CHEMICAL ENGINEERING TRANSACTIONS
VOL. 117, 2025

A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.cetjournal.it

Guest Editors: Fabrizio Bezzo, Flavio Manenti, Gabriele Pannocchia, Almerinda di Benedetto
Copyright © 2025, AIDIC Servizi S.r.l.
ISBN 979-12-81206-17-5; **ISSN** 2283-9216

DOI: 10.3303/CET25117072

Biological Method with *Pseudomonas putida* for Chromium VI Reduction in Chromium Plating Process Wastewater

Elmer Benites-Alfaro [a*], Danny Lizarzaburu-Aguinaga [b], Carlos Cabrera Carranza[c], Jorge Jave Nakayo [c], Guido Rene Suca-Apaza[d], Jose Freddy Atuncar Yrribari[c], Jorge López Bulnes [c], Felipa Muñoz-Ccuro[a]

[a] Universidad César Vallejo, Av. Alfredo Mendiola 6232, Los Olivos, Lima – Perú
[b] Universidad César Vallejo, Av. Argentina 1795, Callao – Perú
[c] Universidad Nacional Mayor de San Marcos, Lima – Perú
[d] Universidad César Vallejo, Carretera Central Km 8.2, Ate – Perú,
ebenitesa@ucv.edu.pe

The issue surrounding wastewater from the industrial chrome plating process is highly relevant due to its negative environmental and public health impacts. In the chrome plating of metals and other substrates, toxic chemicals are used, including chromic acid and heavy metals that contaminate the water used in the process, generating contaminated effluents that must be treated before disposal into receiving bodies. One of the substances presents in these effluents is the highly hazardous and known carcinogen hexavalent chromium, which requires the development of treatment technologies to reduce or eliminate the effluent. The objective of the research was to reduce hexavalent chromium in industrial chrome plating water samples (M1, M2, and M3) using a biological method using the bacterium's *bacteriums*. Three different concentrations of bacterium were tested in volume percentages (30, 40, and 60 %) for 21 days, with the process being monitored every 7 days. At the end of the trial, the concentration of hexavalent chromium (Cr VI) was reduced by approximately 76 %. It is concluded that this methodology is efficient in the non-toxic reduction of Cr VI, economical compared to physical and chemical methods in the long term, and sustainable as it does not generate toxic waste; but above all, it is a promising and very environmentally friendly methodology.

1. Introduction

Currently, the use of bacterial bio sorbents is a very environmentally advantageous strategy for reducing heavy metals found as contaminants in domestic and industrial wastewater (Pham et al., 2022). Good results have been obtained in the treatment of domestic wastewater with various bacteriium, fungi, and algae, but there is still a gap to be filled in the treatment of wastewater, industrial processes, and other services, where effluents contaminated with specific substances such as chromium, lead, mercury, recalcitrant compounds, among others, are generated. Therefore, it is also necessary to establish specific studies for the biosorption of each contaminant, taking into account certain operational conditions, efficient treatment parameters, and the type of microorganisms (Inoue, 2017), capable of using contaminants for their growth (Ayangbenro, 2017). The heavy metal Chromium in its hexavalent state is very dangerous for living beings, even causing cancer, it is very commonly present in wastewater from various industries including tanning, steel industry and metal chrome plating (Sharma, 2022); therefore, preventive measures must be taken against this contaminant that poses a great risk to human health mainly (Georgaki M., and Charalambous, 2023).
Bioremediation is one of the methods that can be used to reduce hexavalent chromium. As a bioremediation technique for chromium (VI), there is the use of biomass in various forms, such as using municipal dried sludge as a permeable bio reactive barrier (Kholisa et al., 2022); also, with the use of *Pseudomonas putida* bacterium, which have already been evaluated with very promising results for the treatment of domestic wastewater, they have also been used for the treatment of wastewater contaminated with Cr (VI) with an efficiency of 98.6% in 26 days (Mendoza-Vela et al., 2022). There is a study where the bacterium *Pseudomona putida* was used to decontaminate wastewater from the tanning industry, achieving 96 % removal of Cr (VI) in 7 days (Liu, et al.,

