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Effects of heavy metals on Antarctic bacterial cell growth kinetics and degradation of waste canola oil

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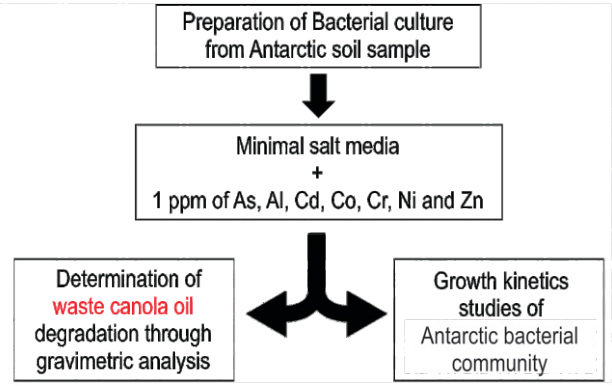
Abstract

Aim: The aim of the present study was to study the effect of heavy metals on growth kinetics of Antarctic bacterial in degradation of waste canola oil.

Methodology: The BS14 Antarctic bacterial community was introduced in the minimal salt media containing 1 ppm of heavy metals (Cd, Cr, Al, Zn, Ni, As and Co) with 1% waste canola oil, and the effects of heavy metals on biodegradation of waste canola oil was analysed by gravimetric analysis. The turbidity of bacteria was obtained through UV-visible spectroscopy at 600 nm of wavelength for every 24 hr within seven days of incubation period, and the data were regressed with linear and non-linear kinetic equations.

Results: The results demonstrated that Co was the most active metal that led to 4.217% increase in waste canola oil and the least active metal in biodegradation of oil was zinc, as it degraded the waste canola oil only to 29.26%. Overall, the bacterial growth was inhibited in increasing order of Al > Cd> As> Zn> Ni> Cr> Co whereas the waste canola oil biodegradation was inhibited in the order of Zn> Cr> Ni> Al> Cd> As> Co. The best fitted-regression model was determined by comparing the kinetic parameters estimated between linear and non-linear model equations, where the R^2 value for non-linear regression was highest at 0.8421, and low sy.x at 0.324 for Ni with a maximum growth rate (0.01131 hr^{-1}) of the Antarctic bacterial in degrading waste canola oil, meantime best-fitted in the linear regression model was Zn with high R^2 and growth constant values (0.9082 and 0.2075 hr^{-1}, respectively) as well as low value of statistical error, which was 0.2075.



Interpretation: The presence of heavy metals in Antarctic bacterial community could suppress the ability of bacteria to degrade waste canola oil, and this can slower the rate of bacterial growth in the kinetics studies. Hence, this work would be helpful in actual bioremediation operations by understanding and manipulating the process of the kinetics parameters.

Key words: Antarctic, Canola oil, Degradation, Heavy metal, Kinetic growth

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Introduction

Canola oil is used most in the station base in Antarctica for food preparation, including butter and margarine (Zahri *et al.,* 2020). Globally, grease and oil are discharged directly from the kitchens, restaurants and food industries, including the waste cooking oil, which can have serious environmental consequences due to their properties that are similar to those of petroleum-based oils (García-Dávila *et al.,* 2017). The oil spillage is one of the biggest issues in Antarctica, together with cooking oil pollution that also could contribute to this issue since both of them are categorised as hydrocarbons. At the same time, oil pollution can affect the environmental ecosystems in Antarctica,including animals (penguins, seal, fish, birds) and humans. Oil can also cause mechanical injuries such as loss of insulation, mobility and smothering (Department of Ecology, 2016). Waste canola oil can be toxic to organisms if the oil is repeatedly heated for cooking, which can lead to the formation of acrolein with thermal oxidation (Falade *et al.,* 2017) known as a precursor to carcinogen (Henning *et al.,* 2017).

