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The relationship between environmental variables and Bacterial community of Al-Kufa River sediments

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Summary:

There is no any study about the sediment quality and bacterial composition in Al-Kufa River, so this study comes to investigation the correlation between the sediment quality and bacterial composition.

Four stations were chosen to sediments collection, for Jun, Aug (Summer 2011), Oct, and Dec (winter 2011). Profiles of bacterial communities were generated using different culture media, and the results were interpreted with multivariate statistical analysis. Findings suggested that microbial communities varied with sample collection sites and seasons. The samples collected from the same sample sites at the same time had more similar microbial communities except the samples of station 3 and 4, which also had unique sediment quality. Canonical correspondence analysis (CCA) revealed that the organic carbon concentration of the sediments accounted for a significant amount of the variability in the bacterial community composition.

Introduction:

Although bacteria are the most abundant life forms on earth, knowledge of microbial community structures and population dynamics is still minimal, especially with sediments community. An estimated 80 to 90% of microorganisms in soil are as yet unidentified (1), and various researchers have detected enormous diversity in such habitats.

Inland freshwater ecosystems, including rivers and streams, lakes and ponds, wetlands and groundwater, are eminently microbial- based, relying on the activity of archaea, bacteria and protists for functioning. Many of these microorganisms are thought to be key components in the biogeochemical cycling of elements such as carbon and nitrogen and, therefore, to play a significant role in the biosphere. Furthermore, they intervene in the decontamination of wastewaters released into the environment from urban, industrial, and agricultural activities, thus contributing to maintaining water quality (2, 3).

However, knowledge of the composition of microbial communities in these systems is still very fragmentary, given the heterogeneity of freshwater habitats in space and time. Various ecological factors, both physicochemical (size, water chemistry and retention time, temperature, irradiation) and biological (organic matter supply, primary producers, predation, viral dynamics) influence to various extents the composition of microbial communities (4,5).

Comparisons of diversity across microbial communities may lead to a knowledge base applicable to a variety of environmental issues. It is necessary to accurately measure changes in populations of microbial community members, especially major components of the community, in response to seasonal, natural, or anthropogenic changes and to identify keystone species (6).

Changes in the diversity and structure of a microbial community could become manifested in the ecological processes (7, 8).

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There is no study about the bacterial composition and sediment quality, so this study comes to investigated the change in bacterial diversity and its correlation with organic matter and sediment quality as indicator for anthropogenic pollutant.

Sampling strategy

For investigation this study, chosen four stations, two upward of the river and two under ward the river, during Jun& Aug (Summer season 2011), and Oct & Dec(winter 2011).

Study site

At Al-Kifl city, the Euphrates subdivided into two parts: Al-Abassia and Al-Kufa river, the last one extends from Al-Kifl city via Al-Najaf government to Al-Diwania city, the total length of Kufa River is about 36 Km , its the capacity about 375 m^3 $.sec^{-1}$, but the actual capacity reach to 552 $m^3.sec^{-1}$. The water level in this river undergoes large fluctuations, the highest level occur during the flooding season (end of March early April), the lowest water level occur in the summer (9).

A lot of villages and farms (animal, crop, and vegetation farms) are found along the River; there are domestic, municipal wastewater and agriculture drainage discharged to the River; in addition to the industrial wastes that which come from: the industrial region in Al-Najaf city, the leather industry, and the cement factory. All of above have affecting the water quality. To investigated this study, chosen four stations as following: **st.1** (control station) beside Imam Ali Bridge, **st.2** beside old Al-Kufa Bridge (Al-Kufa municipal wastewater discharge), **st.3** after buffalo's farm, and **st.4** located in the Last part of Kufa River (near Cements Factory Bridge, after Al-Barakya municipal wastewater treatment plant discharge) (Figure 1).

Figure 1: the studies stations

Material and methods:

1. **Bacterial community:**

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The sediment bacterial sampling were handling collected in sterile disposal tubes, the preparing of culture media of bacterial community (selective media) were done according to Ronald (10) and APHA (11). Table (1), cleared the type of media that used to isolation and identification of the studied bacteria.

Table 1: the type of media that used to isolation and identification of the studied bacteria.

