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Industrial Wastewater Bacteria, Resistant to lead, Selenium and Antibiotics

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Abstract: Environmental pollution with heavy metals is a serious problem facing most countries. This problem is correlated always with misuse of different industrial products and wrong treatment of industrial waste. Presence and prevalence of heavy metal resistant bacteria consider an indicator of contamination with heavy metals.

This work was performed to support the evidence of presence of heavy metal resistant bacteria in polluted environment, and to examine the changes occur in bacterial morphology under the stress of heavy metal ions occurrence. Water samples were collected from industrial waste, Riyadh Saudi Arabia. Bacterial isolates were identified using VITEK System version: 04.01. The antibiotic resistance of bacterial isolates were also examined using the standardized single disk method. The bacterial morphology was examined using scanning electron microscope to clearify whether the bacterial cell characteristics are changed. The MICs (Minimal Inhibition Concentration) were determined in triplicate by growing the bacterial strains on NA plates containing different concentrations of lead and Selenium as well. Three bacterial isolates were identified as *Escherichia coli, Klebsiella pneumonia, Bacillus* sp. The strain of *Klebsiella pneumoniae* was resistant to 15% of used antibiotics, *Bacillus* sp. isolate was resistant to 30%, while *Escherichia coli*, was resistant to 40% of tested antibiotics. The higher lead MIC value was detected with *Klebsiella pneumonia* (1100 ppm). The cell sizes of the isolated bacteria were slightly changed according to scanning electron microscopy.

Growing of *E. coil* on nutrient agar amended with selenium oxide resulted in selenium particles size around 270 nm.

Keywords: Lead, Selenium, Heavy metal, MIC. Nanoparticles.

Introduction

Heavy metals are those include individual metals or metal compounds, for example, arsenic, barium cadmium, chromium, lead, mercury, selenium, and silver which may accumulate in water and soil causing pollution problems. These all are naturally occurring substances which are often present in the environment at low concentration levels, but in large amounts, they can be dangerous to all environmental components.

Environment contamination with heavy metals consider a widespread problem resulting from industrial activities causing permanent toxic effects to human and environment because of miss industrial wastewaters treatment (Rehman *et al*., 2008).

Lead is one of the much important and dangerous heavy metal, which accumulates in the environment because of human activities, such as fuel burning, mining, and manufacturing (Martin, 2009), it may pose a health hazard for children and can lead to mental retardation.

Lead accumulates in skeleton and mobilizes from mother bones during pregnancy and lactation, hence, the lifetime exposure to lead of woman before pregnancy is important.

Selenium is also trace mineral, that occurs in most rocks and soils and accumulates to dangerous levels consequences of release in the environment as it enter many industries, such as electronics industry, glass industry, plastics, paints, enamels, inks, rubber, in the preparation of pharmaceuticals. It also used as a nutritional feed additive for poultry and livestock, in pesticide formulations, it used as radioactive selenium in diagnostic medicine as well (Martin, 2009).

Although selenium is necessary for cellular function in most animals, but in trace amounts, it is toxic in large amount. For humans, selenium is an essential trace nutrient that plays an important role in thyroid gland function.

Not like the other chemicals, heavy metals are harder

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to be chemically or biologically degraded, since they are in the simplest chemical form, and affect the living organisms in this form. Three methods are usually employed to remediate heavy metals contamination in soil and water; (1) Physical removal of these contaminants, (2) stabilization-amendment of these metals in the soil on site, and (3) bioremediation (Usman & Mohamed, 2009).

However, the application of first two methods is restricted due to technological or economical constrains, that is why, bioremediation provide an alternative way for detoxification of these pollutants in the environment (Plion-Smits, 2005), that's why different microbes have been proposed to be efficient and economical alternative in removal of heavy metals from water (Waisberg *et al*., 2003).

The first step in devising a bioremediation strategy is to identify candidate bacterial strains capable of modifying the contaminant, so the objective of this study is to determine and identify the lead and selenium resistant bacteria, determine MIC of these metals, and using electron microscope studies, to exploit these isolates for clean-up of industrial wastewater and sewage.

Materials and method

I. Sample collection

Water samples were collected in clean and sterile bottles from studying area (industrial waste from Riyadh, Saudia Arabia and pH, temperature were measured at the site of collection. For bacterial community analysis, samples were transferred in ice box and stored at 4°C.

II. Bacterial cultivation

Serial dilutions of water samples were placed on the top of Nutrient Agar plates, containing 500 ppm of lead acetate and selenium oxide. Plates were incubated at 37oC for 48 h, and the growing colonies were picked and purified on NA plates.

III. Bacterial Identification.

Bacterial isolates were identified using VITEK System version: 04.01.

IV. Antibiotics susceptibility

The standardized single disk method (Bauer *et al*., 1966) was used for measuring the antibiotic resistance of bacterial isolates. Twenty antibiotics were used in this test: Ampicillin, Amoxicillin/Clavulanic acid, Piperacillin, Piperacillin/Tazobactam, Cefalotin, Cefuroxime, Cefuroxime/Axetil, cefoxitin, Cefpodoxime, Cefotaxime, Ceftazidime, Cefepime, Meropenem, Amikacin, Gentamycin, Tobramycin, Ciprofloxacin, Norfloxacin, Nitrofurantoin, Trimethoprim/Sulfamethoxazole.