Microbial communities were used directly from the Antarctic soils to degrade the waste canola oil, by reason that the introduction of non-native microorganisms is not permitted according to the Antarctic treaty norms (Hughes *et al.,* 2020). In the bioremediation process, the microorganisms can be affected due to the presence of any other pollution or natural compounds present in that particular area (Martínez-Prado and Soto-Álvarez, 2017). In Antarctica, the presence of heavy metals by natural sources and anthropogenic activity (Chu *et al.,* 2019) needs to be considered for degrading oil. Exposure to heavy metals creates a stress condition for bacteria and disrupts their metabolic reactions; therefore, this can be a major challenge for bioremediation of pollutants including waste canola oil. Earlier studies have reported the presence of heavy metals in the soils, marine, snow and Antarctic ice, which were contributed from natural sources, local contamination and anthropogenic activities including cadmium, zinc, aluminium, arsenic, chromium, nickel and cobalt (Neto *et al.,* 2017; Chu *et al.,* 2019). Some of the psychrophilic and psychrotolerant bacteria genera such as Rhodococcus, Pseudomonas, Acinetobacter, Sphingomonas and Arthrobacter are the microbial community commonly isolated from the Antarctic pristine for degrading the hydrocarbons (Aislabie *et al.,* 2001; Ruberto *et al.,* 2005; Roslee *et al.,* 2019).

The growth rate of microbial community is one of the key players in degrading waste canola oil in the presence of heavy metals. Heavy metals can be inhibitory or stimulatory for growth depending on the protection mechanisms of heavy metal resistance by microbial cells (Inigiri *et al.,* 2018). Abatenh *et al.* (2017) stated that metal ion has a direct and indirect impact on the rate of degradation, where the degree and mechanism of toxicity of different type of metals are vary with specific toxicants and exposed microorganisms. Some metals are essential for bacteria for redox processes; these include Co, Cu and Ni. Nevertheless, most of them are non-essential, which have no nutrient value and potentially toxic to microorganisms (Bruins *et al.,* 2000). Toxicity of heavy metals require various mechanisms, which can cause sever fatal enzymatic functions, generate reactive oxygen species, destroying ion regulation, and directly manipulate DNA and protein formation (Inigiri *et al.,* 2018). For example, heavy metals like lead disturb most microbial metabolism and activities, including membrane transport, respiration and ribosome activity; yet, in certain circumstances, these heavy metals can lead to cell death (Murthy *et al.,* 2013).

To predict and estimate the biodegradation efficiency, scientists have developed mathematical models for bacterial growth and decay as well as for the substrate and electron acceptor utilisation (Kim *et al.,* 2005). Various models have been established for the kinetics of microbial growth, which generate several important kinetics parameters for analysis. Moreover, kinetic studies provide a strategy for solving problems of the real application in the industrial scale, including bioremediation operations (Gupta and Yadav, 2017). Keeping in view the above the aim of this study was to investigate the bacterial community growth and degradation of waste canola oil in presence of heavy metals by evaluating and comparing linear and exponential growth curves.

Materials and Methods

Sample collection: Soil samples for isolation of bacterial strains and waste canola oil samples were collected from the research station in Antarctica (Base General Bernardo O'Higgins Riquelme). The samples were stored appropriately in Eco-Remediation Technology Laboratory, Universiti Putra Malaysia until further analysis.

Preparation of bacteria culture: Bacteria from soil samples were cultured in sterile nutrient broth for four days on a 150 rpm orbital shaker at 10°C. The bacterial culture was transferred to 50 ml of nutrient broth and left for shaking on an orbital shaker for another four days. This step was used as a pre-enrichment of bacteria by growing the bacteria in nutrient broth several times and eliminating the solid soil particles. The bacterial community was prepared for glycerol stock and stored at -80°C for long term storage.

Enrichment of waste canola oil utilising bacteria community with heavy metals: The bacteria were cultured from the stock into nutrient broth and incubated for two days on an orbital shaker at 10°C. One milliliter of bacteria from nutrient broth was added to 50 ml of modified minimal salt media; consisting K_2HPO_4 (4.74 g), KH_2PO_4 (0.56 g), $(NH_4)_2SO_4$ (0.5 g), $MgSO_4$. $7H_2O$ (0.5 g) and $CaCl_2$. $2H_2O$ (0.1 g) per L (Ibrahim *et al.,* 2020) and the medium was adjusted with HCl to pH 7. All flasks were supplemented with 1 ppm of different types of heavy metals and1% of sterilised waste canola oil(filtered using a 0.45 µm filter membrane). All flasks were incubated at 10°C on 150 rpm orbital shaker for seven days. The experiment was conducted in triplicate. The bacterial community growth was recorded every day with a spectrophotometer (OD_{600nm}) and the data were further used for

