Bioremediation Ability of the Local Isolate *Enterobacter cloacae* from Disposal Site

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Abstract—**Illegal dumping is a serious problem that needs to be addressed immediately to preserve human health and the environment as if the pollution that arises from it reaches the groundwater, complications of the remediation processes will increase. To decontaminate the organic and inorganic components, bioremediation seems to be the most environmentally friendly and economically viable technique without further treatment as reported by many studies. In this investigation, samples were taken from the soil of the main dumping area in Koysinjaq in Kurdistan Region of Iraq to determine the most potent bacteria to remediate the existed pollutants. The existence of non-essential minerals and organic compounds in the soil sample was detected using X-ray fluorescence device, and ethane and 1,2-dichloroethane solvents separating technique, respectively. Then, from the same samples, three different naturally occurring bacteria were isolated and cultured under optimized conditions then stimulated for a good result. Finally, spectrophotometer was set at wavelength of 600 nm and used to detect the heaviest growth of bacteria after incubating the cultured bacteria on a mineral salt broth medium with the extracted pollutants at pH 7.0 overnight at 32°C. Based on the highest absorbance, the most effective type of bacteria (*Enterobacter cloacae*) was chosen among others to remediate the organic components in which approximately 90% of them are plastics, medical waste, municipal waste, electrical items, and hydrocarbons, and some heavy metals, for instance aluminum and lead, which were found in the soil.**

Index Terms—**Bioremediation, Enterobacter cloacae, Organic matter, Dumping area.**

I. Introduction

Dumping areas cause some significant negative impacts on the environment, therefore, managing and remediating it should be any country's priority (Chandler, 2017). Hence, bioremediation is chosen to be one of the most effective, environmentally friendly, and less expensive treatment options to purify water and soil from organic matters and some metals (Tyagi and Kumar, 2021) (Ganguly, 2018; Jardine and Taylor, 1995; Shen and Wang, 1994; Tyagi and Kumar, 2021), whereas landfills are considered to be necessary to store almost about 55% the partially degradable and undegradable wastes, for example, plastic wrappers, take-out containers, Band-Aids, newspapers and water bottles, and glasses (Chandler, 2017; CHEJ, 2016).

Recently, microbial systems are frequently been used for both soil and water remediation, which can reduce the mass, concentration, and toxicity of the contaminants (Head, 1998) with the consideration of some crucial environmental conditions, for example, the presence of carbon source, pH, temperature, dissolved oxygen, oxidation-reduction potential, and presence of other oxyanions and metal cations (Chandler, 2017; Chen and Hao, 1998; Xia and Boufadel, 2010). Besides, the final product from this process is mainly CO_2 and water (Boopathy, 2000; Thomas, Hughes and Daly, 2006). Thus, there is a possibility of complete mineralization of organic pollutants to inorganic materials (Head, 1998). For an organism to survive, electron donors (BTEX, PAHs, phenols, and cresol) and electron acceptors (oxygen, nitrate, sulfate, manganese, iron, and CO_2) are required (Bewley and Webb, 2001; Kracke, Vassilev and Krömer, 2015). Frische in 2003 also declared that introducing organisms to the soil can enhance bioremediation, as a certain organism can only degrade specific contaminants. The most common applications of soil bioremediation are stimulating the naturally occurring microorganisms to pollutant by enhancing nutrients concentration, oxygen supply, and moisture content (Frische, 2003).

The aim of this study is to remediate the existed pollutants, such as plastics, medical waste, municipal waste, electrical items, and hydrocarbons and some toxic heavy metals in the main damping area in Koya city (Fig. 1) through bioremediation using *Enterobacter cloacae*. In spite of the fact that, the performance of this system with the optimum condition for the bacteria is necessary to be monitored and observed as the rate of the biodegradation of each contamination is different so that the remediation time could be reduced (Margesin, Zimmerbauer and Schinner, 2000). In addition, the microbial activity could be increased due to the association of some other techniques, namely, the addition of biosurfactants, biostimulation, and bioaugmentation, as the mobility and the access to nutrients of the microbes in the soil are limited (de Rizzo, et al., 2010). The eco-toxicological tests can be used to evaluate the environmental toxicity of

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the treated soil using plant or survival earthworm (Hund and Traunspurger, 1994).

II. Materials and Methods

A. Soil Sample Collection

Based on International Atomic Energy Agency in 2004 of soil sampling for environmental contaminants, three samples (100 g) were collected from the depth of 25 cm of the contaminated site in Koya city, in which the upper 5 cm of the soil surface was discarded, then the rest of the sample was kept in a sterilized plastic bag for about an hour.

B. Contamination Found

According to the site investigation, which was done through sampling processes, from digging pits across the site in a regular grid for soil; most of the contaminants exist in the soil caused by continuous dumping, therefore, it's considered to be the main source of the contamination (Fig. 1). These two methods were used to detect the pollutants in the site:

1. X-ray fluorescence (XRF Rigaku 50 KV in Koya University) was used to detect the existence of metals in the soil samples (Fig. 2). This device provides the most accurate compositional analysis that based on the emission of a characteristic X-rays by a specific chemical components (Pinto, 2018).

