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NOVEL AND RAPID TECHNOLOGY FOR DISSECTING AND REMOVINGMICROBIAL COMMUNITY IN AQUATIC ENVIRONMENT

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ABSTRACT:

The present study aims to integrate the benefits of Illumina microbial sequencing approaches using novel prepared copper oxide (CuO) nanoparticles for improving water quality monitoring and management. Our results showed rapid and accurate discovery of novel different microbial compositions in the collected water samples. The domestic drains were had pathogenic microbes as viruses, Alpha-, Beta-Gamma proteobacteria, Bacteroidia, Deinococci, Bacilli also Clostridia with variety quantitive. The class Pseudomonadales were found to be higher in quantity in all drains outfall. Viruses, including Enteroviruses and hepatitis A and E species were found out in high volume of domestic drains in comparison with the agricultural and industrial drains. On the other hand, our results showed that decreasing and removing microbial pathogens using prepared copper oxide nanoparticles (CuO NPs) by quick precipitation method. The highest antibacterial activity was notified for CuO NPs with the optimum concentration 10^2 µg/mL were ranged between 92 to 96% after contact time 24h at 35°C. The shaking during the treatment gave a positive effect removing with CuO NPs. Concluding remarks highlighted the potential of nanoparticles and Illumina mix as accurate, simple method. The study recommended future efforts to apply this as a robust, rapid and powerful metagenomics technology to detecting other disease-causing agents of public health concern to update and control water pollution.

INTRODUCTION

Increasing demands on clean water for many purposes as drinking, industrial and irrigation purposes, Clean water sources is shorted to meet increasing of population, increasing industrial demands and other reasons. wastewater usage is one of the most source available to meet the clean water demands. pathogens and hazard chemicals which come from wastewater had an effects on health **(Silva et al., 2009)**.

Copper (II) oxide is semiconducting material have amonoclinic structure systems, which having useful physical and chemical properties as superconductivity at relatively stable, high temperature, photovoltaic properties, and has antimicrobial effect (**Rene et al., 2009)**. CuO nanoparticles have different technology applications as catalysis **(Zhou et al., 2006)**, batteries due to high electrochemical capacity **(Anandan et al., 2012)**, and gas sensors **(Borgohain and Mahamuni, 2002)**. CuO nanoparticles synthesized by different methods as sonochemical technique **(Anandan et al., 2012)**, electrochemical method **(Borgohain and Mahamuni, 2002)**, high temperature combustion **(Chang and Zeng, 2004)** andnovel quick precipitation method **(Zhu et al., 2004)**.

Removing organic pollutants from sewage water such as nitrogen, sulfur and carbon was considered a hot issue using specific microorganisms. bacterial community exists inactivated microbial state. Many methodologies were used for remediation wastewater of plants such as culture-dependent and culture-independent. Using various technologies for sequenced and analyzed structure and framework of microorganisms as 16S rRNA gene, 454 pyrosequencing, metagenomic sequencing where this tools used for microbial estimation from different domestic agricultural drains **(Ye et al., 2012; Sanchez et al., 2013; Ye and Zhang, 2013)**.

Approach clone library sequencing can give non-accurate results deep-rooted alignment of planning **(Aird et al., 2011; Ye et al., 2012)**. Some technologies evaluated Illumina sequencing technology where are an inventive practice to microbial genome **(Albertsen et al., 2006; Bragg and Tyson, 2014)**. Several studies were evaluated anaerobic microbial structure using method 454 pyrosequencing **(Wong et al., 2013; Li et al., 2013; Sundberg et al., 2013)**.

Illumina sequencing technology is a low-cost and effective method when compared with 454 pyrosequencing investigate microbial community structure **(Mardis, 2008 and Glenn, 2011)** where it has been used for investigation microbial community from water samples **(Mackelprang et al., 2011),** also samples from the ocean **(Mason et al., 2014).** Anaerobic digested sludge **(Ju et al. 2014)**. A study has tried to analyze complete data of microbiome from digested sludge samples **(Yang et al., 2014)**. fecal indicators pollutants such as *Escherichia coli* or *enterococci* is being founded in the effluent part of wastewater samples, there are other disease-causing agents, shuch as bacteria and viruses which may be presented in fecal indicators which transmitted throw different carriers in environment. Ejection of the disease-causing agents is important for reutilization of water with measurement of pathogenic microorganisms in the environment (**Varela and Manaia,2013)**. Pathogenic bacteria “Mycobacteria” has never been observed and monitored to be measure for human and animal infection in wastewater where some researchers have quantified the presence of Mycobacteria in the drainage and surface water samples and they reported that Pathogenic bacteria "Mycobacteria" were not expected to be detected by known parameters. **(Radomski et al., 2011).** Viruses were reported to be non-predictable in the polluted water by given parameters **(Savichtcheva and Okabe,2006)**.