growth kinetic studies. On the other hand, degradation of waste canola oil was determined gravimetrically by measuring the oil mass. After seven days incubation, n-hexane solvent was added to the media (1:1 ratio) and the flasks were shaken vigorously (Brooksbank *et al.,* 2007), allowing the mixture of oil and media to separate into two layers by dissolving the oil with the solvent for 20 min at room temperature. The organic phase was extracted into a petri dish,while oil reduction was determined by the weight of the oil, reflecting the amount of waste canola oil (Ibrahim *et al.,* 2020). The pre-weighed petri dishes containing the solvent were left overnight in the fume hood to allow complete evaporation of the solvent. The volume of extracted crude waste canola oil was deducted from petri dish and the degradation percent was calculated by the formula:

$$\text{Degradation (\%)} = \frac{\text{Amount of crude oil degraded}}{\text{Amount of crude oil added in the media}} \times 100$$

Kinetic growth curve: Non-linear regression was selected as it is more general and can be utilised to estimate model parameters. Bacteria grow exponentially, and the optical density (OD) increases as a function of ln (OD). The growth rate is the change in the number of cells per minute that estimates the change in OD per min (Hall *et al.,* 2003). The growth measurement assessed the cell density at a series of seven time points (seven days) spectrophotometrically and measured the turbidity of cells, in terms of optical density. This growth curve was assessed using the exponential growth equation (curve fit) by statistical software of GraphPad Prism software (Version 7). At the same time, the linear regression curve was analysed to identify the differences between these two models.

Statistical analyses: Statistical analysis was carried out with SPSS software version 22 through One-way analysis of variance (ANOVA) where all the experiments were completed in triplicate. 95% confidence level was used to evaluate differences between group with $p<0.05$ considered as statistically significant. The data acquired are presented as mean ± standard error.

Results and Discussion

Based on Fig. 1, the bacterial growth was inhibited in the increasing order of Al > Cd> As> Zn> Ni> Cr> Co. 1 ppm of Al could inhibit bacterial growth as compared to other heavy metals. The lowest turbidity of bacteria in the media containing Al was at 1.672 nm; as a result, Al can become toxic to microorganisms and plants at low pH. Similarly, Kurniawan *et al.* (2018) stated that Al was able to decrease bacterial growth rate by adjusting or inhibiting some enzymatic reactions (especially disrupt membranes). This is in agreement with the study of (Purwanti *et al.,* 2018) who reported that the growth of *Vibrio alginolyticus* inhibited at 100 to 350 mg l^{-1} of Al concentration.

Cd demonstrated high bacterial growth (1.689 nm), which was slightly higher than Al; however, there were no significant differences between these two conditions ($p> 0.05$). Cd suppressed the Antarctic bacteria community, which might occur due to the properties of this metal that could alter the protein expression in bacterial cells. This is important for determining the number of growth factors and other protein (Abbas *et al.,* 2014a). treated *Pseudomonas strain* RZCD with different concentrations of Cd (50 to 550 µg ml^{-1}) and observed the at 400 µg ml^{-1} Cd the bacterial growth started to decrease rapidly as bacterial cells could not resist the interference of Cd at that particular concentration. Similarly, arsenic also showed low turbidity cell growth due to the interference of proteins in the bacterial metabolism responsible for phosphate uptake and transport, since this metal acts a phosphate analogue (Pandey *et al.,* 2011).