No	Bacteria sp	Type of media*
1	Sphaerotilus Sp	Sphaerotilus CGYA Medium
2	Desulfovibrio Sp	Starkey's Medium C, Modified
3	Escherichia coli	m-TEC Agar
4	Gallionella Sp.	Ferrous Sulfide Agar
5	Klebsiella Sp	Klebsiella Medium
6	Pseudomonas aeruginosa	Malachite Green Broth
7	Staphellococous Sp	Nutrient Agar with 3% NaCl
8	Streptococcus Sp	Nutrient Agar with 3% NaCl
9	Thiobacillus Sp	S6 Medium for Thiobacilli
10	Saccharococcus Sp	Saccharococcus Agar

*With: biochemical test, Simmons' Citrate Agar, SIM Medium, Enrichment Medium, or nutrient agar, with aerobic or anaerobic conditions.

2. Sediment Quality:

The sediment samples were handling collected, preserved using nylon zipper-sealed bags (17.7*20.3 cm), and placed in an ice box until reaching the lab. In the lab, they were dried using the oven at 45°C, then sieved through a 2 mm mesh-size sieve and preserved in new bags to await analysis (12).

***a.* Sediment Organic Matter:**

The method used for the estimation of total organic matters (TOM) in sediments is by oxidizing the organic carbon (TOC) by chromic acid and phosphoric acid and back titration of the remaining acid with a standard solution of ferrous ammonium sulfate using diphenylamine indicator. In order to calculate the amount of TOM in the sediment samples, TOC results were multiplied by a factor (1.74) (11).

***b.* Degree of pore-water reaction (pH):**

A sediment suspension with water was prepared at 1:1 ratio and the procedure was:

1. Weigh 50 gm of sample and put it in 100 ml beaker.
2. Add 50 ml de-ionized water and shake well every 10 minutes for ½ hour.
3. After 1 hour shake the suspension and measure pH by the combined electrode of the pH meter. Put the electrode to 3 cm in the suspension and take the readings after 30 seconds.
4. Pull it out, rinse well with de-ionized water and dry it with a smooth cloth.

***c.* Pore-water Salinity:**

The sediment suspension was prepared just like the suspension in the pH test. In addition:

1. Filtrate the suspension by the filtration apparatus using Watman filter papers No 42.
2. If the filtration is turbid (not clear), the filtration process is redone.
3. Readings are taken from the clear final filtration using the salinity probe in a Multi 340i meter.
4. Readings are in the unit ppt.
5. Pull the probe out of the filtration and rinse it with de-ionized water.

***d.* Sediment Total Phosphorus:**

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Total phosphorus in the sediment samples were determined by digesting 2 grams of sub-samples with perchloric acid at 180ºC for about 40 min. After cooling and filtering the aliquot, 5ml were withdrawn 10ml of vanadomolybdate solution was added and the volume was made to 50ml. The absorption of the developed fainted yellow color was measured at 410nm by a Cintra 5 spectrophotometer (11).

The procedure was:

1. Weight 2 gm of sample and put it in a digestion tube (capacity 250 ml).
2. Add 30 ml perchloric acid ($HClO_4$) and a few Pumice rocks (anti-bumping granules) and mix well.
3. Put the tube in the digestion apparatus that has been heated to 100°c.
4. Raise the temperature to 180°c until the white gasses of the acid appear and the complete digestion process takes place (up to 40 minutes).
5. Cool the mixture down and add de-ionized water reaching to 250 ml and mix well.
6. Filtrate it using filter paper № 41. In case of high organic matter sample, add 20 ml nitric acid before step № 2 and heat it to oxidize organic matter.
7. Take 5 ml from the mixture, put it in a 50 ml volumetric flask, add 10 ml vanadomolybdate solution and add de-ionized water reaching to 50 ml.
8. Absorbance is measured after 10 minutes by a Cintra-5 UV Visible Spectrophotometer at wavelength 410 nm.
9. Prepare the standard curve :

Take 2, 4, 6, 8, and 10 ml from the standard solution that you prepared before and put it in a 100 ml volumetric flask.

Add 10 ml vanadomolybdate solution and complete the volume to 100 ml by de-ionized water to raise the yellow color. Draw the curve between absorbance and concentration of the standard solution.

