EasyChair Preprint

№ 11169

An Environmental Study Showing the Effect of Different Concentrations of Cyanide Compounds on the Growth Rate of Pseudomonas Aeruginosa and Klebsiella Pneumonia Bacteria

Nuha Mohammed, Mohammed Sharqi, Eethar Al-Othman and Mohammed Abood

October 26, 2023

An environmental study showing the effect of different concentrations of cyanide compounds on the growth rate of *Pseudomonas aeruginosa* and *Klebsiella pneumonia* bacteria

Nuha Abdullah Mohammed [1], Mohammed musleh sharqi [2], Eethar M.A.Al-Othman[2] Mohammed Fadil Abood[1]

[1] Department of Biology, College of Education for Pure Science, University of Anbar

[2] Department of Biology, College of Education for women, University of Anbar

Corresponding: mohammed.musleh@uoanbar.edu.iq

Abstract

Background Cyanide compounds are considered dangerous and toxic compounds for human and animal health, and there are many industries that release these pollutants, determine these compounds and analyze (convert) them into non-toxic primary compounds to avoid their danger using microorganisms. **Methodology** The compositional medium for the growth of bacteria on many compounds, taking into account the change in the concentration of cyanide, where four concentrations were used (1.5 , 2, 2.5 , 3 g/L). **Result** the analysis of potassium cyanide compound by *Pseudomonas aeruginosa* and *Klebsiella pneumonia* where four concentrations of cyanide were used or the last treatment was positive control, where sugar was used instead of cyanide compound. The second, third and fourth treatment gave significant differences compared to the control, as the growth rate in these treatments reached 3,5,6 compared to control 7 for *Pseudomonas aeruginosa* bacteria, while in Klebsiella bacteria, the three treatments gave a growth rate of 2,4,5 compared to control 7.**Conclusion** the best concentration of cyanide compound, which gave the best growth is 3 grams per liter and that the best bacteria in The analysis of this compound is *Pseudomonas aeruginosa* bacteria.

Keywords: cyanide compounds, Gram-negative bacteria, Pseudomonas

Introduction

Cyanide is a chemical that does not exist separately but is usually combined with other chemicals to form cyanide compounds.[1,2] For example hydrogen cyanide, sodium cyanide and potassium cyanide.[3,4] Cyanide is found naturally in some bacteria, fungi, and algae that produce cyanide, and it is also found in a number of foods and plants, and we also find it in some edible plants such as almonds, soybeans, spinach, bamboo shoots,

and cassava roots which is the main source of food in countries equatorial.[5,6] Cyanide was found in the form of small granules in the form of white powder.[7,8] All cyanides are insoluble in water except for the alkali metal cyanides such as potassium (KCN), sodium (NaCN) and calcium (CaCN), as well as mercury cyanide that dissolves in water and its boiling point is 630° C.[9] Cyanide is one of the most common toxins used in the fish trade, and cyanide fishing is common in Northeast Asia and the South Pacific.[10] Fishermen use cyanide in coral communities in order to obtain live fish and marine organisms.[11] Cyanide affects the respiratory system of fish, making it easier to catch them after they float to the surface, which are sold to marine organisms traders.[12] Cyanide is found in hydrogen gas or solid form.[13] A person needs a quantity not exceeding 0.2 of a gram to die directly within seconds. Inhaling cyanide leads to poisoning the body by stopping cellular oxidation processes and their death. Before that, a person suffers from multiple clinical symptoms, including: feeling dizziness, nausea, vomiting, loss of consciousness, and eventually cardiac arrest and sudden death.[14]

Methodology

Isolation and identification of bacteria

Isolation of bacteria from different pathological sources and then they were grown on selective media, and then they were diagnosed with APE20, Where *Pseudomonas*

Preparation of synthetic medium for bacterial growth

The compositional medium for the growth of bacteria on many compounds.[4] taking into account the change in the concentration of cyanide, where four concentrations were used (1.5 , 2, 2.5 , 3) g/L (4)