Viruses are dangerous for animals and human health more bacteria, where they diffused easily **(Rosa et al.,2010)**.

This study is important to analyses and dissect the microbiome structure from various parts.Using samples from El-Rahawy, Tala, Sabal drains in Egypt. Detecting concentration of viruses and mycobacteria. Using metagenomic sequencing technology. Pathogenic microbial species present of drainage water.

MATERIALS AND METHODS

COLLECTION OF SAMPLES

According to Standard Methods for Examination of Water and Wastewater water samples the water samples analysis was carried out (**APHA, 2012)**. Samples of water were collected in clean and sterile polyethylene plastic bottles. Various drains outfalls along the River Nile at Rosetta branch were chosen for sample collection and represent the major sector in Egypt, including El-Rahawy drain (R), Sabal drain (S), El-Tahreer drain (E), Zawiet El-Bahr drain (Z) and Tala drain (T) for 24 h after every 30 min. Drains mixed from sewage, agricultural and industrial wastes.

Samples were collected under consistent sampling procedures such that: presence of ample air space in the bottles (at least 2.5 cm) to facilitate mixing by shaking, keeping sampling bottles closed until it is to be filled, avoiding external contamination during sample collection, avoiding internal contamination of stopper or cap and bottle neck and filling container without rinsing. Allsamples collected for either chemical or bacteriological detection and immediately forwarded to the Central Laboratory for Environmental Quality Monitoring (CLEQM) National Water Research Center (NWRC), Cairo, Egypt in an iced cooler.

Preparation of Copper oxide nanoparticles (CuO NPs)

Copper oxide nanoparticles with tetraoctylammonium bromide (TOAB) surfactant were prepared using precipitation method. CuO NPs stabilized with TOAB surfactant. Dissolving about 15.00 g of $CuSO_4.5H_2O$ using 2.34 g of tetraoctylammonium bromide surfactant in 150 mL of ionized water for prepare (CuO-TOAB). Different size of CuO

NPs was prepared at 65, 75 and 85°C using the reflux capacitor. Heating and stirring for about 15 min at 150 rpm. About 2.0M of sodium hydroxide were added to 100 ml of the prepared solution. Black precipitate (ppt) was collected and washing by the ionized water then allowed to dry.

Physico-chemical analysis

All field parameters were measured in the field and rechecked in laboratory to ensure data accuracy; Temperature, pH, dissolved oxygen (DO), electric conductivity (EC) and total dissolved solids (TDS) were measured in water samples by using the multi-probe system, model Hydralab– Surveyor, Germany. Once the samples were received in the lab, they were manually mixed by shaking and examined as follows: Ammonia (NH_3) measured by using Kedah method . Nephelometric turbidity meter HACH using measured turbidity . (BOD) is known by Biochemical oxygen demand measuredd by ORION BOD model 890. (COD) is known Chemical Oxygen Demand tested using potassium permanganate method. Total hardness, Calcium hardness (Ca. hardness), and Magnesium hardness (Mg. hardness) measured by Titrimetric Method. Chloride (Cl^-) measured by Argentometric method. Nitrate (NO_3^-), Nitrite

(NO_2^-), Phosphate (PO_4^{3-}), and Sulphate (SO_4^{2-}) were measured by Ion Chromatography. The concentrations of major cations, Calcium (Ca^{2+}), Sodium (Na^+), Magnesium (Mg^{2+}) and Potassium

(K^+) . heavy metals as arsenic (As^{+2}), cadmium (Cd^{+2}), chromium (Cr^{+3}), copper (Cu^{+2}), zink (Zn^{+2}), lead (Pb^{+2}), nickel (Ni^{+2}), aluminum (Al^{+3}), manganese (Mn^{+2}) and iron (Fe^{+3}) were measured by using ICP-OES Model Varian lab Liberty Series II.

Bacteriological analysis

Collected samples were examined within 6 hours followed the method from Standard book for Examination of Water and Wastewater **(APHA, 2012)**. According to standard method Nos. 9222B, 9222 D total coliforms (TC), fecal coliforms (FC) and fecal streptococci (FS), using membrane filter technique. All media were obtained form Difco-USA. Results were recorded as colony forming unit (cfu $100ml^{-1}$) by using equation:

$$\text{Colonies}/100\ \text{ml} = \frac{\text{Counted colonies}}{\text{ml of sample filtered}} \times 100$$

Factors effect on the NPs antibacterial activityTemperature effect

CuO-TOAB was used at 10^2 and 10^3 concentrations by µg/mL . Samples could be control shaking at 150 rpm for 2 h at 15, 25, 35°C.