Generally, bacterial growth is concomitant and proportional to the degradation of pollutants as pollutants itself (such as hydrocarbons serves as carbon source) act as nutrient for bacterial growth (Al-Hadithi *et al.,* 2017; Samimi and Moghadam, 2020). However, in this study, the effect of heavy metals on the bacterial community exhibited different conditions for the growth and degradation of waste canola oil. Viewing at waste canola oil degradation, inhibition increased in the following order: Zn> Cr> Ni> Al> Cd> As> Co (Fig. 1). Antarctic bacterial community degraded a minimum of 29.26% of waste canola oil in the media comprising Zn metal. This was followed by 31.07% in Cr, 32.19% in Ni, 35.11% in Al, 36.49% in Cd, 37.01% As and maximum degradation at 53.18% in Co media. Although the media containing Zn, Cr and Ni ($p<0.001$) showed high bacterial growth, the rate of degradation was inhibited since these metals have altered the enzymatic function of the bacteria to breakdown the waste canola oil. The bacteria may survive in this condition because the survival of microbial depends on the inherent structural and biochemical properties. Although, this isolate involved different types of community bacterial where the possibilities for Rhodococcus, Pseudomonas and Arthrobacter Antarctic bacterial group are involved in hydrocarbons degradation as reported by several previous studies (Aislabie *et al.,* 2001; Lee *et al.,* 2018).

Zn is an essential trace element and acts as a catalytic or structural cofactor of enzymes and proteins involved in protein synthesis and DNA replication (Zakaria *et al.,* 2020). However, excessive amount of Zn can be harmful to organisms. In this study, 1 ppm of Zn hindered the degradation of waste canola oil where it was capable of inhibiting the enzyme activity through interaction with enzymes directly involved in biodegradation (Abdu *et al.,* 2016). Low concentration of zinc ion reported to reduce biodegradation of crude oil by *Pseudomonas* sp. at 0.43 mg $l^{-1}$and *Micrococcus* sp. at 0.46 mg l^{-1} (Sandrin and Maier, 2003). Nevertheless, strong inhibition of enzyme activity for degrading waste canola oil by heavy metals may occur (Malley *et al.,* 2006; Karaca *et al.,* 2010). Generally, heavy metals causetoxicity by relocating the essential metal ions from native binding sites, hindering the essential functional groups of protein, changing conformation of biological structure, disintegrating essential metabolites and osmotic balance around the cells arechange (Murthy *et al*., 2014).