Paper Received: 30 December 2024; Revised: 27 March 2025; Accepted: 22 May 2025
Please cite this article as: Benites-Alfaro E., Lizarzaburu Aguinaga D., Cabrera Carranza C., Jave Nakayo J., Suca-Apaza G.R., Atuncar Yrribari J.F., Lopez Bulnes J., Munoz-Ccuro F., 2025, Biological Method with Pseudomonas Putida for Chromium Vi Reduction in Chromium Plating Process Wastewater, Chemical Engineering Transactions, 117, 427-432 DOI:10.3303/CET25117072

2021). Scientific literature indicates that, in bioremediation, the bacterium that are most successful in their remediation work are those that have been subjected to an environment with the presence of the contaminant to be treated, since they are bacteriium that are already resistant to the contaminant with maximum stress, which makes them optimal for decontamination processes (Wang et all., 2015). In this sense, it has been found that the bacterium *Bacillus amyloliquefaciens*, isolated from the soil of a chromite mine, with high tolerance to Chromium (VI) (less than or equal to 900 mg/L), achieved a reduction at a rate of 2.22 mg of Cr(VI)/(L*h), under optimized conditions of 100 mg/L of Cr(VI), at pH 7 and temperature of 35 °C in a time of 45 hours, this study by AAS analysis of the culture products treated with Cr(VI) found that the reduced Cr(III) was distributed in the pellet of the microorganisms (bacterial cell surface) the culture supernatant (37.4+/-1.7 and 62.6+/-3.4 respectively) (Das, et al., 2014). Given this background, the research aimed to evaluate the removal of hexavalent chromium from wastewater generated in the chrome plating process (little studied with this method) using *Pseudomonas putida* bacterium at laboratory level.

2. Methodology

The research process followed the following phases:

Characterization of *Pseudomonas Putida*

Pseudomonas putida was obtained from waste tubers from a vegetable and tuber market. It was extracted from corn crowns because this is the area where they are found, also from short-stemmed vegetables from the agricultural area of the Chuquitanta farm. Two bioreactors of 80 cm in length and 15.24 cm (6 inches) in diameter were used.
The vegetable residues were cut in small size and 300 ml of frying oil was added, water was added in a volume of 7 Liters, the reproduction was aerobic and after 10 days the pseudomonas strains were obtained. Simmons Citrate differential agar was added in a volume of 100 ml and then proceeded to evaluate the bacterium colonies formed, identified by their cylindrical shape with celios and in some cases are bright green, proceeded to count them in colony counter that had carbon and ammonium substrates, potassium phosphate, sodium citrate, presenting a pH of 6.8. With Eq(1) the colony number was obtained.

$$\text{UFC} = \frac{\text{Number of colonies per plate} * \text{ dilution factor (inverse)}}{\text{mL of the seeded solution}} \tag{1}$$

Procedure: The colony counter was used to establish the number of colonies, while identification was performed with an electron microscope. The multiple plate method was used.

Treatment of chromium plating wastewater samples with *Psudomona putida* bacterium

In this second phase, the three wastewater samples from the chrome plating process were treated by adding a solution with *Pseudomonas putida* bacterium in three concentrations. The concentrations prepared were 30, 40 and 60 %. See Table 1.

Table 1: Concentrations of Pseudomonas putida used in treatment

Sample	Concentration (%)	Dilution volume with bacterium (mL)	Number of colonies (CFU/mL)
M1	30	240	2080008
M2	40	320	2773344
M3	60	480	4160016

The evaluation and control were carried out 7, 14 and 21 days after the three samples (with three replicates) were subjected to treatment with *Pseudomonas putida*.

Method of physicochemical analysis of wastewater samples

To determine hexavalent chromium in the samples, the spectrometry method was used, considering dilution factors of 1/500 and 10/100. The wastewater samples were coded as M1, M2, and M3.
The potentiometric method was used to determine turbidity, temperature, pH, electrical conductivity, and redox potential. The gravimetric method was used for total suspended solids.

