

Effect of *Eruca Sativa* extract, some heavy metals (Pb, Ni, Cr, and Al), on some soil bacterial isolates

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Abstract

This study was performed in laboratories of Al-Mustansiriya University / College of Science - Department of Life Sciences during the period from 2021 / 3 / 3 till 2022 / 3 / 28 to study the effect of some heavy metals on some microscopic soil organisms. The study included isolation and identification of bacteria from twenty-four samples of elements contaminated soil, the bacterial identification contained macroscopic and microscopic examinations, as well as biochemical tests, the identification then confirmed by VITEK2 system. The ability of biofilm formation of these identified bacteria was performed by using Congo red agar (CRA). The antimicrobial effects of four selected heavy metals (Al, Cr, Ni, and Pb) on the isolated bacterial isolates. Results of bacterial isolation showed that thirteen bacterial isolates were identified (three isolates of *Pseudomonas aeruginosa*, two isolates of *Sphingomonas paucimobilis*, and *Stenotrophomonas maltophilia*, and one isolate of each *Staphylococcus aureus*, *Staphylococcus lentis*, *Serratia ficaria*, *Demacoccus nishinomiyaensis*, *Kocuria rhizophila*, and *Kytococcus sendertarius*), with isolation percentage equal to (54.11%). Results of three concentrations of plant extract (300, 150, 75 mg/ml) showed there wasn't any antimicrobial effects of that extract against any of the isolated bacteria, with no inhibition zone of all the prepared concentrations.

keyword: heavy metals, isolation and identification of bacteria, antimicrobia

1. Introduction

Heavy metals released into the environment pose a risk to organisms exposed to high levels of these pollutants. Minerals are essential for the biological functions of plants, but at elevated levels they interfere with metabolic reactions in living systems (Tekere, 2020). Minerals are naturally present in rocks and loosened soils and are released by weathering. They do not decompose, but rather accumulate constantly in the soil, which in turn is transmitted to plants (Murat and Parvaiz, 2016).

In the last century, a number of studies began dealing with the effect of heavy elements on microorganisms in the soil. The measures that can be inferred from the effect of soil contaminated with heavy elements on the activities and activities of microorganisms in the soil are often indirect measures. It also has a significant role in reducing soil pollution (Romaniuk et al., 2018).

Most microorganisms have biofilm as a defense against the stresses they are exposed to. These membranes are the result of the union of microorganisms surrounded by an extracellular matrix of polysaccharides, secretions and residues, although they can also be made by a single bacterial species (Martin et al., 2020).

2. Methods

Isolation of bacteria.

Using a sterile loop, soil samples were collected

from soil pots were treated with three concentrations (1, 0.1, 0.01) mg/L for all the four selected heavy metals (Al, Cr, Ni, and Pb). These soil samples were decimal diluted with sterilized distilled water and then 0.1 ml of three dilutions (10⁻⁵, 10⁻⁶, and 10⁻⁷) were transferred and cultivated immediately and separately on petri dishes of nutrient agar as well as MacConky medium as a differential media, then the dishes were incubated at 37° for 18 h with daily observing.

Identification of bacteria.

Bacterial isolates were identifying based on their morphological, microscopical, cultural, and biochemical characteristics (such as catalase, oxidase, indol, Methyl red, Voges – Proskauer, and citrate tests) according to a taxonomic basis (Tonso et al., 1995). As well as Vitek 2 System was used to conform the bacterial identification.

Determine the antibiotic resistance of the isolated bacteria.

According to Banoon et al., bacterial susceptibilities to antibiotics were tested (2020). Separately, tenth ml suspensions of active bacterial isolates were planted on Muller-Hinton agar plates. The antibiotic discs were placed on the surface of the media after the bacterial suspensions were equal to McFarland solution, and the plates were left to dry for 10 minutes at room temperature before being incubated at 37°C. After 18-24 hours of incubation, inhibition zones were measured (diameter mm) and compared to the reference table of the National

Committee for Clinical Laboratory Standards (NCCLS) (Sofy et al., 2020).

Determine the biofilm formation ability of the isolated bacteria.