Fig. 1: The main disposal site that located in the city of Koya (Koysinjaq) in Kurdistan region of Iraq.

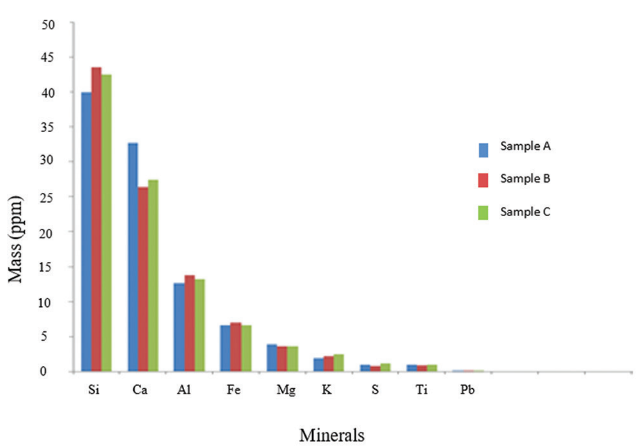

Fig. 2: X-ray fluorescence result analyzes for minerals existed in collected samples from the disposal site in Koya city.

2. Following the methodology of Osman and Saim in 2013 to extract the organic compounds from the soil, the samples were air-dried at room temperature to evaporate water to less than 5% and sieved through a 600 m pore sieve. Then, each sample was split into two equal weight (50 g). Next, each part was mixed with 250 ml of ethane and 1,2-dichloroethane solvents (Ke, et al., 2014). These mixtures were stirred and stored for 48 h in a screw cap glass specimen jar. To extract the organic compounds, solvent was completely evaporated by stirring the soil sample inside the chamber hood for nearly 6 h.

C. Bacterial Isolation

Bacterial isolates were developed on mineral salt broth medium (Zajic and Supplisson, 1972) that was prepared by dissolving 1.8 g K2HPO4, 4.0 g NH4 CI, 0.2 g MgSO4.7H2O, 0.1 g NaCl, and 0.01 g FeSO4.7H2O in 1 L of distilled water.

Using the methods mentioned by Zhang, Pab and Li in 2010, briefly, 1 g of each specimen was added to 10 ml of sterile distilled water and then vortexed carefully to homogenize the soil suspension. Following this, a 1/10 serial dilution of this suspension was prepared from the above culture by taking 1 ml and adding it to 9 ml of sterile media to 10^{-4}, which was used to inoculate mineral salt broth medium agar plates.

From each dilution, 0.1 ml were spread on nutrient agar plates at pH 7.0, to check the growth of bacteria, incubation was conducted in Petri plates at 28 ± 1°C for 72 h. Then the types of the bacteria were classified based on the colony morphology, such as color, shape, and size.

D. Bioremediation Assay

Three different bacterial colonies were primarily selected for further studies, as they grew better compared to the rest of the colonies (Fig. 3). Next, the three different colonies were taken and cultured on slant nutrient agar separately to be used as reference (Fig. 4). To observe which bacterial strain manifests the highest tolerance level, the three isolates were taken from each colony which was resuspended in 10 mL saline to give the inoculums suspension at absorbance of 0.5 and wavelength at 600 nm and then cultured in a mineral salt broth medium

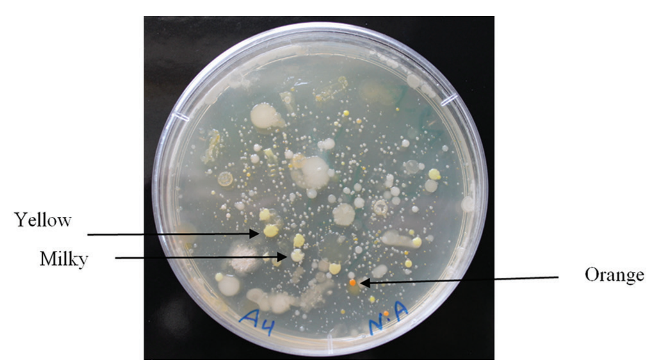

Fig. 3: Bacterial colonies morphology which were extracted from the contaminated soil of Koya city in Kurdistan region of Iraq.

at pH 7.0 with adding the same concentration of the organic matter which was found in the site and incubated overnight at 32°C. The results were recorded after 48 h by spectrophotometer on 600 nm.

E. Bacterial Identification

To characterize the selected bacteria, these techniques were used (by Medya Diagnostic Center in Erbil city).

1. Gram stain test was performed for the isolate.
2. Biomerieux Analytical Profile Index (API) 20E.

III. Results and Discussion

In this present study, the isolated bacteria were Gram-negative rods and Biomerieux API 20E showed that there was a heavy growth of *E. cloacae* (Fig. 5).

