**	**DO(mg/l)**	**BOD5 *(* mg/l *)***
Location 1	26	53	7	6
Location 2	26.6	60.7	8	0.76
Location 3	26	61	7.6	0.7
Location 4	25 C	61	8.4	0.8
Location5	26	61	7.7	1

3.1.2**Microbial tests**

After the microbial tests, the bacteria type and number of colonies were determined. It was observed that the first sample was the highest polluted site. Bacteria such as Escherichia coli, klebsiella spp, proteus, lococcus spp, and other colon bacteria were found. The number of colonies was about 107/100ml In the second and third samples there was less pollution than the first sample. The number of colonies was about 103/100ml . The fourth samples of the bacteria were colonies with about 3 × 103/100ml .

The fifth sample was more than the rest of the samples except the first sample (number of colonies 5 × 103/100ml) because of its proximity to the source of pollution of sewage water in addition to its occurrence near the commercial port, which is one of the sources of pollution in the Bay of Tobruk .

IV. CONCLUSION AND RECOMMENDATION

This study showed that the beaches of Tobruk Bay were not safe for human activities with body contact such as swimming. Wastewater can lead to marine pollution and the absence of wastewater treatment plants and systems,Therefore, discharge the sewage into the sea without any

Treatment, leading to degradation of coastal water quality and disruption of marine ecosystems. Where the bacteria in the waters of Tobruk Bay were higher than the limit, some countries such as the United States, the Philippines and Canada issued E. coli standards for recreational seawater with a maximum of 200 / 100 ml water, while Australia and Hong Kong 150 and 180/100 ml respectively [14] The US Environmental Protection Agency's assessment of bacteriological data indicated that the use of fecal coliforms at 200/100 ml would result in 19 diseases per 1000 swimmers in the American seabed [15].

It is necessary to treat wastewater to reduce the pollutants to acceptable concentrations before dumping them into the Bay water and to collect the wastewater in one long pipeline and direct it to the deep sea away from populated areas to increase the rate of mitigation. Whether wastewater is treated or not ,The transfer of sewers in the sea to a more distant place will be safer for the health of people especially in densely populated places, but the disposal of sewage from the deep sea will always cause environmental problems wherever they are discharged.

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