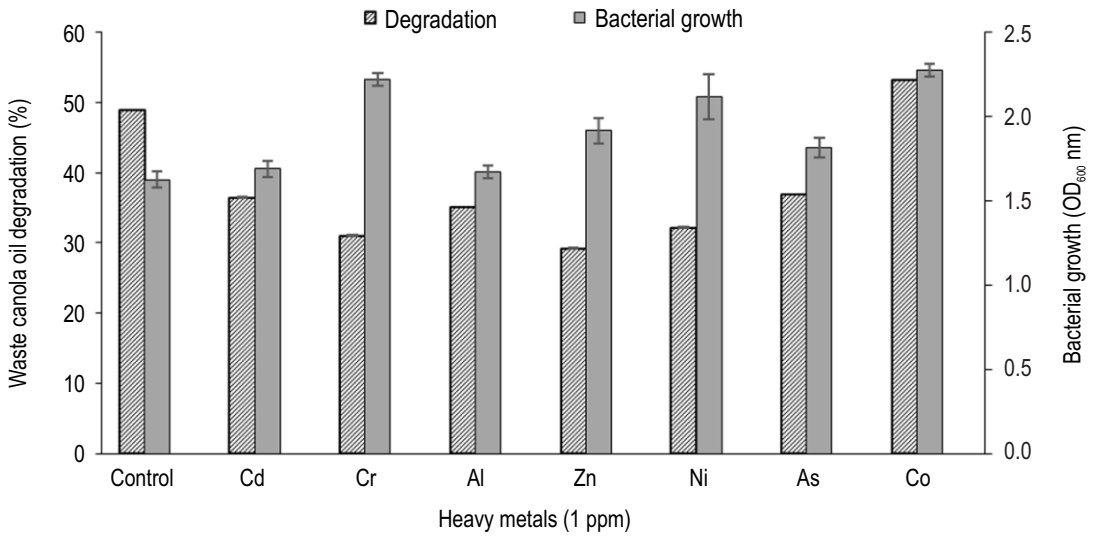
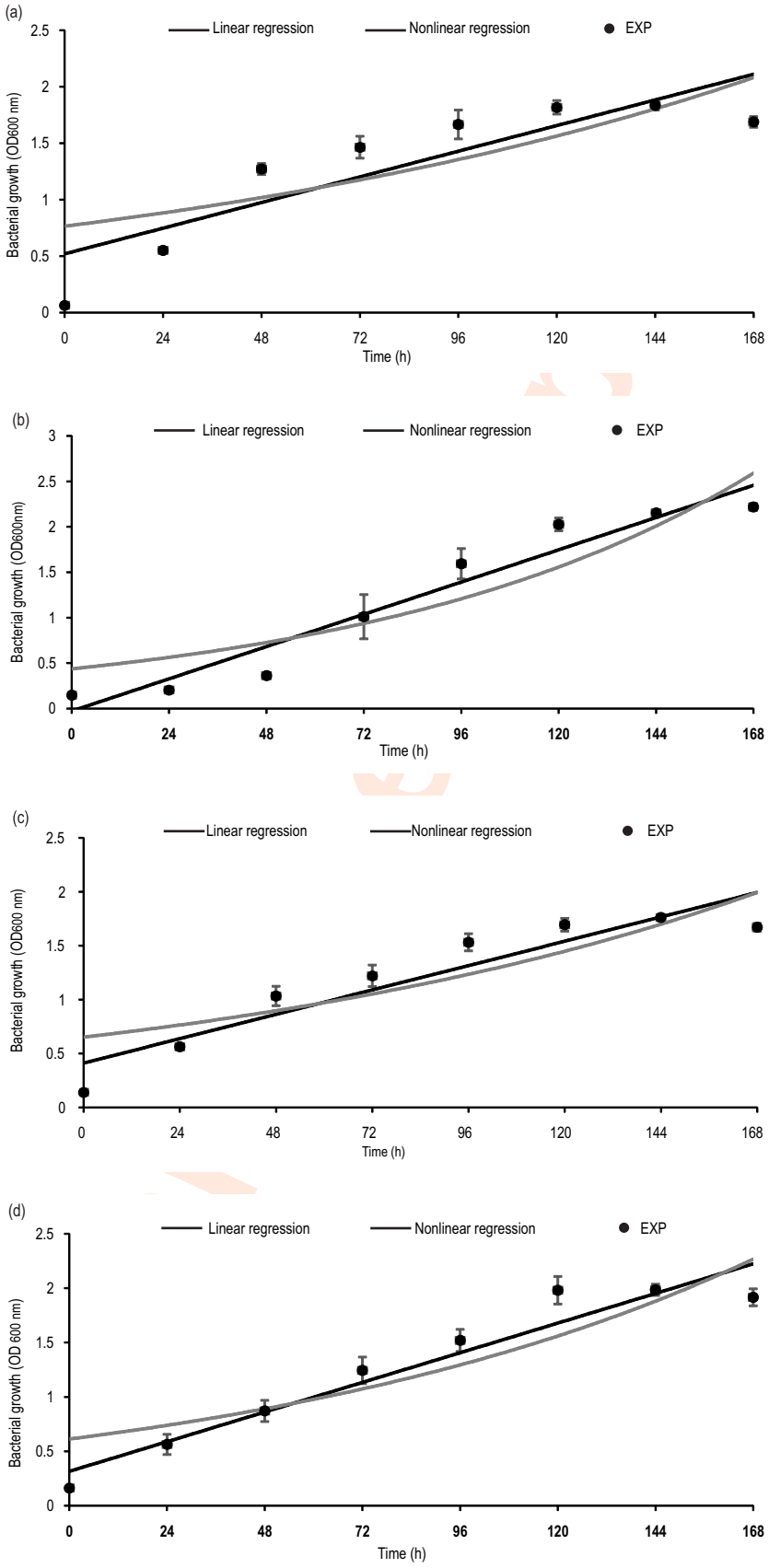


Fig. 1: Growth and waste canola oil degradation effect of various heavy metals (1 ppm) for 7 days. Control indicates no heavy metals. Data represent mean ± SEM (n-3).

