The Microbiome of the Long Island City Coast Water: What is the source of Pathogenic bacteria?

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ABSTRACT

Tidal Straits, such as the Long Island portion of the East River, are characterized for having salinity levels that fluctuate between fresh and saltwater levels, which presents varying osmotic pressures on lifeforms and affects the dissolution of oxygen in the water. Moreover, the natural fertility of brackish water and the contamination with sewer runoff can make the water nutrient-rich. Here we present the results of our experiments determining the physical-chemical characteristics of deepwater samples from LIC, as well as the bacterial class diversity obtained through meta genomic analysis of DNA samples purified from the LIC Deepwater. We also compare our data to the information obtained by another group of students, using Long IslandCity Surface samples. We believe that the diversity of microorganisms found in the LICSurface water is related to the dissolved oxygen (DO) and nitrate(RONO2)levels. The concentrations of pathogenic bacteria found in the water of LIC were unusual for brackish water. We posit that the pathogens must be coming from an unnatural source such as sewage.

Keywords: East River, water quality, Metagenomic analysis, Long Island City, pathogenic bacteria, coliform.

INTRODUCTION

In this study, we examine the characteristics of fall water samples from the surface and deep waters of the Long Island City portion of the East River to determine if the water is contaminated and where this contamination originates from. Chemical and physical factors can influence the concentration of bacteria in a water body, such as levels of Salinity, Dissolved Oxygen, Dissolved Carbon Dioxide, Nitrates, pH and temperature. In estuaries, these indicators fluctuate according to season and tide,

affecting the microbiome. Contaminants can also change the characteristics of the water and bacterial populations, which can be pathogenic. Water contaminated with feces presents the greatest risk for bacterial diseases, making the water a vehicle for diseases (Cabral J. P., 2010). To detect waste contamination, the NYC Environmental Protection Agency considers levels of fecal coliforms and enterococci as determinants of water quality ("New York Harbor Water Quality Report,"2017). The reason for this indicator in discussed in this paper. The major source of pathogenic and non-pathogenic fecal microorganisms are wastewater discharges (2010). Most of NewYork City's sanitary infrastructure follows the Combined Sewer System (CSS), a design that overflows when there is an extra input of water ("Combined Sewer Overflows (CSO)," 2018). This overflow, which contains untreated wastewater and stormwater runoff, goes directly into water bodies, such as the East River. Combined Sewer Overflows (CSOs) and StormwaterRunoff contamination is an established problem in NYC, negatively impacting water quality, through the addition of pathogenic bacteria, nutrients, and reduction of oxygen levels ("NewYork City's Wastewater Treatment System,"2017).The portion of the East River analyzed in our study, Long Island City, was considered Impaired for recreation and fish consumption, due primarily to CSO's and contaminated sediment runoff ("Bronx river/East river watershed,"2011). These indicators do not provide a complete picture of the contamination because they do not include all of the pathogens in the water, and where they come from. To obtain more detailed information, it is necessary to do a Metagenomic Analysis to account for all of the bacteria in the water.In 2011, the East River was determined to have abnormally high levels of pathogenic bacteria at some locations ("Bronx river/East river watershed," 2011). Here, we present our research into the possible sources of pathogenic bacteria. We analyze the physical and chemical characteristics and metagenomic data on the bacterial classes from samples taken from the Surface and Deep waters of Gantry Park. We also analyze possible sources of pathogens: the Wastewater Treatment Plants (Wards Island and Newtown Creek), and CSO outfalls near Gantry Park. Finally, we conclude the Treatment Plants are likely the source of pathogenic bacteria in the LIC Deep. The lack of studies on the need for Metagenomic Analysis to identify

contamination in water sources amplifies the importance of this study.Our study advances the knowledge on the limitations of current water quality testing practices by concluding that the classes of bacteria in the sample reveal their origins.

MATERIALS AND METHODS

1) Physical and chemical characteristics of the LIC Deep water:

Temperature and pH were measured at the time of collection. Salinity was determined by evaporating 10 mL of the LIC water sample on a hot plate and subtracting the weight of the watch glass (figure 1).We also measured the salt concentration by inserting 8 dialysis bags into beakers filled with distilled water (figure 2), calculating the mass changes after 45 minutes and drawing a standard curve to approximate the salinity of the water sample. Six of the dialysis bags had different NaCl concentrations (0.1M, 0.2 M, 0.3M, 0.4M, 0.5M and 0.6 M). There were 2 control bags, one with distilled water and another with our water sample.