The components	Concentration g/L
Cyanide	(1.5 , 2, 2.5 , 3 g/L)
K2HPO4	0.52
KH2PO4	0.41
CaSO4.2H2O	0.20
Na2MoO4.2H2O	0.002
MgSO4.7H2O	0.16
FeSO4.7H2O	0.005

NaCl	0.20
MgSO4.7H2O	0.1

Result and discussion

Table 1 Effect of potassium cyanide on the growth of some types of Gram-negative bacteria

Dependent Variable: growth of bacteria			
type of bacteria	concentration of Potassium cyanide g/L	Mean	Std. Deviation
Pseudomonas	1.5	.240	.0548
	2	.300	.0707
	2.5	.500	.0707
	3	.680	.0837
	control	.700	.1000
	Total	.484	.2055
Klebsiella	1.5	.220	.0837
	2	.260	.1140
	2.5	.480	.0837
	3	.560	.0894
	control	.720	.1304
	Total	.448	.2124

Cyanide is one of the fastest and most lethal poisons ever, and we find it has appeared in a large number of spy movies as one of the types of poison used to kill either enemies or even commit suicide when arresting a soldier for fear of leaking the secrets of his state to the enemies some of his exposure to torture, but What is the secret of potassium cyanide and why is it known as a deadly poison. Table 1 shows the analysis of potassium cyanide compound by *Pseudomonas aeruginosa* and Klebsiella bacteria, where four concentrations of cyanide were used or the last treatment was positive control, where sugar was used instead of cyanide compound. The second, third and fourth treatment gave significant differences compared to the control, as the growth rate in these treatments reached 3,5,6 compared to control 7 for *Pseudomonas aeruginosa* bacteria, while in *Klebsiella bacteria*, the three treatments gave a growth rate of 2,4,5 compared to control

7, we conclude from this that the best concentration of cyanide compound, which gave the best growth is 3 grams per liter and that the best bacteria in The analysis of this compound is *Pseudomonas aeruginosa bacteria*

Table 2 ANOVA table of Effect of potassium cyanide on the growth of some types of Gram-negative bacteria

Tests of Between-Subjects Effects

Dependent Variable: growth of bacteria

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.784[a]	9	.198	24.176	.000
Intercept	10.858	1	10.858	1324.122	.000
bacteria	.016	1	.016	1.976	.168
concentration	1.741	4	.435	53.085	.000
bacteria * concentration	.027	4	.007	.817	.522
Error	.328	40	.008		
Total	12.970	50			
Corrected Total	2.112	49			

a. R Squared = .845 (Adjusted R Squared = .810)

Table 2 shows the meta-analysis of the effect of some concentrations of potassium cyanide on the growth of *Klebsiella* and *Pseudomonas aeruginosa* bacteria, where it was noted that there were significant differences between all treatments except for the fourth treatment in which the potassium cyanide concentration was 3 g per liter did not give a significant difference with the positive control, so it was the best A treatment compared to the rest of the treatments for both types of bacteria As shown in Figure 1 What distinguishes cyanide and makes it one of the most dangerous poisons used is the composition of which it consists. Dangerous, because hydrocyanide acid is a volatile acid that smells very similar to bitter almonds, and poisoning occurs when it enters the body.

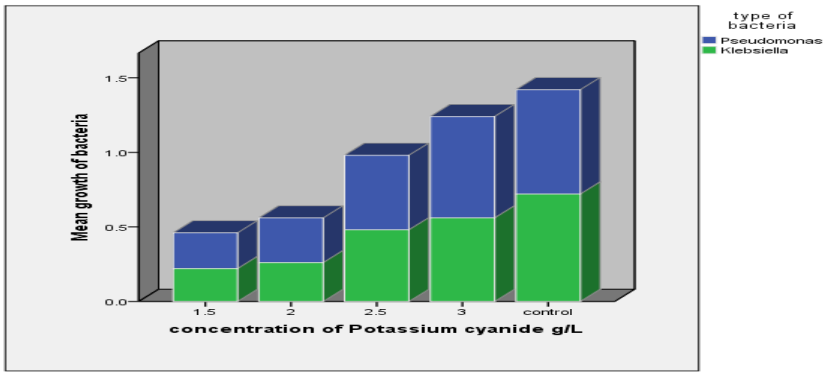

Figure 1 Effect of potassium cyanide on the growth of some types of Gram-negative bacteria

