Journal of Multidisciplinary Sciences
www.multidisciplines.com

Effect of heavy metals on the resistant strain of *Pseudomonas luteola* isolated from polluted sites in El-Kharga city at New Valley Governorate, Egypt

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Received: 27 May 2019; Accepted: 16 August 2019; Published online: 22 August 2019

Abstract. In this study, *Pseudomonas* Z4A2 has been isolated from polluted wastewater of El-Kharga city, New Valley Governorate, Egypt. The bacterial isolate was found to be highly resistant to different concentrations of zinc and nickel. The isolated bacterium was identified according to 16s rRNA as *Pseudomonas luteola* Z4A2. Its minimal inhibitory concentration (MIC) for Zn^{2+} and Ni^{2+} were 700 and 200 ppm, respectively. Growth kinetics, parameters, and the protein content of *Pseudomonas luteola* Z4A2 were determined under $Zn^{2+}$and Ni^{2+} stress. The results indicated that the protein content was decreased under a high concentration of heavy metals.

Keywords: *Heavy metals, resistance, Pseudomonas luteola, MIC, growth kinetics.*

Cite this as: Sidkey, N.M., Hassan, A.S., Hassan, S.H.A. (2019). Effect of heavy metals on the resistant strain of *Pseudomonas luteola* isolated from polluted sites in El-Kharga city at New Valley Governorate, Egypt. J. Multidiscip. Sci. 1(2), 1-7.

1. Introduction

The discharges of heavy metals from different industries have become a global concern for living organisms and environmental pollution. The toxic, harmful heavy metals such as Cd^{2+}, Zn^{2+}, Hg^{2+}, Pb^{2+}, Cr^{6+}, Cu^{2+}, Mn^{2+}, and Fe^{2+} are discharged from many industrial effluents and could be accumulated to food chains [1]. Most of these heavy metals are non-biodegradable, which may cause significant dangerous effects on human health [2]. These metals cause disturbance to the ecological status of biota by inhibition of enzymatic activity, DNA replication, translation, and proteins [3-5].

Several conventional methods have been used in wastewater treatment, such as precipitation, adsorption, membrane filtration, ionic exchange, and flotation [6]. These methods involve several disadvantages such as ineffective for low concentrations of heavy metals in large volumes of solution, expensive, cause secondary pollution, and require high energy [7,8]. The biosorption process is a cost-effective alternative technology for heavy metal removal from industrial wastewater, present in low concentrations [9]. It depends on the ability of biological materials to sequestering heavy metals from wastewater by the physicochemical mechanism of uptake [10]. Fungi, bacteria, algae, and yeast in living or dead form were studied for their biosorption efficacy to remove metal ions from aqueous solutions [11-13].

Zinc and nickel are essential elements to all organisms as a structural element in stabilizing protein folds and as a catalytic cofactor for several enzymes and healthy plants [14,15]. Trace amounts are naturally found in most vegetables, fruits, nuts and slightly more significant amounts in chocolate and wine [16]. However, like most metals, However, inadequate or excessive zinc or nickel intake can lead to toxicity [16-18].

Resistance to heavy metals was recorded in different bacteria, especially in the genus *Pseudomonas*. *Pseudomonas* is known as one of the bacterial indicators for measuring pollution [19,20]. Microbial resistance to toxic chemicals depends on biotic and abiotic factors and genetic adaptation [21,22].

The purpose of this study was to check the resistance of *the heavy metal of Pseudomonas luteola* Z4A2, determining for its minimal inhibitory concentration (MIC) the effect of selected heavy metals on its growth kinetics, growth parameters, and protein content.

2.Materials and methods

2.1. Collection of samples

Different polluted water samples were taken in sterilized bottles from El-Kharga city at New Valley Governorate (Egypt). These samples represented different water sources, namely El-Kharga (eastern area), Ein El-Sheik, the Big waste station, El-Kalaa (western area), El-Kalaa (eastern area), and the dates factory, which are exposed to domestic sewage disposal and industrial effluent.

2.2. Physical and chemical properties of water samples

The pH was determined by a pH meter (Crison meter model pH # GLP21). A conductivity meter (model #CD-3416) has been used to determine electrical conductivity ;then, salinity has been calculated by multiplying the electrical conductivity value in (0.64). Chemical Oxygen Demand (COD) was determined by using Hanna instrument (Romanian # HI830099) COD and Multiparameter photometer After digestion with ($K_2Cr_2O_7$) in the digestive instrument (Italian Thermo Reactor) [23].