3. Results and discussion

The experimental design involved treating three wastewater samples under the same initial conditions with *Pseudomonas putida* bacterium at three different concentrations (30, 40, and 60%). The evaluation was carried out over a period of 7, 14, and 21 days, with hexavalent chromium concentrations in the wastewater being monitored in triplicate.

Initial Hexavalent Chromium (Cr+6) concentration in industrial wastewater

Industrial effluents from the chromium plating process in an industrial plant had a Cr^{+6} concentration of 23975.767mg/L.

Evaluation of Cr^{+6} Concentration at 7, 14 and 21 days of treatment with *Pseudomonas putida*

After 7 days of treatment, it was observed that sample M1 treated with 30 % *Pseudomonas putida* bacterium reduced the average hexavalent chromium concentration to a greater extent (13720.07 ± 17.50) compared to the other samples, followed in reduction level by samples M2 and M3. The treatment temperature was 22.10 °C for all cases (See Table 2), which presents the test results for samples M1, M2 and M3 performed in triplicate. The last column shows the average with the +/- statistical error of the reductions for the three samples.
This mechanism and biosorption capacity of bacterium depend on the nature of the contaminant. In this case, chromium (VI) penetrates the bacterial cells and is reduced to chromium (III), the most stable form. The bacterial cell envelope contains anionic lipopolysaccharide (LPS) molecules, phospholipids, and proteins, facilitating this remedial action (Killic et al., 2010). Therefore, the bacterium exert a significant chromium (VI)-reducing effect.
The results after 14 days of treatment of the same samples M1, M2, and M3 using *Pseudomonas putida* (at 30, 40, and 60 %) show that, for all three samples, the concentration of hexavalent chromium is reduced to levels greater than that of the 7-day treatment; sample M1 showed the greatest reduction. (10881.61 ± 8.6 mg/L) using *Pseudomonas putida* at 30 % (see Table 2).
From the analysis of the average hexavalent chromium reduction results at 7 and 14 days, it increases over time; however, not exponentially, this could correspond to a certain slowdown in the reduction, probably due to the consequence of oxidative damage and denaturation in the cell membrane of the bacterium, which weaken the bioremediation capacity (Igiri et al., 2018).

Table 2: Average hexavalent chromium in wastewater at 7, 14 and 21 days of treatment

Sample	0 Days (Initial)	7 Days		14 Days		21 Days	
	Concentration of Cr^{+6} (mg/L)	Concentration of Cr^{+6} (mg/L)	Reduction (%)	Concentration of Cr^{+6} (mg/L)	Reduction (%)	Concentration of Cr^{+6} (mg/L)	Reduction (%)
M1	23975.767	13720.07±17.5	42.78	10881.61 ± 8.6	54.61	5656.68 ± 7.5	76.41
M2	23975.767	15886.18±29.8	33.74	12674.96 ± 23.8	47.13	5714.002 ± 2.8	76.17
M3	23975.767	17970.43±13.8	25.05	13597.78 ± 29.1	43.29	5743.39 ± 4.2	76.04

The control after 21 days of treatment shows a lower concentration of Chromium (VI) than at the beginning, in the three samples treated with *Pseudomonas putida* (see Table 2). However, it is in sample M1 treated at 30 % concentration of the aforementioned bacterium, where it presents a slightly greater reduction of hexavalent Chromium, the sample presented an average of 5656.68 ± 7.5 mg/L of hexavalent Chromium. At this time, samples M2 and M3 also showed a good reduction that the shorter the treatment time.