Nonetheless, bacteria can adopt different mechanisms to interact and survive in the presence of inorganic metals. Soil microbes have devised several mechanisms to combat and mitigate the effects of heavy metal toxicity on their survival (Abdu *et al.,* 2016). As shown in Fig. 1, the ability of bacterial community to stand with the toxicity of heavy metals to degrade waste canola oil was found to decrease, except for Co that is known to be an essential trace element for bacterial growth. Degradation of waste canola oil on adding cobalt was found highest (53.13%) which induced the degradation of waste canola oil as compared to the control ($p<0.001$). Cobalt mainly occurs as cofactor B12 that plays an important role in the metabolism of microorganisms (Nies *et al.,* 1999). The availability of this metal helps in degrading waste canola oil as the bacterial community was able to resist and survive. Zhou *et al.* (2013) reported that in the presence of Co, as a catalyst, to the bicarbonate-activated H_2O_2 accelerated the degradation rate of dyes and phenol.

Growth kinetic modelling is a useful tool in implementation for process control and field-scale bioremediation designs. The effects of heavy metals in bacterial growth in biodegrading pollutants are among the important environmental factors that may be affect the bioremediation process since the availability of heavy metals are found in the environment either from natural sources or anthropogenic activities. From this study, the microbial growth profile was regressed using exponential and linear models, which allowed the prediction of microbial growth behaviour by knowing the kinetic parameters including growth rate, doubling time and best-fitting models. The parameters for goodness of the curve for exponential regression curve fit were shown, including the coefficient determination (R^2), degree of freedom (DF), as well as the sum of squares (SS) and standard deviation of the residuals (Sy.x). The R^2 value allowed to compare the fit of the model to fit an experimental line through means of all Y-axis values. A high R^2 value indicates that the curve of regression was close to the experimental value, and the possibilities for the curve to the best fit were high. The result exhibited the highest R^2 value of Ni at 0.8421, followed by Cr and Zn at 0.825 and 0.8005, respectively. However, the value of SS and sy.x both for Ni and Cr were higher as compared to Zn (SS: 2.057; Sy.x: 0.3058). The low value of SS shows the regression curve minuses the SS of the distance of the points from the curve, while Sy.x value is the standard deviation of the vertical distances of the experimental point from the regressed line. Ideally, the SS and sy.x values should be lower in any regression model, which indicates a fitted model to the data.

The non-linear regression graph showed different Y_0, as the presence of a different type of heavy metals affected the growth pattern of BS14 (Fig. 2), while k is the rate constant. All data were regressed with the exponential growth equation, where Y_0 also could be observed from the graph, as shown in Fig. 2; at the same time, the best-fit values were determined during the analysis (Table 2). This parameter value was found within the 95% confidence interval of distribution as it indicated the best-fit values for the growth curve of the bacteria. Y_0 is the turbidity of cells when time is zero, while T_d is the doubling period, which is a process that gives rise to significant cells to double in size or value at a certain time especially in lag phase for bacterial growth curve (Todar, 2012). The value of Y_0 for the bacterial growth in the presence of Ni, Cr and Co was low in value and offered faster doubling time *i.e.,* 61.27, 65.45, 71.31 h for Ni, Cr and Co,respectively. Meanwhile, the bacteria community required more time for their population to double in number in Al (104.2 h) and Cd


(a)
Linear regression
Nonlinear regression
EXP
Bacterial growth (OD600 nm)
Time (h)
(b)
Linear regression
Nonlinear regression
EXP
Bacterial growth (OD600nm)
Time (h)
(c)
Linear regression
Nonlinear regression
EXP
Bacterial growth (OD600 nm)
Time (h)
(d)
Linear regression
Nonlinear regression
EXP
Bacterial growth (OD 600 nm)
Time (h)

