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Modelling the Effect of Copper on the Growth Rate of *Enterobacter sp.* strain Neni-13 on SDS

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ABSTRACT

The introduction of tiny amounts of heavy metals into the environment can encourage the growth of a wide variety of microorganisms. The concentration at which enhanced microbial activity is seen, on the other hand, results in a significant decrease in growth rate as well as an increase in lag time (due to the higher lag time). An established link exists between heavy metal toxicity in microorganisms and the process of bioremediation, which has been well-documented. Because heavy metals have an impact on bioremediation, they must be researched, and appropriate countermeasures must be implemented. Copper reduced the growth of the SDS-degrading bacteria Enterobacter sp. strain Neni-13 to a significant extent. Under varying doses of mercury, the SDS-degrading bacteria exhibited a sigmoidal pattern with time periods ranging from 7 to 10 hours. Gompertz's model was used to calculate the growth rates of copper in different concentrations. As the copper concentration rose, the growth of bacteria was suppressed with a concentration of 1.0 g/L, with virtually total stoppage of bacterial development. From the Gompertz model, we got the estimates of growth rates; after which, they were estimated according to the Han-Levenspiel, Shukor, Wang, Liu, Andrews, and Amor models. The modified Han-Levenspiel, Andrews, Liu, and Shukor models could all successfully fit the curve. Results of the statistical analysis showed that the Han-Levenspiel model was the best model based on highest adjusted correlation coefficient (adR^2), the lowest values for RMSE and AICc, and values of AF and BF closest to unity. The parameters obtained from the Han-Levenspiel model were C_{crit} 0.209 mg/L (95%, C.I., 0.199 to 0.219), μ_{max} 0.209 h^{-1} (95% C.I., 0.199 to 0.219) and m 0.472 (95% C.I., 0.383 to 0.561. The results obtained in this study indicate the maximum tolerable copper concentration that the conditions for biodegradation should not exceed.

INTRODUCTION

It was discovered that the presence of toxic metal ions in polluted wastewater had an inhibitory effect on bacterial growth and the utilisation of a toxic substance. The presence of heavy metals can interfere with biodegradation, which can then interfere with the bioremediation process. It is due to the fact that, in contrast to a number of other inhibitors, heavy metal ions cannot be degraded and once accumulated by microorganisms to a toxic level, result in a reduction in the rate of growth of the microorganism in question. Depending on the microorganism, even different strains of the same species, and even different activities of the same microbial species, there can be significant differences in their sensitivity to metals. When soils with similar physical and chemical properties were compared to one another, it was discovered that the sensitivity of the microbiome that is responsible for acetate mineralization in soils with no history of exposure to elevated metal concentrations differed by orders of magnitude between soils with varying physical and chemical properties [1].

In many cases, the addition of trace amounts of heavy metals to the environment of microbial cells stimulates the growth of the microorganisms [2]. The concentration at which enhanced microbial activity is seen, on the other hand, results in a

significant decrease in growth rate as well as an increase in lag time (due to the higher lag time). Heavy metal toxic effects on microorganisms, particularly sulfate-reducing bacteria, has been demonstrated in a plethora of research. The onset of increased metabolic activity is delayed as a result of raised heavy metal concentrations, and the rate of oxygen mass transfer is decreased as a result of increased heavy metal concentrations. Several decades have elapsed since researchers learned that bacteria, like all other forms of life, are extremely vulnerable to heavy metal exposure. Chemical methods were actually used in some of the earliest attempts to control microorganisms, such as the use of copper chloride in plants as a fungicide and mercury salts in the treatment of certain infectious diseases. For example, copper chloride was used in the treatment of fungal infections and mercury salts were used in the diagnosis of certain infectious diseases. A variety of these metals have been shown to be components or cofactors of enzyme systems in laboratory experiments. Gold, silver, platinum, and other precious metals are common components found in jewellery, and they are available in a variety of grades. It is common for factors such as the organism, the metal, and the chemical and physical makeup of the metal to have an impact on the average concentrations at which the processes take place in a given environment. Heavy metals are only weakly reacted with by the majority of species, and such reactions occur at lower concentrations than interactions with alkali and alkaline earth metals. E. coli is a bacteria that can be found in various doses of stimulation and inhibition caused by magnesium and sodium, which are 100-fold and 1000-fold higher than the concentrations of stimulation and inhibition caused by zinc. E. coli can be observed in multiple doses of stimulation and inhibition triggered by zinc.