2.3. Determination of ammonia and nitrite

The determination of ammonia and nitrite was conducted according to the standard methods of examination of water and wastewater APHA [23].

2.4. Determination of some heavy metals in samples

The heavy metals were determined by Atomic Absorption Spectroscopy (AAS) Model 240FSAA, 200 series AA.

2.5. Determination of the Minimal Inhibitory Concentration (MICs) of Zn^{+2} and Ni^{+2} for *Pseudomonas luteola* Z4A2

Minimal inhibitory concentration (MIC) was evaluated on a nutrient agar medium. Different concentrations of zinc ions (100-700 ppm) and nickel ions (100-600 ppm) were prepared in Petri dishes plates and then sterilized at 121°C and1.5 bars for 15 min. The plates inoculated with 250 µl of 48 hours old nutrient broth cultures studied bacterial isolate and incubated at 37 ˚C for 48hrs. The concentration of metal ions that are ultimately preventing growth was termed MIC [24].

2.6. Effect of Ni^{2+} and Zn^{2+} on the growth kinetics and growth parameters of *Pseudomonas luteola* Z4A2

The growth rate of resistant strain *Pseudomonas luteola* Z4A2 was determined according to [25]. An asset of flasks containing 100 ml of NB at different concentrations of heavy metals (10,50,100 ppm) was inoculated with 200 µl of pre-culture of *Pseudomonas luteola* Z4A2. A control was carried out under the same condition without heavy metals and incubated at 37 °C at 150 rpm on a shaker for 12 hours. Heavy metals were added to the samples after 3 hours of their incubation. The bacterial growth was monitored by measuring absorbance at 600 nm with an EMC-11-UV spectrophotometer [26].

2.7. Effect of Ni^{2+} and Zn^{2+} on the protein content of *Pseudomonas luteola* Z4A2

The effect of different concentrations of Ni^{2+} and Zn^{2+} was studied on the protein content of bacterial isolate *Pseudomonas luteola* Z4A2. The bacterial culture was grown in nutrient broth containing 10, 50,100 ppm of Ni^{2+} and Zn^{2+}, which were added after 3 hours of incubation. The proteins were detected according to Lowry et al. [27]. A calibration curve was conducted using egg albumin, and the unit is µg protein/mg bacterial cells.

3. Results and discussion

3.1. Physicochemical properties of wastewater samples collected from El-Kharga at New Valley governorate

In our study, six water samples were collected from different contaminated sites El-Kharga (eastern area), Ein El-Sheik, The Big Waste Station, El-kalaa (western area), El-kalaa (eastern area), The Dates factory). The physical and chemical properties of water samples are shown in Table 1. The pH, Electrical conductivity, Salinity, COD, Pb^{2+}, Cd^{2+}, Zn^{2+}, Ni^{2+}, Nitrite, and ammonia were determined.

The pH ranged from 6.64-7.82, and COD has different ranged from 23-470 mg/L. El-kalaa (western and eastern area) has the highest range of EC (10.34 mS/cm and 12.48 mS/cm), while the salinity ranged from 760-7987 mg/l for the sites El-Kharga (eastern area) and El-Kala (eastern area), respectively. Heavy metal concentrations of Pb^{2+}, Cd^{2+}, Zn^{2+}, and Ni^{2+} in the tested water samples were deficient compared to the value of WHO (2005). The concentration of Pb^{2+} ranged from 0.12 to 5.37 ppb, while the concentration of Cd^{2+} ranged from 0.5 to 4.0 ppb. Other heavy metals were not detected in the tested samples. The nitrite concentrations (NO^{2-}) varied from75-200 µg/L, and ammonia ($NH4^{+}$) was found in traces amount. The high COD content in wastewater reflects the enrichment of organic matter, which is essential to chelate heavy metals.

The toxicity of heavy metals and other particulate pollutants to microbes is affected by environmental conditions. Thus, in assessing the impact of a pollutant on the biosphere, the influence of the biotic physicochemical factors on pollutant toxicity should be considered [28].