Use the equation:

Phosphorus concentration (ppm) = phosphorus concentration from the standard curve (ppm)*250/A*50/B

A= amount of digested solution used, B= weight of the sample digested

Results and dissection:

Over 80 genera of bacteria that are nonpathogenic for humans have their natural habitat in water. In addition, some opportunistically pathogenic bacteria (Pseudomonas, Serratia, Acinetobacter, Chromobacterium, Achromobacter, Aeromonas, etc.) occur naturally in water. Other opportunists (Bacillus, Enterobacter, Klebsiella, Actinomyces, Streptomyces, etc.) are sometimes washed into water from their natural habitat in soil or on vegetative matter. Opportunistic pathogens also may be seeded from regrowth and biofilms in water treatment plants and distribution systems. (11), in this study the researcher identified ten species that lasted in table (2), as shown In figures (2) and (3), st.4 was more bacterial appearance and diversity than others, st.3, st.2 and then st.1. The Aug month was more diversity than other months of study (figures 3 and 4).

According to statistical analysis, the results showed a significance differences between all sites for all months of study, The highest number of bacteria was found in site (4) in June while the lowest number detected in site(1) in December. Also a high abundance of bacterial Sp appeared in Summer season, and Sphaerotilus, Desulfovibrio, Escherichia coli, Pseudomonas, and Streptococcus species considered as a common species in all sites while Klebsiella, Gallionella, Saccharococcus ,

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Staphellococous, and Thiobacillus Sp was fluctuated between study sites, and Gallionella sp. Recorded only during Aug in st.(3) and (4).

The diversity come because of the microbial mineralization of organic matter – which is carried out by microbial communities of variable compositions, using sequentially different electron acceptors (Jones, 1985) – is highly influenced by the presence or absence of oxygen and has a remarkable influence on the chemical composition of the sediment and sediment interstitial water (Berner 1980).

The highest values of salinity, and TP were in st.4 (during Aug), while the lowest values were in st.1 (during Dec and Jun) (figure 5, and 6). As shown in figure (7), the highest values of pH were recorded in st.1, during Jun, while the lowest value of pH were recorded in st.4 during Aug. According to figure (8), the highest value of TOC and TOM was in st.3 during Dec, while the lowest value was in st.2 during Aug.

Ultimately, P-release is the result of complex mechanisms including mineralogical diagenesis, biotic and abiotic redox processes, biological assimilation, and enzymatic and non-enzymatic hydrolysis reactions (Baldwin, 1996). Anaerobic conditions favour P-release from sediments (Mitchell & Baldwin, 1998). Pathways involving C-mediated bacterial activity, SO_4^{-3} and Fe are often implicated (Baldwin et al., 1998). Bacterial activity reduces oxygen levels if oxygen consumption by bacteria exceeds supply. The resultant anaerobic conditions enhance Prelease locally (Holdren & Armstrong, 1980) With higher pH values in the water column, there is decreased P-binding capacity of the sediment and ligand exchanging mechanisms become important by liberating P adsorbed by Fe(OOH) in the sediment (Boström & Pettersson, 1982), and this come agree with this study.

The accumulation of large amounts of organic matter in stations 3 &4 that comes from anthropogenic sources increasing to leaves, and other vegetal material leads to rapid consumption of oxygen and nearly permanent anoxic conditions, particularly in the deepest layers of sediments (with grey dark colour). The result is oxygen deficit very close to the surface and anoxic conditions in the deepest regions, which favors the development of anaerobic communities of bacteria that supplement the system with reduced gases such as methane and hydrogen sulfide.

Usually the increase in PO_4^{3-} concentrations in the function of sediment depth is the reason for the release of the iron bound phosphate and the decomposition of organic matter in reductive conditions(Istvánovics, 1988), that was agree with present study(sediment TP is bound to the organic matter).

Approximately 60 to 80% of all Klebsiella, E. coli, Pseudomonas, and staphylococci species from feces and from clinical specimens, and if they present that are a primary indicators of contamination from animal pets, rodents, stormwater runoff, and human sources.

Conclusion

Significant differences were found between the sites based on the sediment characteristics, which demonstrated that the changes in the parameters of the sediment interstitial water and in the organic matter content and microbial activity of the sediment are strongly interrelated with the condition. The correlation analysis showed that the microbial activity of the sediment was interrelated with organic matter content, pH and redoxpotential of the sediment. As conclusion we can say the sediments of st.3 and 4 were contaminated with animal pets, rodents, stormwater runoff, and human sources.