Although the investigation of heavy metal toxicity to xenobiotic biodegradation in soils and water bodies can be complicated, a better understanding can be gained by first investigating the effects in a simple medium such as water. It is then possible to use the results obtained to judicially modify the soil by applying soil amendment agents in order to achieve the desired results. Nonlinear models must be used in growth studies in culture flasks to determine the effect of heavy metals on the growth of microorganisms on xenobiotics in order to obtain useful growth and inhibition parameters. Numerous models such as the modified Han-Levenspiel [2], Wang [3], Liu [4], modified Andrews [5], Amor [6] have been utilised [7] to evaluate the result of heavy metal on the bacterial degradation of toxic substance. From these models, inhibition related constants, which include C, C_{crit}, μ, μ_{max}, K_c, K_s, K_i and m which represent heavy metal ion concentration (g/l), critical heavy metal ion concentration (g/l), initial growth rate (g/l h), maximum growth rate (g/l h), inhibition constant (g/l), Monod constant (g/l), metal inhibition constant (g/l) and empirical constant values, respectively, can be found. A previously isolated SDS-degrading bacterium was shown to be strongly inhibited by the heavy metals mercury, silver and copper [8,9] and the models were utilized to study the effect of mercury on growth rates on SDS [10]. The aim of this work is to study the effect of copper on the growth rate of this bacterium on SDS through the use of the above inhibition models.

MATERIALS AND METHODS

Growth and Maintenance of SDS-Degrading Bacterium

The SDS-degrading bacterium—*Enterobacter* sp. strain Neni-13 has been previously reported [8] and was isolated in Padang, Indonesia by the late Dr. Neni Gusmanizar. The growth of the bacterium on SDS was characterized in a microtiter plate format [9,11]. The bacterium was grown on a basal salts (BS) medium containing the followings: Na_2HPO_4, (1.39 g l^{-1}), KH_2PO_4, (1.36 g l^{-1}), KNO_3, (0.5 g l^{-1}), $CaCl_2$ (0.01 g l^{-1}), $MgSO_4$ (0.01 g l^{-1}), and $(NH_4)_2SO_4$ (7.7 g l^{-1}) and 1 mL of trace elements. SDS was added into the medium (filter-sterilized) at 1.0 g l^{-1}. The microplates (Corning® microplate) were incubated sealed at 30 °C and was read at 600 nm (BioRad reader, model 680, Richmond, CA).

Primary Growth Modelling on SDS

The maximum specific growth rate on SDS was modelled according to the modified Gompertz model as this model is routinely used in modelling the growth of microorganisms on xenobiotics [12–14]. The equation is as follows;

$$y = A\exp\left\{-\exp\left[\frac{\mu_m e}{A}(\lambda - t)+1\right]\right\}$$

The value obtained from this primary modelling exercise was then used to model the effect of metal as follows;

Effect of metal on growth rate of on SDS

The models utilized in this study is as follows;

Table 1. Models for the effect of metals on rate inhibition

Models	Equation	Authors
Modified Han-Levenspiel	$r = u_{max}\left(1-\frac{C}{C_{crit}}\right)^m$	[2]
Wang	$r = \frac{u_{max}}{1+\left(\frac{C}{K_C}\right)^m}$	[3]
Liu	$r = \frac{u_{max}K_C}{K_C + C}$	[4]
Modified Andrews	$r = \frac{u_{max}C}{K_s + C + \left(\frac{C^2}{K_i}\right)}$	[5]
Shukor	$r = v_{max}\left(1-\left(\frac{C}{S_m}\right)^n\right)$	[15]
Amor	$r = \frac{u_{max}C}{C + \left(\frac{C^2}{K_i}\right)}$	[6]

Fitting of the Data

CurveExpert Professional software was used to fit the nonlinear equations with the Marquardt algorithm (version 1.6). the algorithm searches for the best method that reduces the sum of the squares between predicted and measured values to the smallest amount possible. with the steepest ascent method, the software calculates the starting values automatically.

Statistical Analysis

To choose the best model, numerous statistical methods including the corrected AICc (Akaike Information Criterion), root-mean-square error (RMSE), bias factor (BF), accuracy factor (AF), and adjusted coefficient of determination (R^2) was utilized as before [16].