Contact time effect

Preparing of CuO-TOAB NPs at 75°C. Using CuO-TOAB is better activity. studying carried out concentrations; 10^2 and 10^3µg/mL of CuO-TOAB. We must control some paramters such as shaking at 150 rpm for 24 h at 25°C.

Testing Flow

Preparing CuO NPs is important for water treatment, testing flow using to investigate the antibacterial activity of CuO NPs .Increasing application of preparing CuO NPs for water treatment. Sterile column (L: 44 X D: 12 mm) used to flow 4.00 mL of drainage water. Thickness of CuO-TOAB layer about 1.0 mm. flow rate of 10 mL/min was constant . E. faecalis bacteria and TC.

DNA extraction and library preparation

All collected water samples were stirring for 10 min at 6000g to be concentrated.Filtration using A 0.22 µm filter injection found structure of microbiome and pathogens. Using 250 mg of pellets for DNA extraction. DNA could be isolated (MoBio, USA **(Kaevska et al., 2011)**. using gel electrophoresis was detect DNA quality. Fluorometer (Thermo, USA) finding out quantity of DNA.. Illumina HiSeq 2000 using conducted at the Colors Company, Egypt.

DNA exctraction was diluted to 200–300 ng/µL, for using experimental **(Ali et al., 2019a)**. The procedure of DNA library related to **Campanaro et al.,2016**. The short reads were fragmented by a minimum 30 quality score. no ambiguous nucleotides. parameters were get overlapping about 20 nucleotide length of region was needed. **(Ali et al., 2019b)**.

Metagenomic determine

A prediction for metagenomic computational of DNA was utilized from the international of National Center for Biotechnology Information (NCBI). Prediction server given the metabolic and taxonomic affiliation. analyzing protein as reported **(Meyeret al., 2008)**. About 10^{-5} E value for investigation taxonomic enrollment on MG-RAST **(Huson et al., 2011)**. Capacity of taxonomic as phyla, and genus, was determined for explanation. Hierarchical divide to at E value deducation of 10^{-5} using for gene comments on profile **(Yang et al., 2014)**. The gentics were divided successfully into hierarchical metabolic groups. Entering reads were decoded across the bibliography of the Kyoto Encyclopedia of Genes and Genomes (KEGG) databases.

Bioinformatic analysis

Sequences of DNA were identified using Basic Local Alignment Search Tool (blast) on the NCBI database. Using DNAMAN software performed for many alignments of sequences (Madison, Wisconsin, USA, version 5.2.9). **(Thompson et al., 1994)**. The nucleotide distances were measured alignment gaps and using Juckes and Cantor's method **(Jukes and Cantor, 1969).** Development of Molecular Genetic Analysis (MEGA) software (version 6.0) **(Tamura et al., 2013)**.

Identification for Relationship of Phylogenetic between microbial community. Evaluaion using unweighting two Method with Arithmetic Mean (UPGMA) through MEGA 6.0 software. and boot strap analysis (1000 replicates) was performed to assess the reliability of the constructed phylogenetic. Nucleotide sequences data determined by the National Center for Biotechnology Information (NCBI) GenBank database, USA.

Statistical analysis

Data mean values and percentages were calculated using Minitab 16 statistical software program **(Minitab, 2010)**.

RESULTS AND DISCUSSION

Eco-diversity of collected water samples

The process quality of water is an evaluation of physical, chemical and biological nature of water .Quality of water may effect ont human health and health of the aquatic system . Our area of study, water quality drains was carried out. The results obtained from physical, chemical, microbial community , calculating parameters and statistical analyses. investigation, both of pH, turbidity, DO, BOD and COD concentrations showed variable results according to site nature of pollution. in **Figure 1(A & B)**. water samples collected from drains outlets showed high turbidity, marked depletion in DO values and all COD concentrations exceeded law 48. The maximum values were at El- Rahawy, Sabal and Tala drains while. Minimum values were at Zawiet El-bahr and El-Tahreer drains.

Water quality of drains outlets was improved after treated with by CuO nanoparticles with different percentages especially in water turbidity (67%), DO (43.2%), BOD (85%) and COD (85%). Organic matter concentrations play the important role in water quality and related stronglywith microbial load in aquatic system. CuO nanoparticles used as antibacterial material in many studies which confirmed the ability of this particles for bacterial indicators degradations. **Suleiman et al., 2013,** prepared and apply CuO nanoparticles in bacterial indicators degradation (TC, FC and FS) and microbial removing with concentration less than 1000 µg/mL.