Table 3 Effect of sodium cyanide on the growth of some types of Gram-negative bacteria

Dependent Variable: growth of bacteria			
type of bacteria	concentration of sodium cyanide g/L	Mean	Std. Deviation
Pseudomonas	1.5	.420	.0837
	2	.460	.1140
	2.5	.560	.1140
	3	.640	.1140
	control	.980	.1924
	Total	.612	.2351
Klebsiella	1.5	.480	.1924
	2	.420	.1304
	2.5	.580	.0837
	3	.680	.0837
	control	1.060	.5413
	Total	.644	.3367

A white or colorless crystalline substance that is very toxic and must be handled with caution and kept in a tightly closed place and out of reach. Its chemical formula is NaCN, its molecular weight is 49.01 g/mol, its melting point is 563 degrees Celsius, its boiling point is 1496 degrees Celsius, and its density is 1.59 g/cm3. It is hygroscopic and soluble in water and ammonia solution (ammonium hydroxide) and sparingly soluble in ethanol. Table 3 shows the growth rates of *Pseudomonas aeruginosa* and *Klebsiella bacteria* in different concentrations of the sodium cyanide compound, where three concentrations of

sodium cyanide were used (1.5, 2, 2.5, 3 and the latter was control). The growth rates of *Pseudomonas aeruginosa bacteria* were 0.48, 0.42, 0.58, 0.68 Compared to the control, it was 0.9. As for the growth rates of Klebsiella bacteria, the growth rates were 0.42, 0.46, 0.56 and 0.64 compared to the control 0.98. Therefore, we conclude that the best concentration that gave the best growth rate was a concentration of 3 grams per liter, and that the best bacteria in the sodium cyanide analysis were *Pseudomonas aeruginosa bacteria.*

Table 4 ANOVA table of Effect of sodium cyanide on the growth of some types of Gram-negative bacteria

Tests of Between-Subjects Effects

Dependent Variable: growth of bacteria

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2.285[a]	9	.254	5.718	.000
Intercept	19.719	1	19.719	444.126	.000
bacteria	.013	1	.013	.288	.594
concentration	2.251	4	.563	12.673	.000
bacteria * concentration	.021	4	.005	.119	.975
Error	1.776	40	.044		
Total	23.780	50			
Corrected Total	4.061	49			

a. R Squared = .563 (Adjusted R Squared = .464)

Table 3 shows the meta-analysis of the effect of some concentrations of sodium cyanide on the growth of *Klebsiella* and *Pseudomonas aeruginosa* bacteria, where it was noted that there were significant differences between all treatments except for the fourth treatment in which the sodium cyanide concentration was 3 g per liter did not give a significant difference with the positive control, so it was the best A treatment compared to the rest of the treatments for both types of bacteria As shown in Figure 2 Sodium cyanide reacts quickly with strong acids to release hydrogen cyanide. This dangerous process presents a significant risk associated with cyanide salts. The most efficient detoxification is done with hydrogen peroxide (H2O2) to produce sodium cyanate (NaOCN) and water.

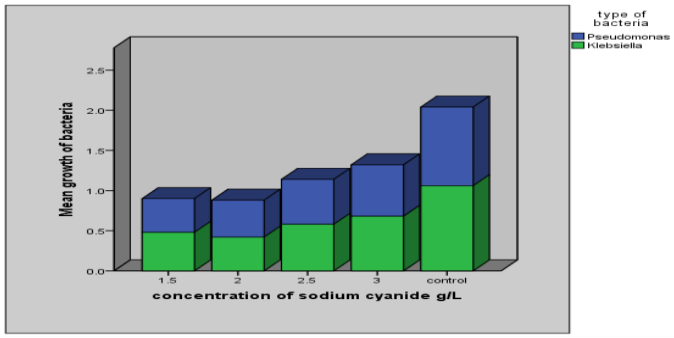

Figure 2 Effect of sodium cyanide on the growth of some types of Gram-negative bacteria