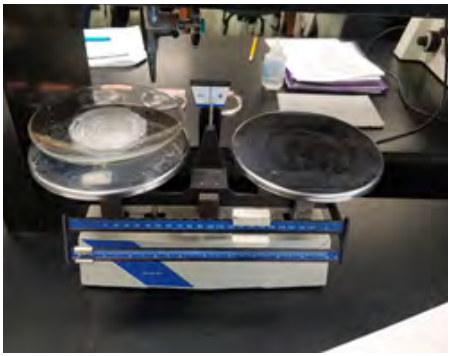

Figure 1. Watch glass with salt on Balance.

Figure 2. Dialysis bags submerged in distilled water filled beakers.

Dissolved carbon dioxide was determined using the LaMotte titration assay kit. This was done using 23 ml of the water sample, to which we added a drop of phenolphthalein indicator and 19 drops of sodium hydroxide until we obtained a light pink color. Dissolved oxygen was also obtained by

using the LaMotte oxygen titration kit. We began by filling the titration tube with 20 mL of the water sample, then inserting the titrator into the plug at the top of the sodium thiosulfate (0.025 N titrating solution).Next, we inverted the bottle slowly removing the plunger without generating bubbles in the titrator barrel until the large ring was opposite to zero and we removed the titrator. Afterwards, 8 drops of starch indicator were added (figure 3) until the water sample turned blue. We continued titrating until the solution became colorless.

Figure 3. Number of drops of Starch indicator added to the solution until color change from yellow to colorless was measured to determine DO.

Nitrates concentration was tested through the HACH Nitrate Test Kit. Coliform bacteria presence was indicated by the inoculation of three Lauryl Tryptose Broth (LTB) fermentation tubes: two with the water sample (1 mL and 0.5 mL) and one control tube with distilled water. The tubes were left for one week and then observed for color and turbidity. Red color and transparency would indicate no fermentation, yellow color and turbidity would indicate high fermentation due to coliform presence.

2) DNA Isolation

DNA was isolated using the PowerWater DNA Isolation kit. First, the water sample was filtered using as filter funnel (Figure 4). Then, we removed

the filter and put it into a 5mL PowerWater Bead Tube. 1 ml of PW1 lysing solution was added to the tube, which was then vortexed for 5 minutes and later centrifuged at 4000 x g for one minute at room temperature. The supernatant was transferred to a clean 2 mL collection tube (figure 5) and centrifuged at 13000 x g for 1 minute. 200 microliters of PW2 solution were added, the tube was put in the vortex to mix, incubated at 4 °C for 5 minutes, and centrifuged at 13000 x g for a minute; the same rate was used throughout the rest of the experiment. 650 microliters of PW3 solution was added to make the DNA fall out of solution, mixed in the vortex, and the tube was centrifuged. The filtrate was discarded. 650 microliters of PW4 solution were added to obtain a higher DNA purity. The tube was centrifuged. The spin filter was removed and placed into a clean tube. The flow was discarded and the tube centrifuged for 2 minutes. 100 microliters of PW6 were added to the tube with the spin filter to have a more efficient release of the DNA from the silica spin filter membrane, then centrifuged. The DNA obtained in the filtrate was then sent for analysis where 16S rRNA sequences were amplified using the Polymerase Chain Reaction (PCR) and sequences were compared using the illumine software. Further analysis of the genetic sequences were done by using the Basic Local Alignment Search Tool (BLAST).