Figure 1. Physicochemical properties of collected water samples

A. pH, turbidity, DO, BOD, COD values before treatment with CuO nanoparticles.

B. Improvement percentage after treatment with CuO nanoparticles.

Different factors effect on NPs antibacterial activity

Assessment for water quality process is an assessed of physical, chemical and biological nature of water ., This process effecsts human health and health of aquatic system.

CuO NPs were prepared with and without TOAB surfactant. It investigatedin a real drainage water samples. Many parameters that may affect the antibacterial activity .We taked the optimum conditions to have NPs with high activity used in drainage water disinfectant.

The most factors affecting bacterial growth in aquaticenvironment is Temperature. Weather of Palestine that temperature almost range from 10-35°C. Areflection of the slow water response to the weather temperature. Different incubation effect on temperature; 15, 25 and 35°C, were studied a factor in antibacterial activity of CuO NPs at concentration (10^2 µg/mL).

Studying area antibacterial activity of CuO NPs was studied at different contact time of 0, 1, 2 and 24 hr at 25°C. Determination appropriate contact time. Studingfor five samples and our results showed the best concentration (10^2 µg/mL) of drainage watersamples were 90, 92, 95, 96, 98%.

Testing flow at fixed flow rate of 10 mL/min was cleared antibacterial CuO NPs. This study needed optimum conditions to investigation such as pHand temperature. Degradation of bacteria percent reach to 99%. Indicators of bacteria using in drainage water samples passing CuO-TOAB. Drainage water samples passing CuO non-stabilized. Percent degradation for E.faecalis bacterial and TC were 79, 83, 75, 89 and 81%, successively . CuO NPs stabilized with TOAB is enable effective degradation of bacteria than using CuO NPs without stabilization.

Biodiversity of Microbial Community

About 35 thousand active sequences abundant quality were collected for evaluation. Homogeneous sequences with different quantities were found in about four parts of drain Zawiet El-Bahr. Concentration comparative active steps was evaluated in studying **(Kaevska et al., 2016 and Azzam et al., 2017)**. Quantity of microbes is high.Viruses was get in both of El-Rahawy and Sabal drain outlet. Fewer in Tala and El-Tahreer drain outlet . **Figure 1**. The sewage sample was reported 1730 species where agriculture drain about 1490 species was discovered in class-level classification. Ye and co-authors reported a similar finding when studding wastewater samples **(Ye and Zhang, 2013)**. In accordance some studied from researches noticed diversification were higher in domestic drains when comparison with agriculture drains **(Lee et al., 2015)**. Data agreement to our investigation to statistical data. Mixing domestic and agricultural drains samples were found like samples from agricultural drainsas reported from statically analysis. Similar variance in the microbial structure founded in El-Rahawy drain (R), Sabal drain (S), El-Tahreer drain (E) drain, Zawiet El-Bahr drain (Z) and Tala drain (T).

Bacteria represent the most percent in domestic drains with 97.2% specially in El-Rahawy, Sabal and Tala drains. Concentration ratio of archaea, eukaryote, and viruses was measured in the five drains. publishing studies reported a similar Concentration **(Yang et al., 2014)**.

In El-Rahawy drain the concentration of viruses , accounting 5.82% ,was about three times higher than the other drains. A smaller bacterial concentration. Analysis details in **Figure 2**.