V. MIC determination (Ghosh *et al*., 1997).

The MICs (Minimal Inhibition Concentration) were determined in triplicate by growing the bacterial strains on NA plates containing different concentrations of lead and selenium (500 ppm, 600ppm, 700ppm, 800ppm, 900ppm, 1000 ppm, 1100ppm, 1200ppm, 1300ppm, 1400ppm, 1500 ppm).

VI. Scanning electron microscope.

The bacterial specimens were mounted on aluminum stubs with conducting silver paint, coated with gold-palladium (60:40), and examined in a Hitachi model SU-3500 scanning electron microscope operated at an accelerating voltage of 15 keV and a 10-mm working distance.

Results

Strain Identification

Using VITEK System, three bacterial isolates were identified as the following: Isolate no 1 which grown on 500 ppm of lead was identified as *Klebsiella pneumoniae*, isolate no. 2 was identified as *Bacillus* sp strain and grown on 500 ppm of lead, and isolate no. 3 was identified as *Escherichia coli* and grown on 500 ppm of selenium.

Antibiotics susceptibility

Klebsiella pneumoniae strain was resistant to Ampicillin, Piperacillin and Nitrofurantion, which represent 15% of used antibiotics, while was sensitive to all other antibiotics.

Bacillus sp. strain was resistant to 30% of tested antibiotics as following: Ampicillin, Amoxicillin/ Clavulanic acid, Cefuroxime, Cefuroxime/Axetil, Cefpodoxime, Nitrofurantoin while, *Escherichia coli* strain, was resistant to the following antibiotics, which represent 40% of tested antibiotics: Ampicillin, Amoxicillin/Clavulanic acid, Cefalothin, Cefuroxime, Cefuroxime/Axetil, cefoxitin, Cefpodoxime, Nitrofurantoin.

MIC of heavy metals.

The higher lead MIC value was detected in the culture of *Klebsiella pneumonia* as shown in **fig.1**.

Morphology

Figures 2, 3, 4 showsd SEM image of *Klebsiella pneumoniae*, *Bacillus* sp., *E. coli* and their corresponding cell length and width. Scanning electron microscopy of *Klebsiella pneumonia* showed that they exist as colonies of rod-shape bacteria in control

culture with an average cell length of 1.66 µm and cell width of 0.752 µm (fig. 2A). The size of *Klebsiella pneumoniae* has decreased to reach 1.2 µm length and 0.707 µm width (fig. 2B). *Bacillus* sp. showed an average of cell length of 2.5 µm and cell width of 0.632 µm in control culture (fig.3A) but changed to be 2.3 µm length x 0.881 µm width in treated culture (fig. 3B). Fig. 4 showed that the cells have enlarged in size when treated with selenium.

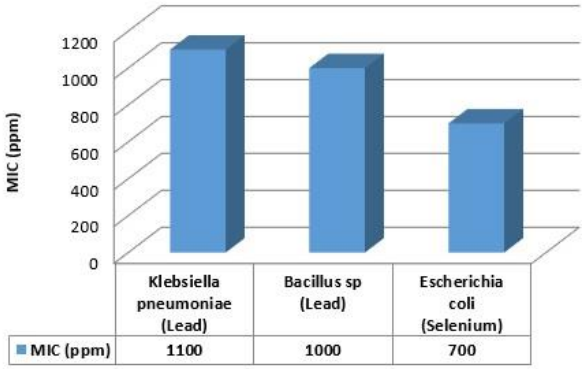

Fig. 1: MICs of Lead and Selenium for isolated bacteria.

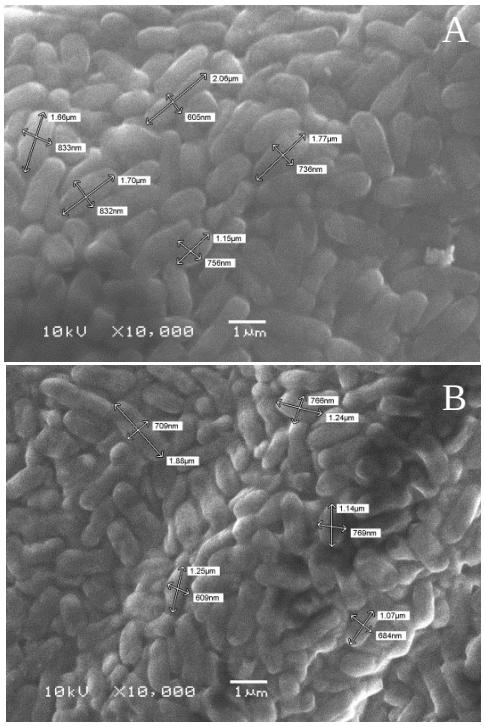

Fig. 2: Scanning electron micrographs of *Klebsiella pneumoniae* cells (A: *Klebsiella pneumoniae* grown on Lead free medium (1.66µm x 752nm, B: *Klebsiella pneumoniae* grown on 500 ppm of Lead (1.2 µm x 707 nm).