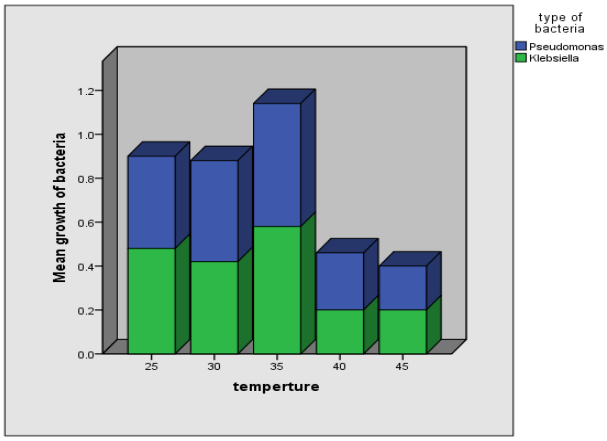

Figure 3 The effect of temperature on the growth of *Pseudomonas aeruginosa* and *Klebsiella bacteria*

It is known that bacteria cells cannot develop at temperatures greater or less than those prevailing in their native surroundings. With regard to the impact of heat and the adaptability of bacterial cells, we assume that bacterial cells and their developing environment are of identical temperature, and that the heat that is emitted in cells is a result of distinct metabolic processes The cell loses it via heat radiation, transformation, or both. The study of the effect of heat on bacteria is summarized in knowing their ability to grow strongly or slowly or stop growing at different temperatures (high and low), and it also includes studying the ability of cells to withstand maximum and minimum degrees of heat when exposed to them for short periods. Figure *3 depicts the influence of temperature on the development of Pseudomonas aeruginosa* and *Klebsiella* bacteria. We observe that growth happened at different temperatures, yet

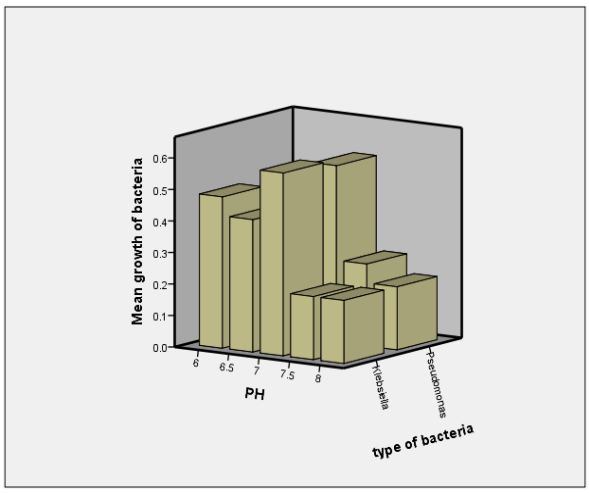

Figure 4 The effect of pH on the growth of *Pseudomonas aeruginosa* and *Klebsiella bacteria*

It is a numerical measure of the pH or alkalinity of various liquids or solutions. This numerical measurement ranges from 1 to 14. As the PH value = 7 represents the state of neutrality, and less than 7 indicates an increase in acidity, and a higher than 7 indicates an increase in alkalinity Microbial growth is affected by changes in the hydrogen ion concentration in the growing environment. Microbial growth stops at high acidity and alkalinity, which affects enzymatic activity and metabolic processes, Each microbial species has an optimum degree of hydrogen ion concentration at which growth is greatest. Maximum degree, which is the maximum degree at which growth occurs. Each microbial species has a minimum degree, which is the degree to which if the hydrogen ion concentration drops, growth stops completely. Figure 4 shows the effect of pH on the growth of Pseudomonas aeruginosa and Klebsiella bacteria. The growth of these types of bacteria with different pH ranges, but gave the best growth rate at pH 7