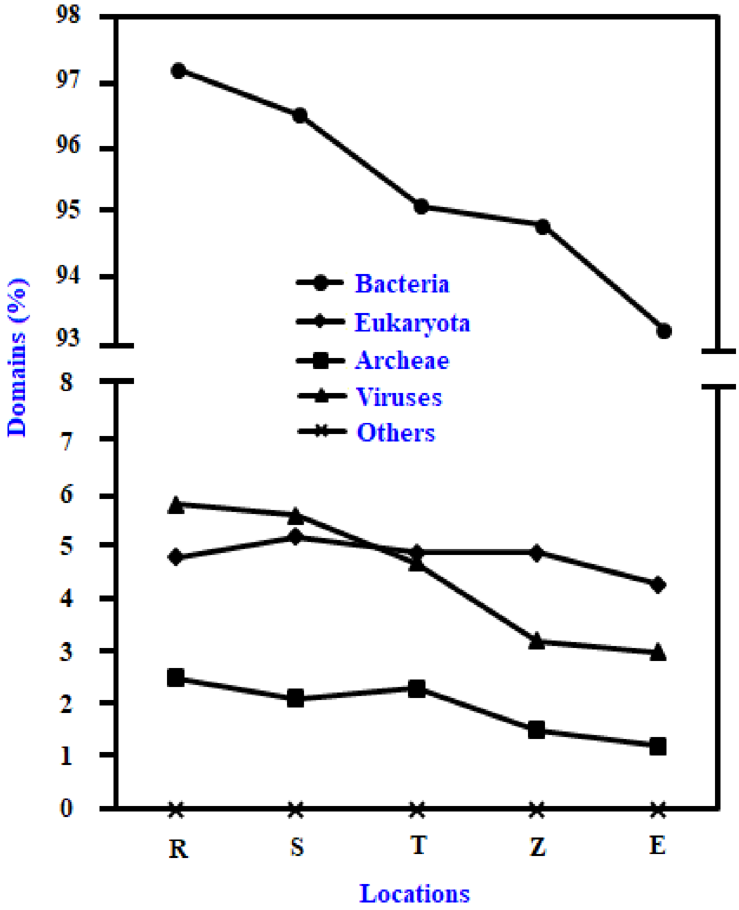

Figure (2): Taxonomic profiling at the domain level of the studied Egyptian five mixed drains. sequences of DNA were assigned to bacteria, eukaryote, archaea, viruses, other sequences.

Detecting the whole microbial structure and also the functional profiling of the (DS) digestion sludge, data were showed in **Figure 3.** represented short reading where analyzed according to the KEGG category database.

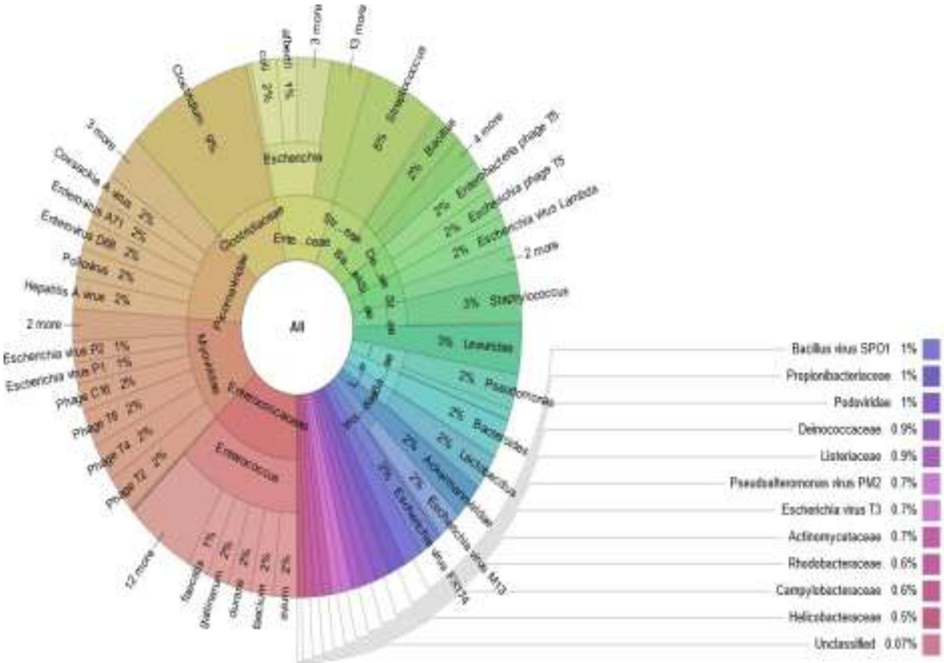

Figure 3. Krona taxonomy chart for all bacterial and viral strains discovered in water samples.

In the sewage drains outlets the precent of phyla Proteobacteria, Bacteroidetes, Firmicutes-associated bacteria were the highest, , as shown in **Table 1**.

The founded results were correlated to previous report which reported by Lee's group **(Lee et al. 2015)**. In agreement with our results a high number of Actinobacteria were found as reported by McLellan's group **(McLellan et al., 2010)**. Delta-proteobacteria and alpha beta **(Table 1).** Significant concentration reporting **i**n all outlets domestic presented Epsilon and Gamma proteobacteria less concentration. Lee and co-authors proving agreement investigation **(Lee et al. 2015)**.

Previous reports reported that Gamma-proteobacteria, Deinococcus and Clostridiales were dominant classes **(Ye and Zhang 2013)**. Parameters difference may cause a variance in microbial composition of aquatic systems. climate zones, industry , agriculture wastes and human activity **(McLellan et al. 2010)**.

Table 1: Dominant precent class in major phylum from bacteria and archaea found inwater samples.