RESULTS AND DISCUSSION

With increasing copper content, the total growth was reduced, with 1.0 mg/L resulting in an almost complete halt of growth. The growth of the bacterium at varying copper concentrations follows a sigmoidal pattern, with lag times ranging from 7 to 10 hours between each phase. (**Fig. 1**). The modified Gompertz model then was used to derive growth rates at varied copper concentrations (**Fig. 2**). The model also reveals that with higher copper content, growth rates have been reduced and the lag

period has been increased as well. The modified Gompertz model growth parameters at each copper concentration (**Table 3**) will then be utilized for secondary modelling.

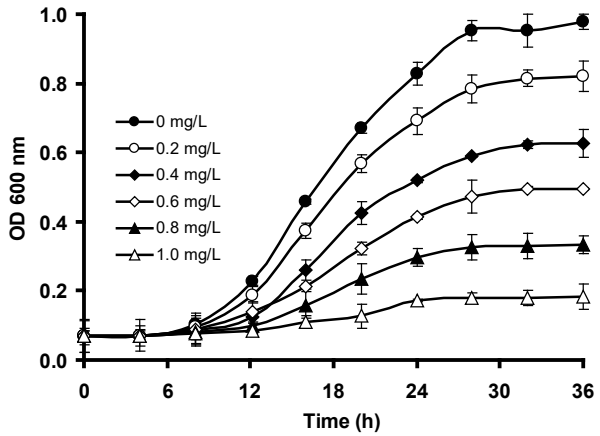

Fig. 1. Growth of *Enterobacter* sp. strain Neni-13 at 1.0 g/L SDS under various concentrations of copper (from 0.2 to 1.0 mg/L). The error bars represent mean ± standard deviation of triplicates.

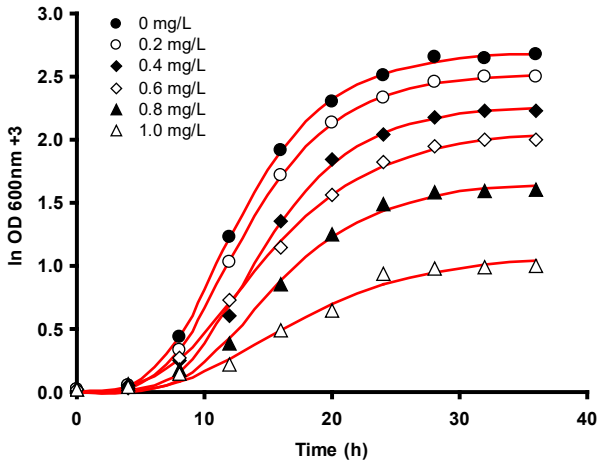

Fig. 2. Growth (ln transformed) of *Enterobacter* sp. strain Neni-13 at 1.0 g/L SDS under various concentrations of copper (from 0.2 to 1.0 mg/L) as modelled using the modified Gompertz model (red curves).

Table 2. modified Gompertz growth parameters for growth (ln transformed) of *Enterobacter* sp. strain Neni-13 at 1.0 g/L SDS under various concentrations of copper.

	0 mg/L	0.2 mg/L	0.4 mg/L	0.6 mg/L
y_{max}	2.695	2.528	2.275	2.076
μ_m (h^{-1})	0.205	0.191	0.169	0.135
lag (h)	6.025	6.579	7.927	6.376

The growth rates at varying copper concentrations were then simulated using the metal inhibition models that were available at the time. Among the five models tested, only the Wang, Shukor, Andrews, modified Han-Levenspiel, and Liu models were successful in fitting the curve (**Figs. 3** to **7**) and Amor model was not able to fit the model. The Han-Levenspiel model was the best model based on the highest adjusted correlation coefficient (adR^2), RMSE and AICc lowest values, and values of accuracy and bias factors closest to one (**Table 3**).

Table 3. Error function analysis.