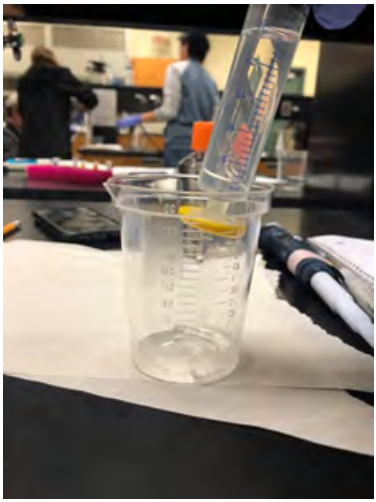

Figure 4. Water sample filtered through the spin filter

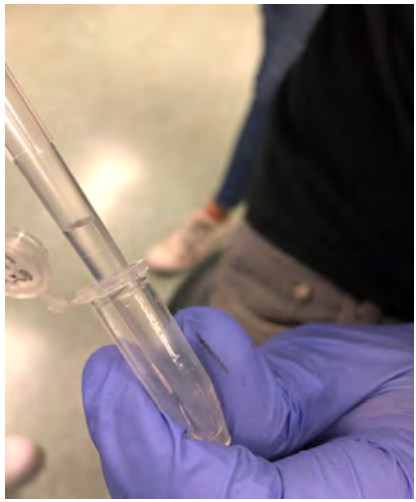

Figure 5. Collection of Supernatant with pipette

MATERIALS AND METHODS

I. Physical and Chemical characteristics of the LIC water sample

Most of the physical and chemical characteristics we obtained denoted a normal estuarine environment, devoid of drastic imbalances indicative of high contamination. The pH of the samples was 8, closer to the pH of freshwater which ranges from 6 to 8. This result is coherent with the dissolved carbon dioxide levels (23.75mg/L), which were not that of an acidic environment. Temperature (from 21°C to 23°C) and dissolved oxygen (5.4 ppm and 5 ppm) were normal for estuaries in the fall. Nitrate concentration (4 mg/L) was within the levels of drinking water (<10 mg/L). The salinity of the samples (26,200 mg/L for surface sample, 28,300 mg/L for deep sample) was normal for tidal straits (between 1,000 mg/L and 35,000 mg/L). These results are shown on Table 1.

Sample	Collection Time & Date	Temperature	pH	Salinity	Dissolved O2	Dissolved CO2	Nitrates
LIC Deep	10/02/2019 at 7:50am	22.00°C	8	28,300mg/L	5.4ppm	-	-
LIC Surface	10/02/2019 at 7:45am	23.00°C	8	26,200mg/L	5.0ppm	-	-
LIC Deep	10/15/2019 at 7:50am	21.00°C	8	-	-	23.75mg/L	4.0mg/L

Table 1. Data on the Physical and Chemical Characteristics of water samples from Gantry Park

When compared, the LIC surface and deep water samples presented differences in their physical-chemical characteristics. The deep sample presented slightly lower temperature, higher salinity and higher dissolved oxygen. The difference in temperature between the samples is normal since surface waters are in direct contact with the environment, constantly exchanging heat to meet temperature changes. In contrast, deep waters are slower in meeting environmental changes, doing so through interaction with tides which allow for water of differing temperatures to interact. The data on the ambient temperature in New York City in the early morning of the day of collection of the water samples, October 2nd (77°F, or 25°C), and the previous day, October 1st (68°F, or 20°C) support this explanation for the temperature distinction between the samples. The surface water temperature was higher because it approximated the new warmer ambient temperature

faster, while the deep water gained heat slower, which resulted in colder temperature. Moreover, colder waters above freezing point are denser, thus having greater solubility to salts and oxygen. These discrepancies led us to question what bacterial classes could be found in the water, and how the microbiome of the LIC surface and deep waters were affected by such unique conditions.

II. Differences in Classes of Bacteria in each sample

Metagenomic Analysis of the bacteria in the LIC Surface (Figure 6) and Deep (Figure 7) samples collected on 10/12/19 from Gantry Park revealed a substantial difference in bacterial class diversity and population numbers. In general, the Surface water presented a greater number of bacteria, and a greater diversity of bacterial classes than the Deep sample. The result conflicts with the fact that the Deep sample presented the greatest dissolved oxygen and nitrates concentration (Table 1), an environment that would be most favorable to the growth of aerobic bacteria than the Surface. This suggests that the bacteria must come from an unnatural source. The Surface of the water is where contaminants first reach the estuary, and, therefore, where most of it is concentrated. If the pollutant brings bacteria into the river, the Surface would retain most of them.