Phylum	Class	R Abundance(%)	S Abundance (%)	T Abundance (%)	Z Abundance (%)	G Abundance (%)
Proteobacteria	Alphaproteobacteria	26	22.1	19.2	17.3	15.4
	Betaproteobacteria	26.3	21.9	19.3	17.1	15.4
	Gammaproteobacteria	26.5	21.6	19.5	17.3	15.1
	Epsilonproteobacteria	25.9	22.2	19.3	17.4	15.2
	Others	26.7	18.1	24.9	13.1	17.2
Actinobacteria	Actinobacteria	27	23	19	17	14
	Others	0.50	0.41	0.30	0.24	0.19
Bacteroidetes	Bacteroidia	32	26	17.1	14.4	10.5
	Others	1.90	1.86	1.90	1.80	1.65
Deinococcus	Deinococci	28	22.8	16	12	8
	Others	6.40	5.87	6.02	3.60	4.01
Firmicutes	Bacilli	20	20	19	13	15
	Clostridia	45	39	32	21	28
	Erysipelotrichia	2.01	2.01	1.99	1.67	1.50

In El-Rahawy, Sabal and Tala drain outlets a high concenteration of viruses with percent 8 % while less concentration was found in El-Tahreer, Zawiet El-Bahar drains of samples. Some studies detected a small concentration of viruses in their investigation **(Hu, 2012 and Safaa and Azzam, 2020)**.

A high concentration of Proteobacterial and virus's classes to clostridia, actinomycetes, Bacteriodes, Deinococcus and Firmicutes were reported in the sewage drains when comparing to another drains **Figure 3**. Several species which belonging to strain Pseudomonades causing opportunistic disease agents in some humans, plants and animals.

Firmicutes strains are deathly to humans and plants and also Closteridia species are pathogens which causing some effects to humans, animals **(Godoy et al., 2003)**. Bifdobacterium considered as Gram-positive and branching. anaerobic bacteria are wide speed in their inhabitants as gastrointestinal tract and mouth of mammals such as humans **(Schell et al., 2002; Mayo and Sinderen 2010)**. To understand the complete microbiota of all parts in the drain outlets the species -level classification was applied. many species as Staphylococcus, Salmonella, Shigella, Pseudomonas were found to be in an enhanced concentration in domestic drains outlets along the Rosetta branch as compared with the industrial and agricultural drains samples, in **Figure 4**.

In some publishing literature the same concentration of species was discussed **(Ye and Zhang 2013)**. aFish technology were conducting by some researchers to input Actinobacteria and Betaproteobacteria are primary bacterial in domestic drains and surface water **(Muszynski et al., 2015)**. About 38% of the strains were existing in the river Nile. Several Publishing research decide the presence of Betaproteobacteria and Gammaproteobacteria in samples of activate sludge **(Kwon et al., 2010)**.