3.2. Comparison of percentage reduction of Chromium VI in samples treated with *Pseudomonas p.*

The treatment on sample M1 reached the highest reduction at the level of 76.41 % after 21 days, in the other samples a similar value was obtained slightly lower 76.17 % and 76.04 % for samples M2 and M3 respectively. According to the analysis of the trend lines (slope) in Figure 1, it can be established that, the reduction of chromium in sample M3 is more accelerated, continuing in sample M2 and then in sample M1 (it would have to be verified by extending the investigation to a longer treatment time), therefore, it is not ruled out that at a time greater than 21 days, it is sample M3, treated with *Pseudomonas putida* at a concentration of 60 %, where the greatest reduction of hexavalent chromium occurs, this being logical, since it presents the highest concentration of bacterium present.
The use of *Pseudomonas putida* bacterium is a viable method for remediating contaminated water with many environmental advantages; however, the relatively long time must be overcome to be as successful as possible. This requires further research to optimize treatments, primarily taking into account the characteristics of the

contaminants. Sánchez-Ríos et al., (2020) tested the reduction of hexavalent chromium to Chromium III with *Pseudomonas putida* isolated from wastewater through a bio electrochemical process (where the bacterium generate electrical energy), obtaining a 98 % reduction, a value higher than that achieved in this research (76.41 %), verifying the theory that bacterium isolated from wastewater have better reducing activity than bacterium obtained from plants (used in the present investigation). Another investigation achieved 95 % reduction of chromium (VI) with strains isolated from wastewater and 85% for strains from plants (Amini et al., 2021); Echevarría et al. (2001) achieved a 91 % reduction using microorganisms isolated from seawater. The increased efficiency of wastewater bacterium is due to their development of adaptive mechanisms to survive under stressful conditions due to the high concentrations of heavy metals present. These results suggest that the use of Pseudomonas strains isolated from wastewater contaminated with Cr(VI) may be an effective strategy for the bioremediation of this contaminant.

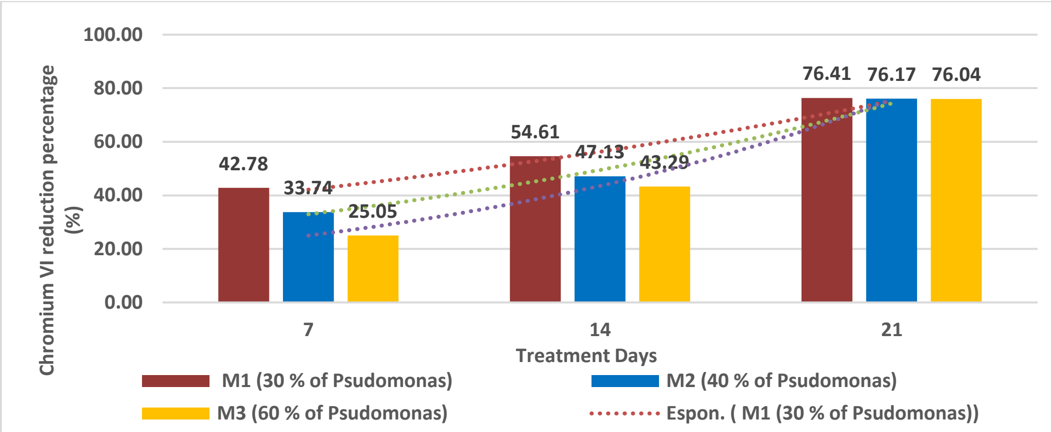

Figure 1: Comparison of percentage reduction of chromium VI in samples

Evaluation of the physicochemical properties of the samples at 7, 14 and 21 days of treatment

In the research, in parallel with the reduction of chromium (VI) in wastewater with bacterium, the physicochemical properties of the water samples M1, M2 and M3 were also monitored, with the results presented in Tables 3, 4 and 5. Sample M3, after 21 days, treated at an average temperature of 21 ° C, obtained a better result in chromium (VI) reduction, as shown in Table 8, compared to the M2 treatments (20 ° C); this corroborates the theory that at higher temperatures, Pseudomonas putida increases its capacity to reduce chromium (VI). Research indicates that at 40 ° C the bacterium is capable of reducing chromium (VI) at concentrations of up to 100 mg / L by 90 % (Singh, et al., 2023). Another aspect to take into account is the pH, at 7, 14 and 21 days the lowest pH values were when there was less chromium reduction, but as the days passed the pH in the samples rose at the same time there was more chromium reduction; this verifies the influence on chromium reduction, so it can be said that at low pH *Pseudomonas putida* has a low capacity to reduce chromium VI (Khan et al., 2024).