References

1. MOHAMED, K. S.; ELBIALY, E. E.; FADDA, A. A. Application of N-(Aryl)-2-oxo-2-(arylamino) acetohydrazonoyl Cyanide in Synthesis of Some Novel Triazole Derivatives and Their Biological Activity. *Russian Journal of General Chemistry*, 2021, 91.8: 1592-1603.
2. PATHAK, Uttarini, et al. Biodegradation efficacy of coke oven wastewater inherent co-cultured novel sp. Alcaligenes faecalis JF339228 and Klebsiella oxytoca KF303807 on phenol and cyanide—kinetic and toxicity analysis. *Biomass Conversion and Biorefinery*, 2021, 1-16.

3. SOLEYMANI-BABADI, Susan, et al. Simple synthesis of novel magnetic silver polymer nanocomposites with a good separation capacity and intrinsic antibacterial activities with high performance. *Dalton Transactions*, 2021.
4. PINKERT, Lukas, et al. Antibiotic Conjugates with an Artificial MECAM-Based Siderophore Are Potent Agents against Gram-Positive and Gram-Negative Bacterial Pathogens. *Journal of Medicinal Chemistry*, 2021.
5. LAI, Yi-Hui. *The uptake mechanism of synthetic siderophore conjugate in Gram-negative bacteria*. 2021. PhD Thesis. Hannover: Institutionelles Repositorium der Leibniz Universität Hannover.
6. SINGH, Kumar Sachin, et al. IspH inhibitors kill Gram-negative bacteria and mobilize immune clearance. *Nature*, 2021, 589.7843: 597-602.
7. DA'SAN MM, Jaradat, et al. Human glucose-dependent insulinotropic polypeptide (GIP) is an antimicrobial adjuvant re-sensitising multidrug-resistant Gram-negative bacteria. *Biological Chemistry*, 2021, 402.4: 513-524.
8. MION, Sonia, et al. Disrupting quorum sensing alters social interactions in Chromobacterium violaceum. *NPJ biofilms and microbiomes*, 2021, 7.1: 1-16.
9. ROPPONEN, Henni-Karoliina, et al. Mastering the gram-negative bacterial barrier–chemical approaches to increase bacterial bioavailability of antibiotics. *Advanced Drug Delivery Reviews*, 2021.
10. HEGAB, Mohamed Ibrahim, et al. Derivatization and biological activity studies of 3-chloro-3-chlorosulfenyl spiro tetrahydropyran/tetrahydrothiopyran-4, 2'-chroman-4'-one. *Indian Journal of Chemistry-Section B (IJC-B)*, 2021, 60.11: 1502-1510.
11. 11. HUSSEIN, Najeeb Mohammed, et al. Isolation and diagnosis of bacteria causing urinary tract infection in children. *Systematic Reviews in Pharmacy*, 2020, 11.1: 76-79.
12. 12. MUSA, Farkad Hawas, et al. Effect of some plant extracts on the Pyocyanin Production from Pseudomonas Aeruginosa which Isolated from clinical samples. In: *IOP Conference Series: Materials Science and Engineering*. IOP Publishing, 2020. p. 012041.

13. HUSSEIN, Najeeb Mohammed, et al. Effect of Sonchus oleracea Extract on Some Virulence Factor of Klebsiella pneumonia Which Isolated from Urinary Tract Infection in Ramadi Hospital. *Indian Journal of Forensic Medicine & Toxicology*, 2021, 15.3.

14. AL-JANABI, Abbas Obaid Farhan; ABDULMAJEED, Bashar Amer; SHEHAN, Mayada Abdullah. Ginkgo biloba extract effect on the proteus mirabilis virulence factor that extracted from urinary tract infections. *Materials Today: Proceedings*, 2021.

15. KADHIM, Mohammed Abed; ALANI, Abdullah Sh Abdullah; HUSSEIN, Najeeb Mohammed. Synthesis, characterization and biological evaluation of some phthalazine derivatives. *Materials Today: Proceedings*, 2021.