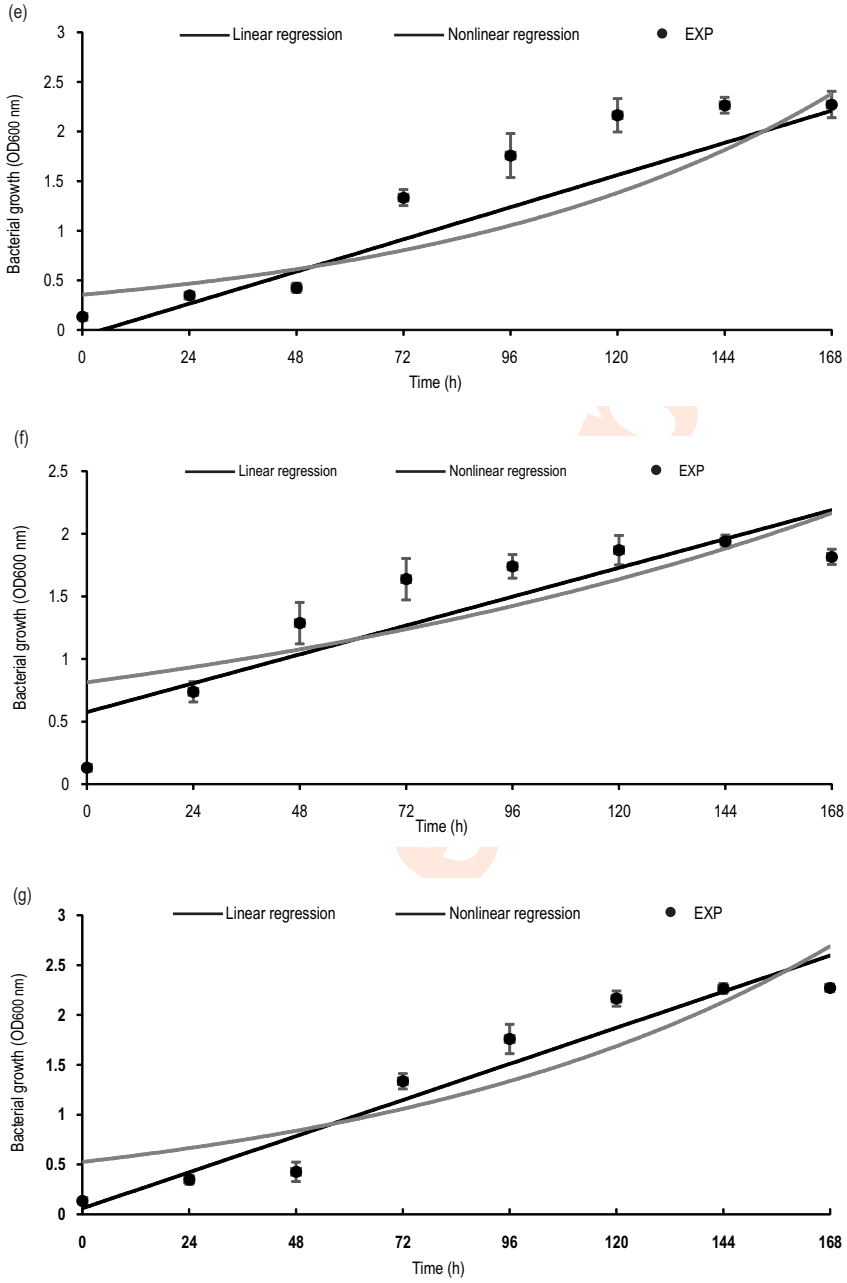


Fig. 2: Exponential regression model. (a) Cadmium. (b) Chromium. (c) Aluminium. (d) Zinc (e) Nickel. (f) Arsenic and (g) Cobalt. EXP indicate to experimental data. Data represent mean ± SEM (n-3).

(116.4 h) media. The slower T_d may have caused the bacterial to grow and degrade at a lower percentage at the end of incubation time (Fig. 1). Generally, low T_d value will exhibit a high rate of bacterial growth (k) under any circumstances.