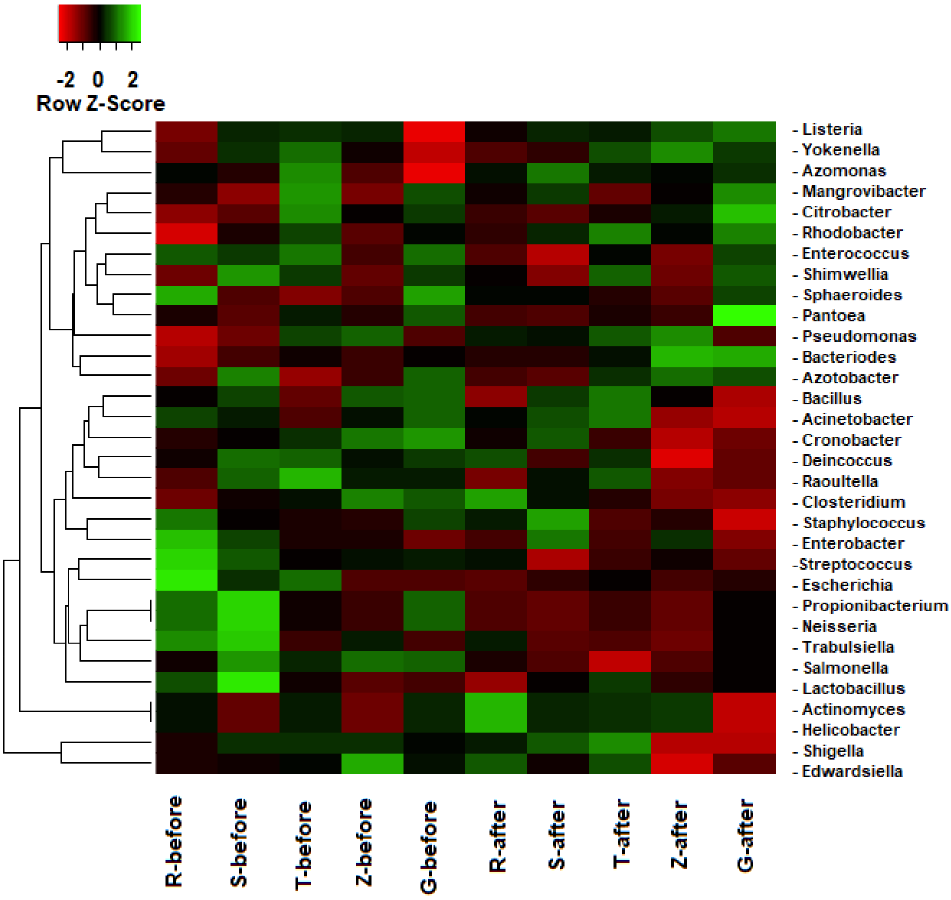

Figure 4. Heat map and genetic distance for novel bacterial species discovered in water samples.

Many of bacterial structure in drains outlets depending locality, source of pollution, water effluent, and long of the drains effects the evaluation and removing capacity of microbial pathogens. Enormous investigation under process dependence cofactor differences of the bacterial community was behaved **(Helbling et al., 2015; Johnson et al., 2015)**. bacterial species range existing in mixed drains and agricultural drains. critical role in the nanoparticle size.Method treatment for water reporting of many researchers **(Hu et al., 2012)**.

Dominant steps reporting in the case study belonge to the phylum Proteobacteria and no effect in drains outlets. Case study proving interesting discovery for a comprehensive pathogenic detection and removal in various stages along the drains. The domestic drains both of industrial and agricultural drains. samples had like composition; a less concentration of Actinobacteria species was reported.

Variety and causing diseases of domestic drain were reported to be higher in comparison with other types of samples **(Figure 5)**. Result revealed that the self- purification and precipitation steps is not complete entirely, microbiota exists in the drains outlets. Transferring in the surface water. Observing in agreement with beforehand publishing. Concentration of pathogenic species as Actinobacter and Salmonella species effects **(Bibby et al. 2010; Ye and Zhang 2013)**. Using Illumina sequencing technology in casing study proved a potential method to measured pathogenic microbes.