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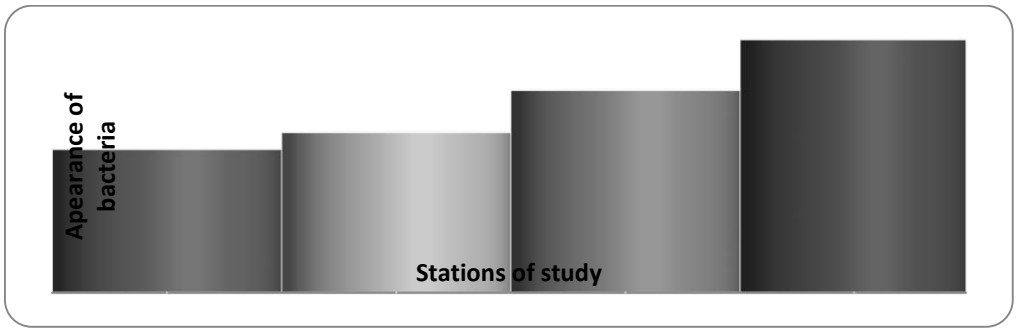

Figure (2): Appearance of bacteria in stations of study

Figure (3): Shannon diversity according to stations and periods of study.

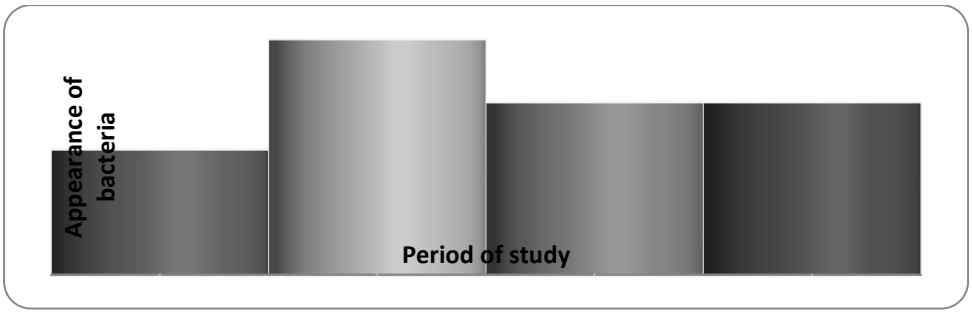

Figure (4): Appearance of bacteria according of period of study.

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Table (2) the Bacterial Composition in Sediment of Al-Kufa River

Table of Bacterial Composition in Sediment of Al-Kufa River																				
No	Bacterial species	St.1					St.2					St.3					St.4				
		Jun	Aug	Oct	Dec	total	Jun	Aug	Oct	Dec	total	Jun	Aug	Oct	Dec	total	Jun	Aug	Oct	Dec	total
1	Sphaerotilus Sp		1			1		1			1		1	1		2		1	1		2
2	Desulfovibrio Sp		1		1	2		1			1				1	1	1	1		1	3
3	Escherichia coli		1	1		2		1	1	1	3	1	1	1	1	4	1	1	1	1	4
4	Gallionella Sp.					0					0		1			1		1			1
5	Klebsiella Sp		1			1					0	1	1	1	1	4	1	1	1	1	4
6	Pseudomonas Sp		1		1	2		1	1		2	1	1	1	1	4	1	1	1	1	4
7	Saccharococcus Sp		1	1	1	3	1	1	1	1	4					0	1		1	1	3
8	Staphellococous Sp					0	1	1	1		3	1	1	1	1	4	1	1	1	1	4
9	Streptococcus Sp	1		1	1	3		1			1	1	1	1	1	4	1	1	1	1	4
10	Thiobacillus Sp		1	1	1	3	1	1	1	1	4					0				1	1
	Total appearance	1	7	4	5	17	3	8	5	3	19	5	7	6	6	24	7	8	7	8	30