Table 3: Physicochemical characteristics of the wastewater after 7 days of treatment

Physicochemical Parameters	7-day average		
	Sample M1	Sample M2	Sample M3
Temperature (° C)	22.10±0.00	22.10±0.00	22.10±0.00
pH	1.17±0.033	1.60 ± 0.013	2.14 ± 0.010
Electrical conductivity (µS/cm)	3540 ± 80	2540 ± 20	1520 ± 13.33
Redox potential (mV)	486.67 ±4.44	427.33±8.22	354.33±4.66
Total suspended solids (mg/L)	453±233.33	495±218	495±218
Chemical oxygen demand (mgO2/L)	1988.6±0.038	1986±0.038	1984.01±0.178
Dissolved oxygen (mgO2/L)	0.03 ± 0.001	0.04 ± 0.001	0.04 ± 0.001

Table 4: Average physicochemical characteristics of wastewater after 14 days of treatment

Physicochemical Parameters	14-day average		
	Sample M1	Sample M2	Sample M3
Temperature (° C)	20.20±0.004	20.20±0.03	20.20±0.007
pH	1.44±0.007	2.02±0.017	2.62±0.013
Electrical conductivity (µS/cm)	3684.33±2.889	2742.66±2.333	2007.66±2.333
Redox potential (mV)	322.00±0.667	266.66±1.667	187.00±2.00
Total suspended solids (mg/L)	2749.33±92.222	2654.00±93.00	3111.00±93.00
Chemical oxygen demand (mgO2/L)	1980.22±0.284	1976.53±0.150	1973.27±0.043
Dissolved oxygen (mgO2/L)	0.042±0.001	0.054±0.000	0.077±0.000

Table 5: Average physicochemical characteristics of wastewater after 21 days of treatment

Physicochemical Parameters	21-day average		
	Sample M1	Sample M2	Sample M3
Temperature (° C)	21±0.00	21±0.00	21±0.00
pH	1.87±0.011	2.11±0.000	2.68±0.013
Electrical conductivity µS/cm	3739.68±3.778	2845.33±1.333	2078.67±2.333
Redox potential (mV)	322.00±0.667	266.68±1.667	189.68±0.333
Total suspended solids (mg/L)	5608.33±69.111	2897.33±120.667	1411.00±11.00
Chemical oxygen demand (mgO2/L)	1980.23±0.284	1976.53±0.150	1973.27±0.043
Dissolved oxygen (mgO2/L)	0.042±0.001	0.054±0.000	0.077±0.000

At the end of the 21-day process, comparing the physicochemical properties of the chromium-containing water before and after 21 days of treatment with Pseudomonas putida, the greatest reduction in hexavalent chromium was obtained in sample M3 (see Table 6). The electrical conductivity (EC) of the wastewater decreased as a result of ionization and ion exchange between bacterium and chromium (VI) (Volesky and Holan, 1995); this is related to the reducing activity of the bacterium, which explains the variation in the redox potential, i.e., the chemical oxidation-reduction energy (Schaller, n.d.). The treatment also improved dissolved oxygen (DO) in the water and reduced chemical oxygen demand (COD), and also decreased total suspended solids (TSS).