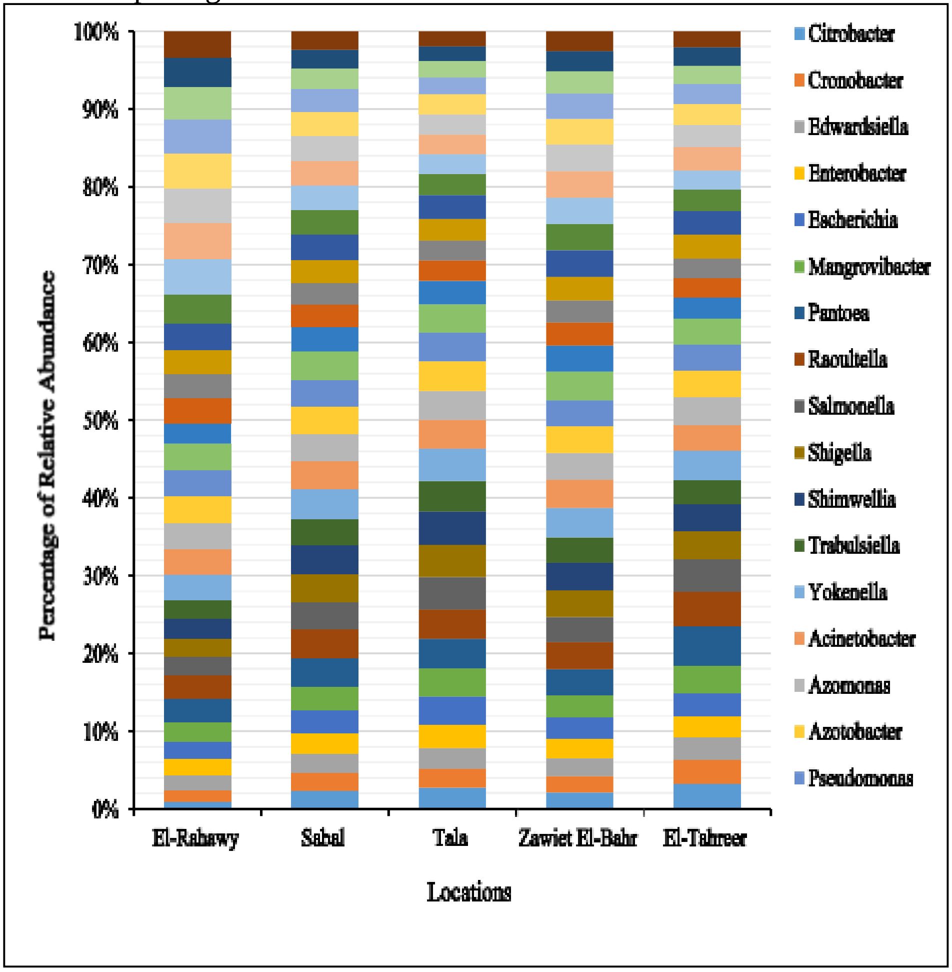

Figure 5. Abundance of microbial community in drainage water before treatment by CuO nanoparticles.

CONCLUSIONS

The synthesized CuO NPs by a rapid precipitation method in absence and presence of (TOAB). Using a stabilizer to control nanoparticles size. CuO NPs was tested to examine their antibacterial activity using (TC), (FC) and (E. faecalis) bacteria in wastewater samples. several parameters were evaluated to having the optimum wastewater disinfection factors, as nanoparticles concentration, and temperature, contact period, pH value, shaking conditions of wastewater. High antibacterial activity appeared from CuO-TOAB stabilized NPs more than without TOAB surfactant. the antibacterial activity of both CuO NPs has slightly effect by the Contact period, where high activity of CuO happened in wastewater samples when were treated at 25 ºC and 35 ºC, respectively. Noticed that, decreasing the pH values The antibacterial activity of CuO increasing.When shaking conditions increase from 70 and 90% the activity of CuO NPs affect. Positive bacteria E. fecals more effects than gram negative (TC and FC) by studied conditions. Using a novel technique using for wastewater. Including Ilumina sequencing technology is a potential and robust way to investigate microbial structure. In future we could remove and eliminate the pathogens from the wastewater treatment process. Illumina sequencing technology detect disease-causing agents in wastewater.

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التكنولوجيا الجديدة والسريعة في ازالة وتشريح المجتمع الميكروبي في البيئة المائية

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الهدف من البحث : يقوم بإ زالة البكتيريا مع التقليل من نسبة المواد العالقة والعكارة من الاوساط المائيه الملوثة وايضا الاكسجين الذائب والكميائي باستخدام مادة نانو اكسيد النحاس.

نتائج البحث: يمثل نتائج التجارب المعملية ومناقشتها وتم فى هذة الدراسة اختيار تأثير العوامل المختلفة المؤثرة على كفاءة إزالة البكتريا من البيئات المائية الملوثة وهي تشمل مصارف (الرهاوي– سبل – تلا وكفر الزيات) وتلك العوامل تشمل دراسة:

1– تأثير عامل الوقت وفى وجود مادة نانو اكسيد النحاس الممتزة للوصول الى الوقت اللازم لحدوث اعلي ازالة للبكتريا وتم دراستها فى البداية استخدام الوقت من 1ساعة الي 24 ساعة

في وجود نانو اكسيد النحاس تكون معدل الازالة للبكتريا تزداد مع زيادة الوقت فتكون اعلى قيمة تصل الي 95 فى المائه عند 24 ساعة .

2- تأثير درجة الحرارة من 10-35 درجة سيلزيوس فى إزالة البكتريا باستخدام مادة نانو اكسيد النحاس وكان احسن درجة هي 35 لمعدل الازالة.

3- تأثير تركيز نانو اكسيد النحاس على إزالة انواع البكتريا محل الدراسة حيث وجد ان احسن تركيز هو 1 جرام في اللتر

4-تاثير درجة الحموضة على معدل الازالة للبكتريا محل الدرسة كانت اعلى معدل للازالة في وجود **Cuo -TOAB**

5- تأثير معدل الرج على معدل الازالة للبكتريا بزيادة معدل الرج تزداد معدل الازالة مع زيادة الرج ويكون معدل النشاط البكتيرى **10ml/min** ومن انواع البكتريا الموجودة (بكتريا القولون الكلية:بكتريا المكورة المعوية البرازية: بكتريا القولون البرازية: متقلبات الفا:متقلبات بيتا سلبية الجرام وجاما ايضا) وكان اسم التنقية المستخدمة في تحليل النتائج هي **Novel Swage Water Bacterial Disinfection technique** و استخدام ايضا تقنية **Illumina Squencing Technology** وهو يحدد الامراض المسببة في البيئة المائية الملوثة وكذاك كمية الحامض النووي الموجود في العينة .