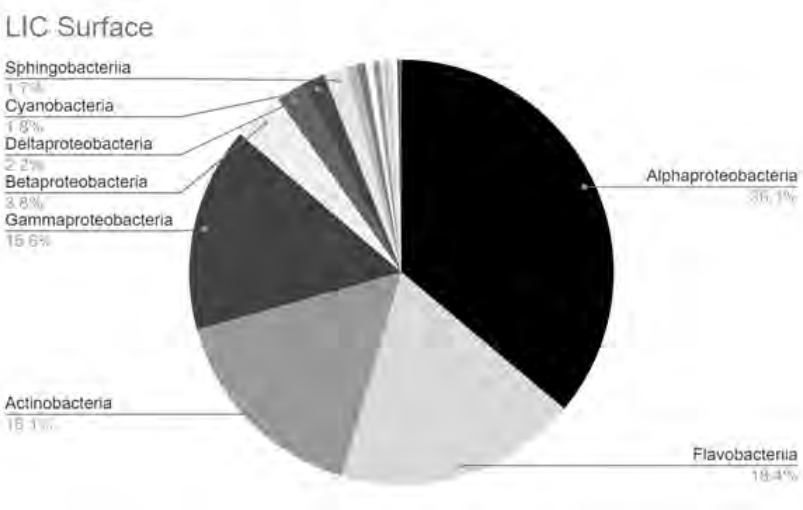

Figure 6. Classes of bacteria in the LIC Surface: more biodiversity despite lower DO and $RONO_2$ levels.

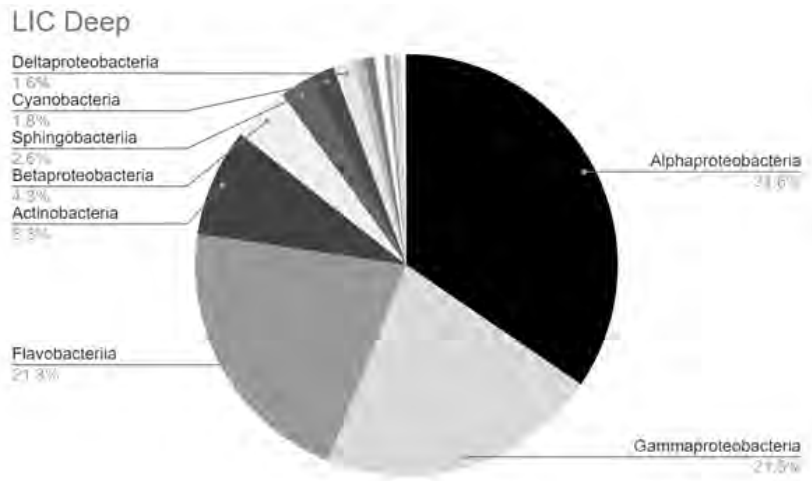

Figure 6. Classes of bacteria in the LIC Deep: more biodiversity despite lower DO and $RONO_2$ levels.

III. Metagenomic Analysis Suggests Contaminants Account for:

A. The Abundance of Bacteria from Waste or Treatment Plant

To determine the source of pollution, we separated the Classes of bacteria obtained through Metagenomic Analysis by isolating the coliform, and organizing the remaining bacteria by natural habitat (Figure 8). We separated the data to compare the limited information used by the government to analyze water quality, the presence of coliform bacteria, with the wealth of insight available through the analysis of every bacterium in the water body. By determining the natural habitat of the bacteria in the water, we can analyze where they probably originated from. Both the Surface and the Deep samples presented the same order of the greatest counts of bacteria respectively: Aquatic or Natural to Estuaries, Waste or Treatment Plants, Environmental, Soil, and Coliform. We already expected that most of the bacteria would be Aquatic or Natural to Estuaries, since these are supposed to exist in great quantities in the water. Our expectations were also confirmed by the fact that the greatest counts of bacteria had the biggest increase from the Deep to the Surface samples, since bacteria reproduce exponentially. The second largest count of bacteria in both samples came from Waste and Water Treatment Plants, indicating contamination. Coliform

were also present in the samples, though in smaller quantities, suggesting waste contamination.