Discssion

E. coli was the much resistant bacteria towards used antibiotics, since it resisted to 40% of used antibiotics, whereas Klebsiella pneumonia was the less resistant bacteria (15%). Daniel *et al*., (2012), have studied the antimicrobial drug resistance in Escherichia coli from humans and food animals in the United States in the period of 1950–2002. They concluded that, multidrug resistance (≥3 antimicrobial drug classes) in *E. coli* increased from 7.2% during the 1950s to 63.6% during the 2000s. This may due mainly to evolution of resistance as a result of introduction and misuse of new antimicrobial against pathogenic bacteria. This resistance may transfer even to the other bacteria in the ecosystem during known genetic transfer (Lorenz & Wackernagel, 1994). Since *E. coli* is a symbiotic enteric bacterium that exposed always to different antibiotics it represents the much resistance bacteria.

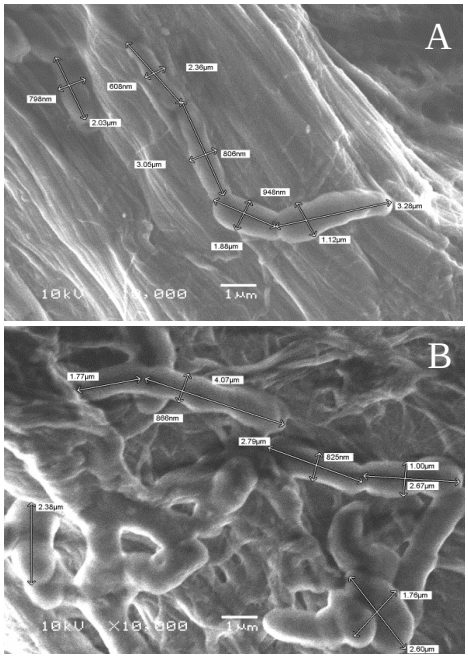

Fig. 3: Scanning electron micrographs of *Bacillus* sp. cells (A: *Bacillus* sp. Grown on Lead free medium (2.5 µm x 632 nm), B: *Bacillus* sp. Grown on 500 ppm of Lead (2.3 µm x 881 nm).

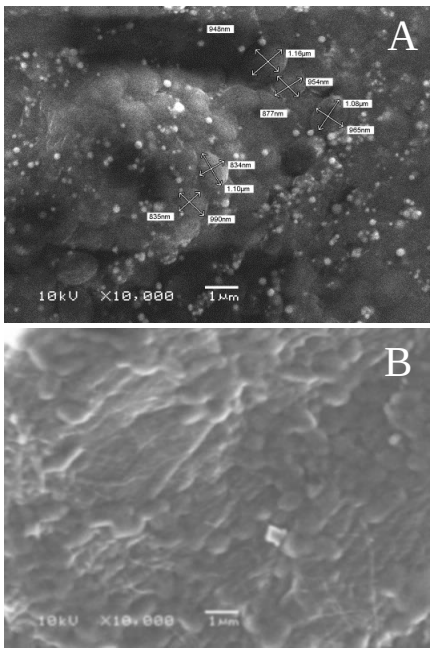

Fig. 4: Scanning electron micrographs of *Esherichia coli* strain (A: *Esherichia coli* grown on 200 ppm of Selenium, B: *Esherichia coli* grown on Selenium free medium).

According to the study of Watkinson *et al*., (2007a), 41% of E. coli isolated from wastewater were resistant to cephalothin. This also was previously reported, with ranges from 8 to 98% and 21 to 100%, respectively by Edge & Stephen (2005); Parveen *et al*., (1997).

Khalid *et al*., 2017, considered that microbial remediation is one of the most promisisng methods of bioremediation compared to physical and chemical methods. The biological techniques have the greatest advantages, since it much economic, eco-friendly, field applicable, low time and coast efficient.

The MIC concentration of lead reported in this study was very high comparing with those reported in some studies (Trevors *et al*., 1985). This may be due to the polluted environment, that the samples were collected from. The high metal values resisted by bacteria may alarm a serious environmental pollution.

Samaneh *et al*., 2020 isolated different bacterial isolates from soil and food that the MIC for Pb ranged from 50 to 3500 µg ml^{-1}. In thier experiment, the number of resistant strains decreased with an increase in the heavy metal concentration.

Bacteria can adopt to cope with environmental stresses through cell morphological changes. The decrease in cell size after incubation in heavy metal including media reported in fig. 2b and 3b, are considered as an adapting strategy.

Chakravarty & Banerjee (2008) reported that the maximum size alterations in Acidiphilium symbioticum happened when the bacterium was exposed to sub-inhibitory concentrations of Cu and Cd. They have concluded that cell shape changed only when subjected to sub-inhibitory concentrations of the metals, but no difference than normal cells was reported when grown at metal inhibitory concentrations.

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