The k value is the rate constant, also known as the maximum specific substrate rate (Talaiekhozani *et al.,* 2015). The rate constant can be expressed through the relationship between the rate of a reaction (growth of bacterial) and the concentration of reacting substance (heavy metals). According to Table 2, the availability of nickel shown the highest rate constant at 0.01131 h^{-1}, and this revealed the reaction is faster as compared to other condition. This exhibits the reasons for doubling time for the bacteria in the presence of nickel is fast (low in value). Simultaneously, the low rate constant indicates slow reaction, for instance, the bacteria in the Cd and As medium at 0.005957 and

Table 1: ANOVA table (goodness of fit) for exponential regression curve fit of BS14 consortium in a different type of heavy metals availability

Heavy metals	Goodness of fit			
	R^2	DF	SS	Sy.x
Cadmium	0.6396	22	3.059	0.3729
Chromium	0.825	22	3.016	0.3703
Aluminium	0.7402	22	1.936	0.2967
Zinc	0.8005	22	2.057	0.3058
Nickel	0.8421	22	2.309	0.324
Arsenic	0.6365	22	3.226	0.3829
Cobalt	0.7964	22	3.712	0.4107

Abbreviation: Degree of freedom (DF), Coefficient determination (R^2), sum of squares (SS), standard deviation of the residuals (Sy.x)

Table 2: ANOVA table (best-fit values) for exponential regression curve fit of BS14 consortium in a different type of heavy metals availability

Heavy metals	Best-fit values		
	Y_0 (nm)	DT (h)	k (h^{-1})
Cadmium	0.7654	116.4	0.005957
Chromium	0.4371	65.45	0.01059
Aluminium	0.6522	104.2	0.006653
Zinc	0.6125	88.98	0.00779
Nickel	0.3561	61.27	0.01131
Arsenic	0.8136	119	0.005827
Cobalt	0.5256	71.31	0.00972

Abbreviation: Initial concentration (Y_0), doubling time (T), rate constant (k)

Table 3: ANOVA table for linear regression curve fit of BS14 consortium in a different type of heavy metals availability

Heavy metals	R^2	Sy.x	F	k (h^{-1})
Cadmium	0.7666	0.3001	72.26	0.009469
Chromium	0.921	0.2488	256.4	0.01479
Aluminium	0.8631	0.2154	138.7	0.009414
Zinc	0.9082	0.2075	217.6	0.01136
Nickel	0.9062	0.2497	212.5	0.01351
Arsenic	0.7554	0.3141	67.93	0.009610
Cobalt	0.9082	0.2758	217.7	0.01510

Abbreviation: Coefficient determination (R^2), standard deviation of the residuals (Sy.x), F-value (F), rate constant (k)

0.005827 h^{-1}, respectively. The growth rates can be arranged in the decreasing order of Ni> Cr> Co> Zn> Al> Cd> As.

On the other hand, linear regression also was observed in this study. Linear regression fits a straight line through the experimental data to find the best-fit value of the slope and intercept. According to Fig. 2, the bacterial growth for BS14 with Zn (Fig. 2d) showed the best fitted model as compared to others. The value R^2 (0.9082) values was high while sy.x value, was low (0.2075). This can be considered that the growth pattern of bacteria in the presence of Zn shows as a good model. Although Cr displayed the highest R^2 value at 0.921, the error of the regressed line was high (0.2488). The rate constant (k) of this regression curve can be arranged in decreasing order of Co> Cr> Ni> Zn> As> Cd> Al, where the highest growth kinetic rate was at 0.01510 h^{-1} in Co media. Yet, the bacterial growth in the availability of Zn exhibited the growth rate at 0.1136 h^{-1}, which is considered to be in a high range of growth rate. F-value is an important kinetic parameter for the determination of best-fitted data to the kinetics equation as F value indicates whether or not the linear regression model provides a better fit to the data. The analysis of linear regression also shows 95% confidence interval for the slope. All media with a different type of heavy metals used in this study fell on the range of 95% confidence

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interval. This range value of the slope contain true value of the slope, and the width of the confidence intervals was determined by the number of data points (GraphPad, 2007).

The results of this study suggested that each of the heavy metals affected the growth of bacteria and were able to inhibit or stimulate waste canola oil degradation. Each growth curve can perform different regression fit with different best-fit values for the results as presence of heavy metals changes the behaviour of cell bacterial growth. The obtained results for different growth kinetic regression curves indicate that linear regression curves can better understand the growth kinetics of Antarctic bacterial community with faster rate of bacterial growth, high values of R^2 and low values of error. These kinetics parameters are important in understanding the biodegradation process, including measuring the speed of bioremediation and improvement of efficient clean up for a waste oil contaminated environment.

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