Table 1. Characteristics of samples collected from El-Kharga at New Valley Governorate

Locations	pH	*EC (µS/cm)	Salinity (mg/l)	*COD (mg/l)	Pb^{2+} (ppb)	Cd^{2+} (ppb)	Zn^{2+} (ppm)	Ni^{2+} (ppm)	Nitrite (ppb)	Ammonia
El-Kharga (eastern area)	6.64	1188	760.32	21	0.18	0.505	Nil	Nil	100	Nil
Ein El-Sheik	7.51	1373	878.72	3	4.13	2.724	Nil	Nil	200	Nil
The Big Waste Station	7.49	1255	803.2	18	5.37	3.986	Nil	Nil	75	0.0001
El-kalaa (western area)	7.82	10340	6617.6	334	1.90	1.344	Nil	Nil	185	0.00001
El-kalaa (eastern area)	7.72	12480	7987.2	470	0.12	1.166	Nil	Nil	80	Nil
The Dates factory	7.59	3150	2016	2	0.22	1.174	Nil	Nil	90	Nil

*Abbreviations: * COD: Chemical Oxygen Demand, * E.C. Electrical conductivity*

3.2. Determination of heavy metal resistance and MIC for *Pseudomonas luteola* Z4A2 to different concentrations of Zn^{2+} and Ni^{2+}

The MIC for *Pseudomonas luteola* Z4A2 collected from El-Kharga in the New Valley governorate is represented in Table 2. Table 2 shows the minimum inhibitory concentration (MIC) for *Pseudomonas luteola* Z4A2 with different concentrations of (Zn^{2+}) and (Ni^{2+}). The maximum level of tolerance was recorded against zinc, while the lowest level was recorded against nickel. *Pseudomonas luteola* Z4A2 can grow at low concentrations of both metals, but growth inhibition is higher. It can grow at (600 ppm), but there is no growth (700 ppm). In contrast to (Ni^{2+}), bacteria cannot grow at (200 ppm), which means that (Ni^{2+}) is more toxic than (Zn^{2+}).

The results showed high *Pseudomonas luteola* Z4A2 from different wastewater places in El-Kharga at New Valley Governorate. The degree of resistance to heavy metals depends on the growth of *Pseudomonas luteola* Z4A2. A high concentration of (Zn^{2+}) and (Ni^{2+}) indicates the higher toxicity of Ni^{2+} than Zn^{2+} to bacteria.

3.3. Effect of $Zn^{2+}$and Ni^{2+} on the growth of *Pseudomonas luteola* Z4A2

The growth curves of resistant strain *P. luteola* Z4A2 to different concentrations of zinc and nickel were shown in Figure 1 (A, B). The strain can grow at the highest concentration (100 ppm) with excellent tolerance to it. There is no adverse effect on bacterial growth on media with little metal concentration (10 ppm), but there is growth retardation compared to control. Growth inhibition occurs at high concentrations depending on the heavy metal type and its toxicity. Bacterial cell production is lower at high heavy metal concentrations than lower concentrations. Bacterial growth patterns also affected by heavy metal concentrations compared to control.

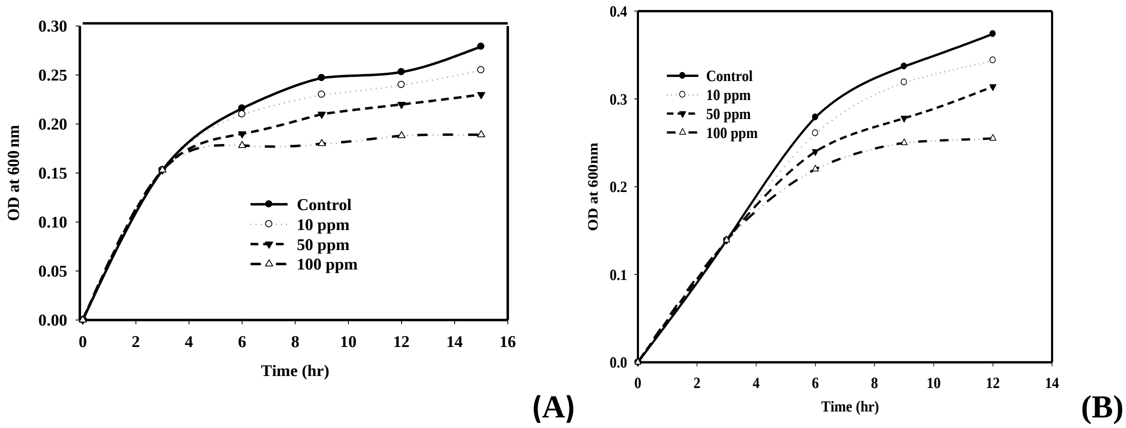


Figure 1. The growth curves of resistant strain *P. luteola* Z4A2 to zinc (A) and nickel (B) at different concentrations grown in nutrient broth medium at 37 °C with agitation at 150 rpm for 12 h.