Model	*P*	RMSE	R^2	ADR^2	AF	BF	AICc
Wang	3	0.02	0.95	0.91	1.16	1.07	-28.04
Han-Levenspiel	3	0.00	1.00	1.00	1.01	1.00	-48.43
Liu	2	0.04	0.49	0.23	1.34	1.17	-30.65
Andrews	4	0.12	0.96	-4.89	1.27	1.16	42.77
Shukor	3	0.01	0.99	0.99	1.05	1.02	-41.73

NOTE:
P: no of parameter
ADR^2: adjusted correlation coefficient
RMSE: root mean square error
AF: accuracy factor
BF Bias factor

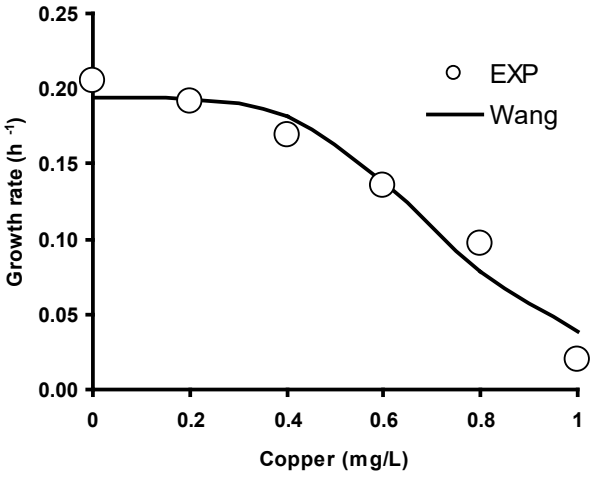

Fig. 3. The effect of increasing concentrations of copper to the specific growth rate of *Enterobacter* sp. strain Neni-13 on SDS as fitted to the Wang model.

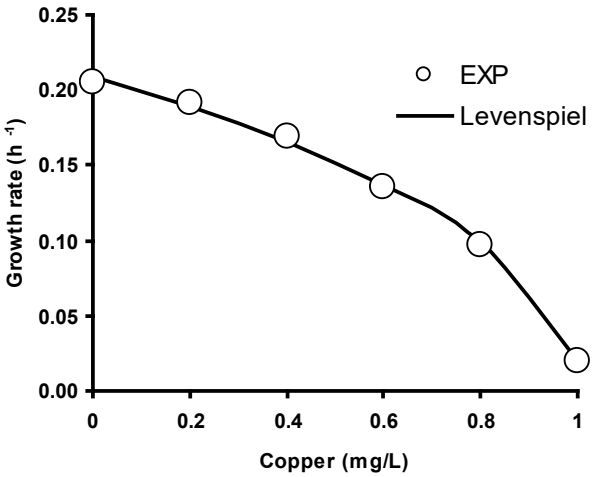

Fig. 4. The effect of increasing concentrations of copper to the specific growth rate of *Enterobacter* sp. strain Neni-13 on SDS as fitted to the Han-Levenspiel model.

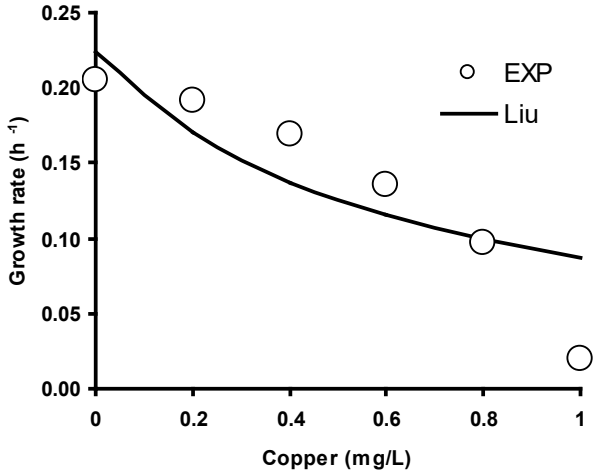

Fig. 5. The effect of increasing concentrations of copper to the specific growth rate of *Enterobacter* sp. strain Neni-13 on SDS as fitted to the Liu model.