The detection of biofilm producer bacterial isolates were done according to (Harika et al., 2020) by using congo red method, which is a simple qualitative method for detecting biofilm. This approach calls for the use of specialized media, namely Brain Heart Infusion (BHI) agar with Sucrose and Congo red in the following proportions: 52 gram of Brain heart infusion agar; 36 gram of sucrose; 10 gram of agar; as well as 0.8 gram of Congo red, all these contents were dissolved in one liter of distilled water. After collect all the contents, Congo red was autoclaved after being made as a concentrated solution. When agar is chilled to 55°C and placed into petridishes, it is added to the medium. Plates were infected and incubated at 37°C for 24–48 hours. Black colonies with dry crystalline appearance were deemed positive for biofilm forming organisms, whereas pink colonies indicated weak or non-biofilm producing species. The experiment was carried out in triplicate (Andhale, 2016).

Determine the antibacterial plant extracts against the bacterial isolates.

The cold aqueous extract of the plants under study was prepared according to the method (AL-Joboory and AL-Rawi, 1994), with a weight of 40 gm of dry powder for each plant, and 160 ml of cold distilled water was added to it. The aqueous samples were left for 24 hours in the refrigerator, then on Shake device for 24 hours as well, filter the extract by several layers of gauze to get rid of large parts of the plant and then using Whatman No.1 filter paper then concentrate the extract through water evaporation using a rotary evaporator at a temperature not exceeding 40 °C until a concentrated extract is obtained, after which it is kept in a sterile and opaque vial in the refrigerator until use. Drills were made in the middle of the dishes containing Muller Hinton's medium after culturing the bacteria in this dish and incubated for 24 hours. The prepared extracts of watercress containing heavy metals were added to the inside of the pits and the dishes were incubated for 24 hours at 37°C, then the results were read by measuring the diameter of the inhibition area using a standard ruler.

3. Results and Discussion

Results of bacterial isolation

The results of isolation and identification showed numbers of bacterial species in soils contaminated with heavy metals and their nano-oxides as shown in table 1. The results indicated that the appearance of these bacteria indicates the resistance of bacteria to heavy metals and/or and their nano-oxides in

contaminated soils. This agrees with the results of Boros et al. (2021) in his study of soil contaminated with heavy metals and cultivated in *Brascia napus* if the aim of the study was the microbial diversity in the contaminated soil, as the impact of metal pollution on the microbiological disruption in the soil and increased the values of indicators of colony development and pollution led to changes in diversity Microbial. The results of the effect of heavy metals on bacteria isolated from soils contaminated with heavy metals indicated that the use of *Eruca sativa* extract grown in soil contaminated with heavy metals on isolated bacteria did not give any result. Studies have shown that the ability of plant extracts to act as antibacterial agents is due to the fact that plant extracts contain active elements such as tannins, flavonoids and saponins (Chetwani et al., 2017). Although the aqueous extract of *Eruca sativa* contained these compounds did not give an inhibition result. . In general, the lack of effect of aqueous extracts may be due to a decrease in the concentration of active chemical groups such as tannins, phenols and saponins, or the absence of other active groups of carbohydrates, flavonoids, alkaloids and (combined) coumarins. (Chetwani et al., 2017).

Contaminated source	Bacterial isolates
Ni	<i>Burkholderia cepaci</i> group
Al	<i>Sphingomonas paucimobilis</i>
Cr, pb	<i>Stenotrophomonas maltophilia</i>
Cr	<i>Dermacoccus nishinomiyaensis</i>
Ni	<i>Kytococcus sendertarius</i>
Pb	<i>Kocuria rhizophila</i>
Pb	<i>Staphylococcus aureus</i>
Pb	<i>Staphylococcus lentis</i>
Al	<i>Pseudomonas aeruginosa</i>
Pb	<i>Serratia ficaria</i>

Results of antibiotic sensitivity test

A sensitivity test of the selected antibiotic was performed against the isolated bacterial isolates to determine the resistance of bacterial isolates to the most effective antibiotic families as shown in Table 2. The results showed that the antibiotic cefotaxime did not inhibit any bacterial growth, which indicates the resistance of the isolated bacteria to this antibiotic, which explains that the isolated bacteria possess self-defense mechanisms for antibiotic resistance, such as decreased membrane permeability, the presence of multiple drug flow systems, and their great ability to acquire resistance mechanisms, As in *Pseudomonas* it is a multi-metabolic bacterium, which makes it highly resistant to antibiotics, (Youenou et al., 2014). The results in Figure 4-3 showed that both the antibiotic levofloxacin and ciprofloxacin had inhibited bacterial growth. It was proved that *Pseudomonas aeruginosa* isolated from soil contaminated with lead element is completely resistant to chloramphenicol and amoxicillin, in

addition to being resistant against tetracycline, it can resist *P. aeruginosa* isolates, ciprofloxacin, kanamycin, trimethoprim, and gentamycin. This is consistent with (Muhsin,2022).In line with Martins, et al., (2014) in a study conducted in Brazil to test the resistance of *Pseudomonas aeruginosa* to isolate from soil, it showed high resistance to azithromycin and tetracycline due to its ability to automatically acquire resistance by plasmids, as well as resistance to ciprofloxacin, gentamicin, especially third generation cephalosporins by 30% and was sensitive to for tetracycline (kaszab, et al., 2011).