The growth parameters of resistant strain *P. luteola* Z4A2 to different concentrations of zinc and nickel were shown in Figure 2 (A, B). It was shown that the generation time (T_h) has increased by increasing heavy metal concentrations. The generation time (T_h) for *Pseudomonas luteola* Z4A2 was 3.69 hr for control of Zn^{2+}, while it was 3.85 hr at concentration 10 ppm of Zn^{2+}. Moreover, it was 4.73 hr at concentration of 100 ppm of zn^{2+}. The same result has been shown in Ni^{2+}. The generation time (T_h) has increased by increasing heavy metal concentrations. The generation time (T_h) for *Pseudomonas luteola* Z4A2 was 2.90 hr for control, while it was 2.99 hr at concentration 10 ppm of Ni^{2+}. Moreover, it was 3.39 hr at a concentration of 100 ppm of Ni^{2+}.

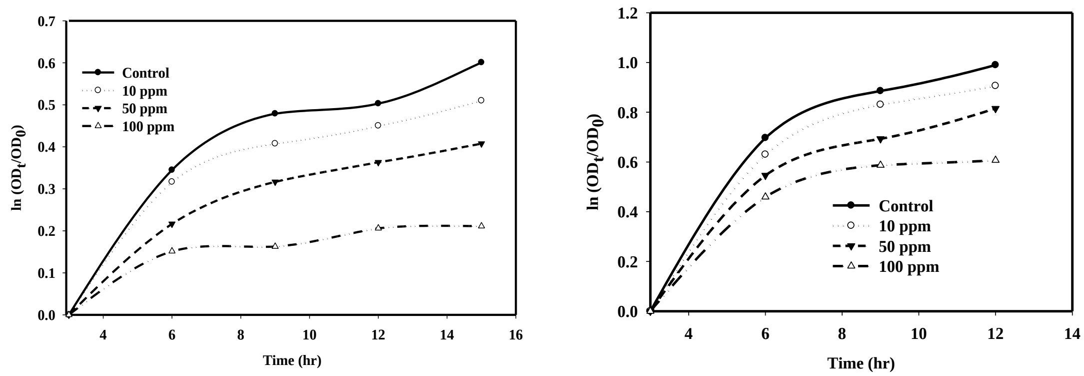
The differences in toxicity of metals could be attributed to the locality of the polluted environment and the selectivity of microbial culture techniques in each study, particularly the nature and specificity of growth media [29]. It was reported by [30] that the strains of *Pseudomonas* isolated from coastal water of Primorye in Russia have MIC (300- 2200 ppm) for chlorides of Cd^{2+}, Co^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, and Pb^{2+}. Otherwise, [31] showed that *P. aeruginosa* could tolerate (3.8-11.9 mM) Pb^{2+}, Cd^{2+}, Cr^{6+}, and Ni^{2+}, respectively, on LB medium. The growth rate of *Pseudomonas luteola* Z4A2 to Ni^{2+} and Zn^{2+} varied with the Ni^{2+} and Zn^{2+} concentration in the media., Generally, the growth rate and parameters decreased with a high concentration of Ni^{2+} and Zn^{2+}. The maximum specific growth rate (K) and protein decreased, while the generation time increased by increasing Ni^{2+} and Zn^{2+} concentrations. This result is in agreement with many investigators [25,32,33]. [34] conclude that heavy metal toxicity results mainly from their ability to denature a protein molecule of the bacterial strain.