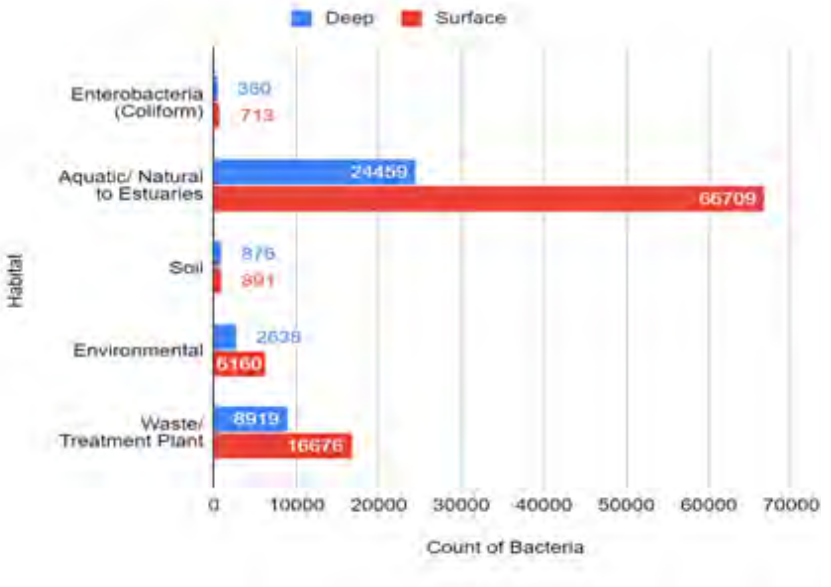

Figure 8. Coliform bacteria vs. other Bacteria divided by Habitat.

B. The Predominance of Classes of Bacteria with some Pathogens

We separated the Classes of Bacteria found in both samples from 10/02/2019 by pathology: all pathogenic, some pathogenic, and not pathogenic. The great majority of bacterial classes presented some genus that were pathogenic, followed by classes that were entirely pathogenic (Figure 9). When we compared Figure 8 to Figure 9, the difference between the counts of Coliform bacteria (360, in the Deep, and 713, in the Surface) and the solely pathogenic classes (9159, in the Deep, 18000 in the Surface) stood out. This was surprising since coliforms are used in indicating water contamination because they exist in greater quantities in feces than pathogenic bacteria, which was not the case. However, the LIC waters suggest that coliform are not the most reliable indicator of the level of water contamination.

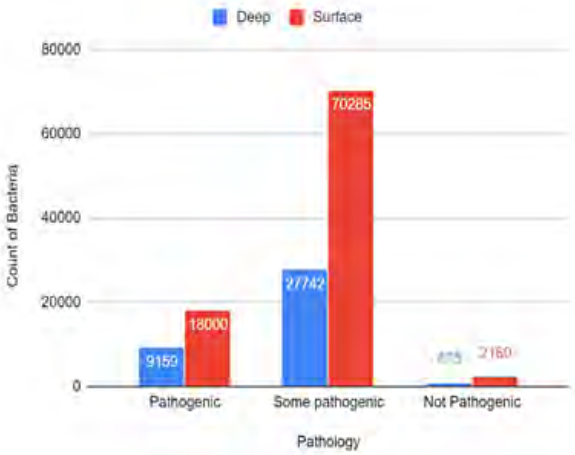

Figure 9. Results from Metagenomic Analysis by Pathology.

IV. Wastewater Treatment Plants Outfall

We looked at the concentration of CSO outfalls and WWTPs near Gantry Park to determine the likeliness of contamination. In the government website, we discovered several CSO outfalls around Gantry Park and two WWTPs closest to the collection site: Newton's Creek and Wards Island. This suggested that the most likely sources of the bacteria were either the CSOs or WWTPs.

Figure 10. New York City Wastewater Treatment Plants Locations and Combined Sewer Overflow Outfalls by Millions of Gallon per Day (https://www1.nyc.gov)

DISCUSSION AND CONCLUSIONS

This study revealed the inefficacy of current water quality determination tests. This is important since it presents an immediate danger to the population of Long Island City, and can call into question the quality of the water accessed by the entire population. The high presence of pathogenic classes of bacteria exposed on this study present a dangerous situation for the people that come into contact with the LIC waters. The dimensions of the estuary, its location in a highly populated area, and the fact that many parks follow its coastline raise many possibilities for direct human exposure to the contaminated water. If used for recreational activities and accidentally ingested, the water can become a source of infectious diseases (Naidoo S, Olaniran AO., 2013). The use of coliform bacteria to discern the safety of a water source is not sufficient to prevent pathogenic bacteria to go undetected. This danger is potentialized since the inefficacy of the method that is used by the government may extend to other water bodies, negatively impacting people's health.