Figure 1: susceptibility of *P. aeruginosa* against antibiotics



Figure 2: susceptibility of *S. ficaria* against antibiotics

The results in Figure 4-4 showed that *Serratia ficaria* isolated from soil contaminated with lead and nickel was sensitive to ciprofloxacin and kanamycin, and the sensitivity was 100%, and the sensitivity to chloramphenicol, tetracycline, amoxicillin, and ampicillin, 50%. This agrees with (Muhsin,2022).

The results *Burkholderia cepacia* group isolated from contaminated soils resistant to pvoxfloxacin, chloramphenicol, tetracycline, amoxicillin, kanamycin, ampicillin, and gentamicin. It has high levels of antibiotic resistance to cephalosporins, due to it having a Pom-reflex mechanism. Researchers have found a genetic affinity for *Bacillus*, a genera that possesses a broad spectrum

of resistance to many antibiotics, (Johnning et al., 2013).

Table 2: Antibiotics effects against the isolated bacteria

Bacterial isolates	Am p	IM P	Ci p	CK	Le v	Ge n	CA Z	A X	T E	T R	A Z M
<i>S. ficaria</i>	S	S	S	S	S	S	S	S	S	S	S
<i>S. auras</i>	R	S	S	S	S	S	S	S	S	S	S
<i>S. maltophilia</i>	R	I	S	S	S	S	S	S	S	S	S
<i>B.cepacia</i> group	R	S	S	S	S	S	S	S	S	S	S
<i>S.paucimobilis</i>	S	I	S	S	S	S	S	S	S	S	S
<i>S. maltophilia</i>	R	I	S	S	S	S	S	S	S	I	I
<i>K. rhizophila</i>	S	S	S	I	S	S	S	I	S	30	S
<i>P. aeruginosa</i>	S	R	S	S	S	S	S	R	S	S	S
<i>K.sendertarius</i>	S	S	S	S	S	S	S	I	S	I	S
<i>S. lents</i>	I	S	S	I	S	S	S	I	I	I	8

Where: AX Amoxicillin , TE Trimethoprim , CiP Ciprofloxacin, Gen Gentamycin ,TR Tetracycline, Imp Imipenem, LEV Levofloxacin , CAZ Cefotaxim , AMP Ampicillin , AZM Azithromycin , C Chloramphenicol, K Kanamycin

The results indicated that the majority of bacteria isolated from soils contaminated with heavy metals are Gram-negative as in *Kytococcus sendertarius*/*Dermacoccus nishinomiyaensis*, *Kocuria rhizophila*, Unidentified organism and *Staphylococcus lents*, where these bacteria showed resistance against the antibiotics used in the study .Studies indicate the high ability of antibiotic-negative bacteria due to the activity of outer membrane proteins (Pagu et al., 2020); The outer membrane contains open channels filled with water that allow hydrophilic drugs with a molecular weight less than 600 kDa to passively flow. Purines are the proteins that make up these pores (Fernandez and Hancock, 2012).

The results also revealed gram-positive bacteria, *Sphingomonas paucimobilis*, and *Stenotrophomonas maltophilia* has lower resistance. A study conducted by Bengtsson-Palme scientists in 2018 showed that the origin of bacterial resistance to antibiotics is that these bacteria have internal adaptive resistance mechanisms that stimulate exposure to antibiotics and the transfer of resistance genes from one strain to another.

Results of bacteria biofilm formation

The results of bacterial biofilms formation of bacteria isolated using TCP showed that out of 34 bacterial isolates, 10 bacterial isolates were recorded as biofilm-forming bacteria as in Figure 3. Antibiotics are often less effective against biofilm-forming bacteria than other bacteria. low penetration of antibiotics. The binding of antibiotics to biofilm materials, the high bacterial concentration, and the low growth of bacteria within the biofilm may have contributed to the increase in antimicrobial resistance. This result may help explain the pattern of antimicrobial susceptibility to isolates that showed a high level of resistance (Sannathimmappa, et al., 2021).



Figure 3: ability of *Staphylococcus aureus* to produce biofilm.

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