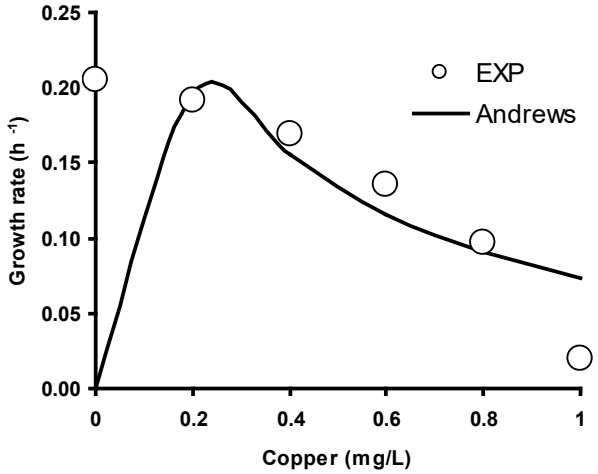

Fig. 6. The effect of increasing concentrations of copper to the specific growth rate of *Enterobacter* sp. strain Neni-13 on SDS as fitted to the Andrew model.

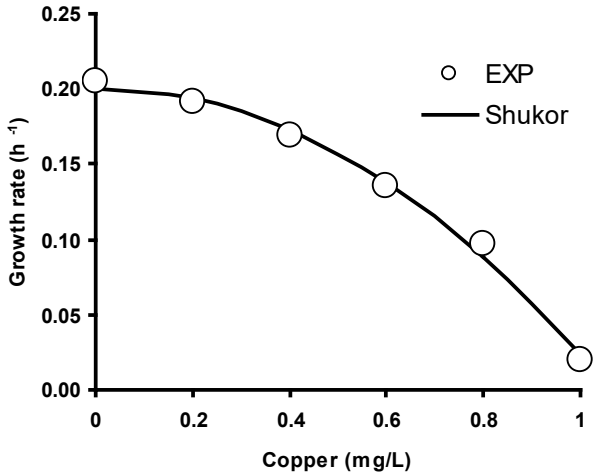

Fig. 7. The effect of increasing concentrations of copper to the specific growth rate of *Enterobacter* sp. strain Neni-13 on SDS as fitted to the Shukor model.

In a previous publication, the modified Gompertz model was used to determine the effect of this bacterium growth rate on SDS at various concentrations of mercury [10]. The only models that could fit the curve were modified versions of Wang, Han-Levenspiel, and Liu. The Andrews and Amor models could not. The parameters obtained from the Han-Levenspiel model (**Table 4**) were C_{crit} 0.209 mg/L (95%, C.I., 0.199 to 0.219), μ_{max} 0.209 h^{-1} (95% C.I., 0.199 to 0.219) and m 0.472 (95% C.I., 0.383 to 0.561. Because of its ability to forecast the threshold heavy metal concentrations that will fully limit bacterial growth, the Han-Levenspiel model has been widely used to model the inhibitory effect of metals to growth rate of microorganisms on xenobiotics [7,10,15,17].

In a specific example, using a batch photobioreactor, researchers investigated the removal of Cu(II) by *Nostoc muscorum*, a cyanobacterium isolated from a hazardous metal-polluted site in Meghalaya, with the goal of elucidating the removal mechanism and the impact on nitrate absorption by the cyanobacterium in the process. The Han-Levenspiel and Andrew models were the most closely matched to the experimental data. The Han-Levenspiel constant; the critical Cu(II) concentration was determined to be 32.5 mg/L [18]. In another study, using a mutant of the bacteria *Pseudomonas* sp., it was determined that there is an inhibitory effect by heavy metal ions on the biodegradation of Congo Red. The critical heavy metals concentrations obtained from the Han–Levenspiel inhibition model for Cr, Zn and Cu were 895, 302 and 204 mg/L, respectively [7].

In the study on the inhibitive effects of heavy metals on Reactive Black 5 decolourization by *Pseudomonas aeruginosa* strain Gb30, the best model modelling the inhibitory effect of heavy metals on the decolourization rate was Han-Levenspiel with C_{crit}, μ_{max} and values of 3496 mg/L (50 mM), 2.013 h^{-1} and 1.193, respectively, for zinc and 280 mg/L (2.491 mM), 1.991 h^{-1} and 0.882, respectively, for cadmium [19].

Table 4. Parameter values for the Han-Levenspiel model.