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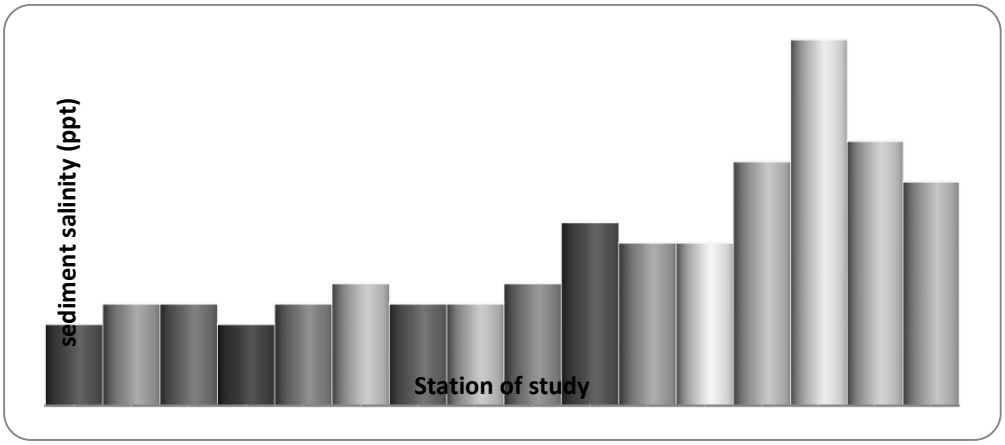

Figure (5): The sediment Salinity (ppt) during the period of study .

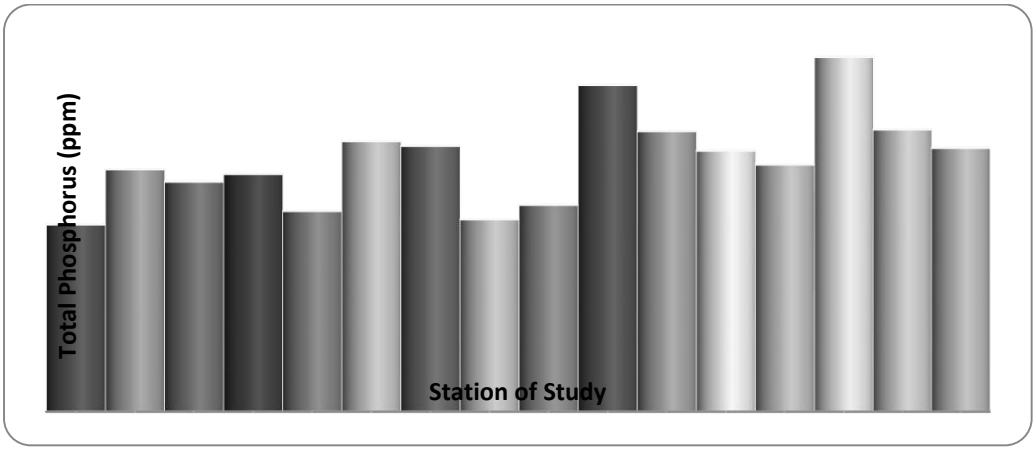

Figure (6): The TP (ppm) in sediments samples during the period of study.

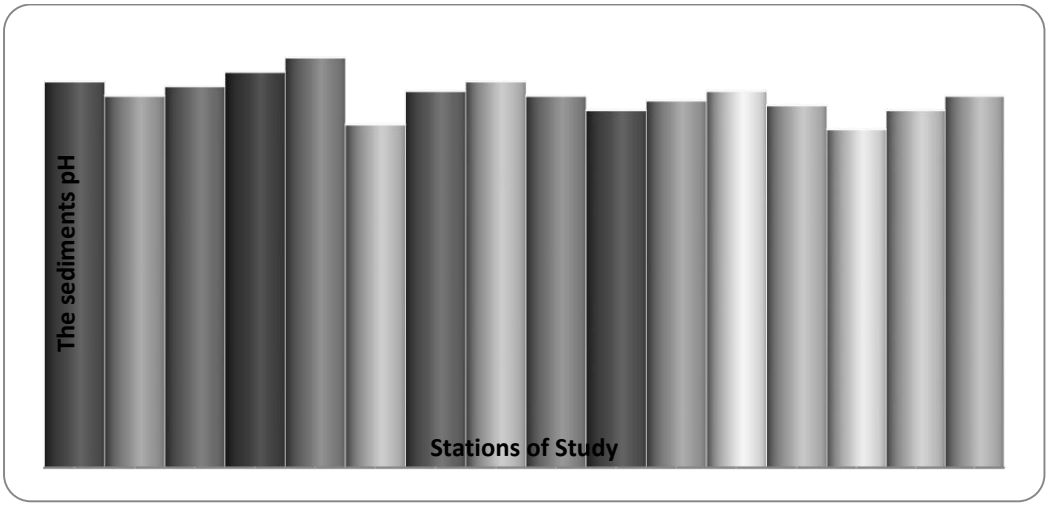

Figure (7): The sediments pH value during the period of study.