Table 3. Growth parameters of *Pseudomonas luteola* Z4A2 to different concentrations of Zn^{2+} and Ni^{2+}

Heavy metal	Microorganism	Heavy metal conc. ppm	T_{λ} (h)	$T_{\acute{\alpha}}$ (h)	A	K	T_h (h)
Zn^{2+}	*Pseudomonas luteola* Z4A2	Control	3	15	0.60	0.050	3.69
		10	3	15	0.51	0.042	3.85
		50	3	15	0.40	0.033	4.08
		100	3	15	0.21	0.017	4.73
Ni^{2+}	*Pseudomonas luteola* Z4A2	Control	3	12	0.989	0.109	2.90
		10	3	12	0.906	0.100	2.99
		50	3	12	0.814	0.090	3.09
		100	3	12	0.606	0.067	3.39

T_{Λ}(h): Lag period, $T_{\acute{\alpha}}$(h): The end exponential phase, a: Asymptotic level = ln ($OD\alpha/OD_0$), k: The maximum specific growth rate = a / $T_{\alpha} – T_0$, T_h: Generation time ln2/k, conc: concentration

The next growth curves in Figures 2 explain the relationship between time and growth parameters with different metal concentrations.

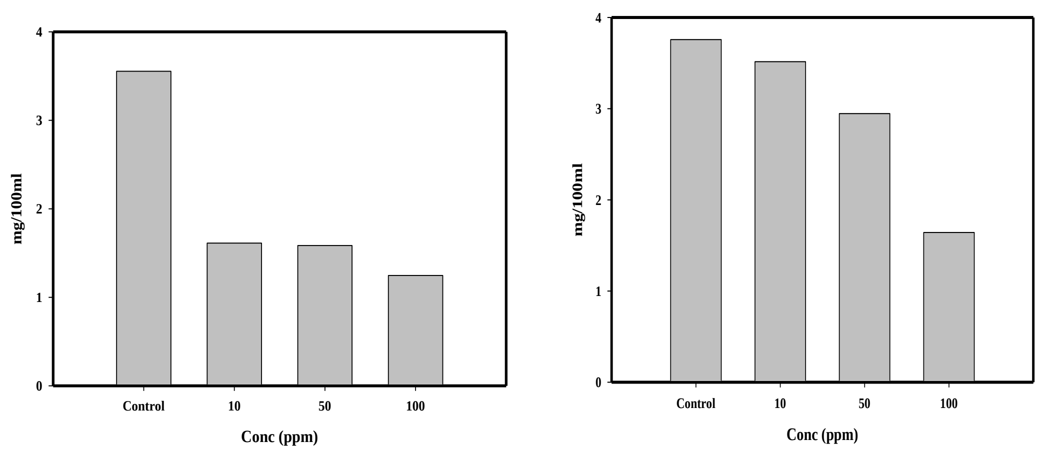


A B

Figure 2. The growth parameters of resistant strain *P. luteola* Z4A2 to zinc (A) and nickel (B) at different concentrations grown in nutrient broth medium at 37 °C with agitation at 150 rpm for 12 h.

3.4. Effect of Zn^{2+} and Ni^{2+} on the protein content of *Pseudomonas luteola* Z4A2

The effect of Zn^{2+} and Ni^{2+} on the protein content of *Pseudomonas luteola* Z4A2 was studied as stated. Figure 3 shows that different concentrations of Zn^{2+} and Ni^{2+} inhibit the protein synthesis compared to the respective control. We can conclude that either Zn^{2+} or Ni^{2+} decreases the total protein content of bacteria, mainly at a high concentration of both metals. The results showed that the toxicity of Ni^{2+} was higher compared to the toxicity of Zn^{2+}.



(A) **(B)**

Figure 3. Effect of different heavy metal concentrations on the protein content of *Pseudomonas luteola* Z4A2 to zinc (A) and nickel (B)

4. Conclusion

Pseudomonas luteola Z4A2 isolated from different wastewater locations in El-Kharga city at New Valley Governorate showed high resistance to Zn^{2+} or Ni^{2+}. The high concentrations of Zn^{2+} or Ni^{2+} decrease bacterial cell production and affect the bacterial growth pattern, and high concentrations of both metals decrease the total protein content of the bacterial cell. The toxicity of Ni^{2+} was higher compared to the toxicity of Zn^{2+}.

Acknowledgments
The authors are highly grateful to the National laboratory for water analysis in the New Valley governorate for their help in our study.

Conflicts of interest. There is no conflict of interest.

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