Parameters	Value	95%, confidence interval
C_{crit} (mg/L)	1.007	0.995 to 1.019
μ_{max} (h^{-1})	0.209	0.199 to 0.219
m	0.472	0.383 to 0.561

Note
C_{CRIT}: critical heavy metal ion concentration (mg/L)
μ_{max}: maximum growth rate (g/Lh)
m : empirical constant

However, despite the fact that heavy metals and organic pollutants are both ubiquitously present in polluted waters, the use of metal inhibition models is underrepresented in the literature. Few studies have investigated the effect of heavy metals on the growth rate of bacteria that are growing in the presence of a toxic substance. According to one study, zinc and nickel inhibited the biodegradation of monoaromatic hydrocarbons by *Bacillus* sp. and *Pseudomonas aeruginosa* significantly, and the effect of these heavy metals on the degradation rate was successfully modelled using the Andrews model [6]. Metals such as gold and silver interact with functional groups in enzymes such as the sulfhydryl group, which is frequently found at the active sites of enzymes, and this is most likely the mechanism of inhibition [7].

Metals can have bactericidal or bacteriostatic effects on bacteria, and these effects can be either positive or negative in nature. When administered at sub-lethal levels, a variety of biochemical and morphological consequences have been

reported. Copper has been shown to transform *E. coli* into spherical forms, whereas platinum has been shown to create exceptionally long filamentous forms in *E. coli* [20,21]. Inhibitory metal interference appears to be causing disruptions in the processes of cell wall formation and cell division, according to preliminary findings. Several organisms develop in the presence of cobalt or copper, and the biochemical makeup of those organisms changes due to a different ratio of macromolecular constituents, notably nucleic acids, and a lower activity of certain oxidative enzymes, particularly porphyrin-containing enzymes, among other factors. In accordance with the presented idea, metals may have harmful impacts on human health because they form strong bonds with various ionic groups on the surface of our cells [22–27].

Increased toxicity results from the metal being more electronegative, which strengthens its bonding and increases its binding strength. Because metals have the ability to form complexes with metal-binding molecules in cells, this concept explains how metals may be detrimental to cells. When cells are growing and developing in a medium containing such compounds, the toxicity of the media is substantially reduced. The amino acids histidine and cysteine, which are both excellent complexing agents, are effective in reversing the growth inhibition of the bacterium Proteus [28,29].

In order to account for this, when cells are grown in the presence of cobalt in basic glucose-salts growth medium, the toxicity of the cells is 1000 times more than that of cells grown in the presence of organic acids. Cobalt and copper have been shown to reverse their inhibitory effects on a variety of bacteria when they are in the presence of organic ligands such as citrate, glutamate, and EDTA, in a similar manner. Chelation may be able to reduce metal toxicity in natural environments, which would be beneficial. If a bacterium that would normally be hindered by metals can survive and grow in ground water or marine sediments that contain complexing agents, it is said to be able to do so. According to recent research, certain organisms may release complexation organic acids into the surrounding environment, which has the effect of detoxifying the environment [28,30–34]. Metals can be removed from solutions in order to make them non-toxic, if desired.

In this application, the precipitation of insoluble metal sulphides in the presence of H_2S is the most conspicuous phenomenon observed. The addition of inorganic ions such as phosphate and thiosulfate to the growth medium resulted in a substantial reduction in the toxicity of copper [35,36]. When manganese is present, cobalt toxicity is reversed in humans; when calcium and copper are present, cobalt toxicity is reversed in yeast; and when zinc is present in lactic acid bacteria, zinc toxicity may be counteracted by the presence of either manganese, magnesium, or calcium, respectively [37]. It is still unclear how these connections function at this time. Many scientists, on the other hand, believe that they represent a direct competition between the inhibitory minerals, such as manganese and magnesium, and the required minerals, such as manganese and magnesium, for enzyme activation sites in the cell [38].

CONCLUSION

After all is said and done, metal inhibition models are only used in a limited number of situations to mimic the impact of metal ions on bacterial growth rates on hazardous substances, which is a pity because this knowledge is critical to a well-functioning biological system. Several metal inhibition models were tested in this work in order to predict the impact of mercury on the growth of an SDS-degrading bacterium, and the Wang model was determined to be the most accurate model. Bacteria will not flourish in Wang because the required heavy metal concentration has been determined in advance of the experiment. Because the bacteria must be able to survive the toxicity of both types of toxicants, it is expected that the pace of development will be significantly slowed when heavy metals exist in the environment. It is possible that the findings of this study will have a significant influence on SDS bioremediation field trial operations when mercury contamination in co-polluted regions is sought.

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