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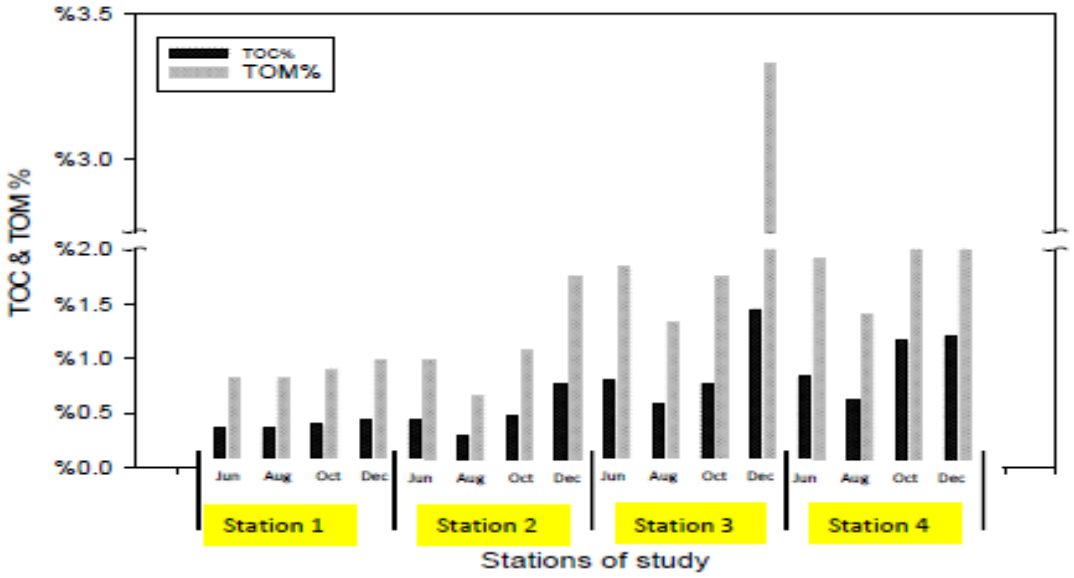

Figure (8):The TOC & TOM (%) in studies stations during the period of study.

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علاقة المتغيرات البيئية و المجتمع البكتيري في رواسب شط الكوفة

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الخلاصة:

لا توجد اي دراسات سابقة حول مواصفات رواسب شط الكوفة او التركيبة المجتمعية للبكتريا في الرواسب ، لذلك جاءت هذه الدراسة لتوضيح العلاقة ما بين مواصفات الرواسب و التركيبة المجتمعة للبكتريا في الرواسب.

لتحقيق الهف من الدراسة اختيرت اربع محطات تمثل الاولى محطة السيطرة والمحطات الثلاث الاخرى تمثل التدرج في كميات الملوثات التي تنزل الى النهر من مدينتي النجف والكوفة، استمرت الدراسة لمدة اربعة اشهر (حزيران و اب و تشرين الاول و كانون الاول) من عام 2011 والتي تمثل فصلي الشتاء والصيف. تم تشخيص البكتريا وعزلها حسب الاوساط المخصصة لكل نوع , اما مواصفات الرواسب الفيزيائية والكيميائية فقد حللت حسب ما ورد في المصادر العالمية المعتمدة. وقد عوملت النتائج احصائياً لمعرفة مدى تاثير محافظة النجف الاشرف على رواسب الشط ومدى العلاقة ما بين المتغيرات المختلفة.

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اوضحت النتائج ان هناك تاثير واضح للمخلفات والنشاطات المختلفة على التنوع البكتيري وعلى تراكيز المواد العضوية مواصفات الرواسب الاخرى عند المقارنة مع المحطة المرجعية، فقد وجد ان هناك اختلافات في خواص الرواسب والتركيبة المجتمعية للبكتريا في المحطتين 3 و 4 واثبت التحليل الاحصائي لمعامل الارتباط ان هناك معامل ارتباط معنوي قوي ما بين الكاربون العضوي و التغايرات في التركيبة المجتمعة للبكتريا.