Enterobacter is a Gram-negative, facultatively anaerobic, rod-shaped, non-spore-forming bacteria, which can grow best at the temperature of 37°C (Mezzatesta, Gona and Stefani, 2012). Since this microorganism is oxidase negative but catalase positive and facultative anaerobic, therefore, for making ATP, it can switch between aerobic respirations to fermentation in the absence of oxygen (Ren, et al., 2010).

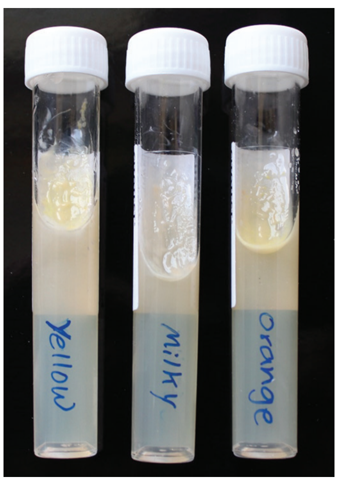

Fig. 4: Three different types of bacteria colonies cultured on slant nutrient agar, which were extracted from the contaminated site in Koya city.

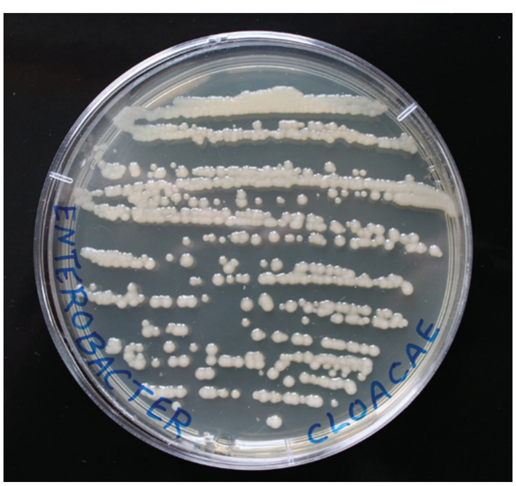

Fig. 5: *Enterobacter cloacae* local isolate from Koya city dumping area on nutrient agar.

In our study, we have isolated one potent organic matter and heavy metal-resistant bacteria in polluted soil from one of the main dumping areas at Koya city to be used in the process to treat the existed pollutants. That bacterium was chosen as it has the heaviest growth in a mineral salt broth medium with the added pollutants from the soil at pH 7.0 after incubating overnight at 32°C. The analysis identified the bacterial isolate as *E. cloacae*.

Since there is a great possibility of leaking the organic compounds in which nearly 90% of them come from plastics, medical waste, municipal waste, electrical items, and hydrocarbons that are found in the site can reach the water bodies eventually, besides, heavy metal toxicity and municipal wastes in the environment due to global industrialization are increasing significantly, for instance, lead (Pb) and cadmium are existed in the site. These two heavy metals are biologically nonessential and non-degradable and tend to accumulate in exposed organisms which are the two most abundant toxic heavy metals in the environment that are reported in the Priority List of Hazardous Substances on the 2nd and 7th places, respectively (Kirillova, et al., 2017). Therefore, without treating and processing the waste, there is a major possibility of releasing a colossal amount of toxic heavy metals into water bodies (Batta, et al., 2013; Hookoom and Puchooa, 2013; Kafilzadeh, et al., 2012). Mechanical treatment of the pollutant can be costly and time consuming, hence, bioremediation is a great alternative (Head, 1998). Thus, developing and applying an efficient and promising technology, such as bioremediation to remediate pollution, are urgent (Chandler, 2017; Olga, et al., 2008).

IV. Conclusion

As dumping areas which caused by the low rate of waste collection and dispersing the garbage in the streets were never been a solution to the clean environment, therefore, some suitable techniques should be applied to deal with this problem that most developing countries might face recently due to the improvement in living standards of people and the proliferation of suburbs. Hence, biodegradation was found to be one of the most effective techniques in Koysinjaq in Kurdistan region of Iraq to destroy hazardous contaminants or transform them into less harmful forms before it was too late to deal with.

In this study, the biodegradation efficiency for the organic compounds in which about 90% of them come from plastics, medical waste, municipal waste, electrical items, and hydrocarbons (as Iraq economically depends mainly on petroleum) that were extracted from the soil samples using ethane and 1,2-dichloroethane solvents organic compounds separation technique, and some of the toxic heavy metals, such as aluminum and Pb, which were detected by XRF analysis, indicates that the proposed remediation technique using local isolated bacteria *E. cloacae* reflects the treatment costs and minimizes the health and environmental risks associated with prolonged exposure to those residues. However, pollutant bioavailability and biodegradation are critical to the success of this technique. Along with that, the operational conditions (pH, temperature, aeration, and moisture content) are essential to the possibility of the effectiveness of this process. Hence, introducing additives to the soil (water, microorganisms, surfactants, nutrients, pH corrections, and cosubstrates) are important as it can enhance bioremediation.

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