The government should use Metagenomic Analysis in determining water quality so as to better detect contaminants to prevent the spread of diseases. Coliform are currently used as the bacterial indicator of water quality primarily because they are cheap to detect, do not multiply outside of the body, and exist in greater quantities in feces than pathogenic bacteria (Cabral J. P., 2010). This would make coliform a reliable detector of the degree of human waste contamination. Nevertheless, the danger of pathogenic bacteria is not restricted to untreated waste contamination, and, therefore, there is a need for testing that reflects that. By looking at the classes and genus of microorganisms in the water, contamination sources other than untreated waste might be more easily identified before there is an outbreak of disease in the population downstream of the dumping site, potentially saving lives.

Moreover, the most likely source of contamination identified in this study were the Wastewater Treatment Plants located near the collection site:

Newtown Creek and Wards Island (Figure 10). The sewer water collected in these facilities go through a series of processes to filter solid contaminants, balance Nitrates and DO levels before it reaches the estuary. In WWTPs the contaminated water is disinfected, eliminating pathogenic organisms before the water is released into water bodies. As indicated by the DO and Nitrates results, the first part of the waste treatment is functioning correctly, however, the abundance of pathogenic bacteria in the water suggests that disinfection is not being efficient. This can be due to the fact that these facilities also use coliform bacteria to determine the adequacy of the treated water. Pathogenic bacteria might also have recently become resistant to the methods of disinfection used in WWTPs, going undetected by the traditional testing method. The performance of a Metagenomic Analysis of the water that is being released from Newtown Creek and Wards Island Treatment Plants into the LIC tidal strait can determine if they are indeed ineffective in disinfecting the water, and if so, fix the issue.

This study also sheds light into the necessity of considering the physical and chemical properties of the water when evaluating contamination in a waterbody. The connection between disciplines is especially useful, since one factor, such as temperature, affects another, the dissolution of salt in water, which influences multiple others, such as the dissolution of oxygen, carbon dioxide and pH. All of these aspects helps researchers understand the environment and isolate possible sources of contamination that could be disrupting the equilibrium of the microbiome in it. This interconnectivity helped us reach the conclusion that CSOs were not the primary source of the pathogenic bacteria in the LIC waters. Therefore, the physical and chemical characteristics of the water are indispensable when evaluating the quality of a water source.

The study also helped us see how the political environment in which a city was constructed impacts the engineering behind it. The NYC sewers started being built in the second half of the 19th century, when green infrastructure was not yet in vogue. This cumulated in the Combined Sewer System that provides a systematic contamination of the city's water bodies with CSOs and, potentially, the water from WWTPs.

This faulty system allowed for a level of contamination that we did not expect in the LIC samples, revealing that even the waters near one of the most important tourist attractions of the world, Manhattan, are in dire condition. The high amount of Combined Sewer Overflow outfalls were also surprising, since environmental consciousness has become a priority in recent times. This level of pollution has only been maintained because of the lack of societal awareness of the issue, which allowed the population to trust that the authorities will protect the city's environment and the wellbeing of people that live there. However, every person in NYC should strive to keep the government in check to make sure that the city they live in does not limit activities inside and near the water sources due to health risk concerns due to contamination.

Our research made us think deeply about the environment and how we can improve it. Now, we realize that the authorities are not as efficient in protecting the population against pathogenic bacteria, and we must supervise their work. This can be done by pursuing further research to convince authorities to adopt Metagenomic Analysis as a protocol in the determination of water quality in order to detect and placate more sources of contamination to improve the quality of water of the East River. This can be done by supplementing the data analyzed in this study to reach more exact conclusions.

Further sampling is necessary to ensure that there are no outliers in the data analyzed, which would skew the accuracy of our conclusions. We plan to gather the data from inspection reports on the bacterial counts of the water purified on the Wards Island and Newton Creek Treatment Plants to determine if the facilities are indeed letting pathogens into LIC waters. The possible contamination sources analyzed in this study are also limited, and other possibilities must be explored, such as if pathogenic bacteria are coming from the waste discarded by businesses in the LIC area. This can be done by researching the dumping permits of companies that operate near Gantry Park. Further research should be on the genus instead of the classes of bacteria present in the water samples to get more specific measures of the quantities of pathogenic bacteria.

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