Table 6: Variation of the physical-chemical characteristics of water

Time (días)	Sample	Temperature (° C)	pH	Redox potential (mV)	COD (mgO_2/L)	DO (mgO_2/L)	TSS (mg/L)	EC (µS/cm)
0	M0	21	0.9	540.00	1994.24	0.023	5023.00	4985.00
21	M3	21	2.66	189.67	1973.27	0.077	1411.00	2078.67
Variation (%)		0	195	65	1	235	72	58

*M0=Original sample; **M3 (sample treated with 60 % *Pseudomonas putida* bacterium)

4. Conclusion

It was established that the use of *Pseudomonas putida* bacterium is an efficient biological method for reducing hexavalent chromium present in wastewater from the chrome plating process in the metal coatings industry. The research achieved an average reduction of approximately 76 % of this contaminant, and it also allows for the improvement of other physicochemical parameters. These results are promising in the field of bioremediation of effluents containing hexavalent chromium and should be taken as a reference for large-scale implementation. Although the method has environmental advantages, the main disadvantage is the treatment time, since better results are obtained with longer application times. However, this bioremediation method is validated by the minimal generation of waste or polluting products compared to other chemical methods. An advantage is the potential use of biofuels generated by the microorganisms.

Acknowledgments

The authors and the GITA Group thank the Vice-Rectorate for Research at César Vallejo University for the execution of this research work.

References

Amini S.M., Shashi M.A., Mousavi M.S., 2021, Comparison of Cr (VI) reduction ability of Pseudomonas strains isolated from wastewater and vegetables, Journal of Environmental Science and Health, Part A, <10.1080/10934529.2020.1868176.>.

Ayangbenro A.S., Babalola O.O, 2017, A new strategy for heavy metal polluted environments: A review of microbial biosorbents, Int. J. Environ. Res. Public Health, 14, 94, < doi.org/10.3390/ijerph14010094>.

Das S., Mishra J., Das SK., Pandey S., Rao DS., Chakraborty A., Sudarshan M. Das N., Thatoi H., 2014, Investigation on mechanism of Cr(VI) reduction and removal by Bacillus amyloliquefaciens, a novel chromate tolerant bacterium isolated from chromite mine soil, Chemosphere, 96: 112-121, < 10.1016/j.chemosphere.2013.08.080>.

Echeverri G., Ramirez L., Saez J., Escorcia A., 2021, In Vitro Evaluation of Bio Reduction of Hexavalent Chrome by Marine Microorganisms Isolated in the Cartagena Bay for Wastewater Treatment, Chemical Engineering Transactions, 86, 655-660.

Georgaki M., and Charalambous M., 2023, Toxic chromium in water and the effects on the human body: a systematic review, J Water Health, 21 (2): 205–223, <doi.org/10.2166/wh.2022.214>.

Igiri B.E., Okoduwa S.I.R., Idoko G.O., Akabuogu E.P., Adeyi A.O., Ejiogu I.K., 2018, Toxicity and Bioremediation of Heavy Metals Contaminated Ecosystem from Tannery Wastewater: A Review, J Toxicol, doi: 10.1155/2018/2568038. PMID: 30363677; PMCID: PMC6180975.

Inoue K., Parajuli D., Ghimire K.N., Biswas B.K., Kawakita H., Oshima T., Ohto K., 2017, Biosorbents for removing hazardous metals and metalloids, Materials, 10, 857, <doi.org/10.3390/ma10080857>

Khan M.A., Naqvi S.A.., Khan M.F., 2024, Effect of pH on the Cr (VI) reduction capacity by Pseudomonas putida, Environmental Pollution, < 10.1016/j.envpol.2024.118674>

Kholisa B., Matsena M.T., Chirwa E.M.N., 2022, Biotreatment of Hexavalent Chromium in a Rectangular Column Using Municipal Wastewater Sludge as a Bioreactive Barrier, Chemical Engineering Transactions, 94, 1405-1410.

Kiliç N. K., Stensballe A., Otzen D. E., and Dönmez G., 2010, Proteomic changes in response to chromium(VI) toxicity in Pseudomonas aeruginosa, Bioresource Technology, 101(7), 2134–2140. <10.1016/j.biortech.2009.11.008>

Liu Y., Zhang Y., Wang X., Li Y., and Zhang H., 2021, Removal of hexavalent chromium from tannery wastewater by immobilized Pseudomonas putida, Journal of Hazardous Materials, 398, 125237.

Mayta Mendoza-Vela J., García-Pérez C., and Pérez-López V., 2022, Biological treatment of chromium (VI) with Pseudomonas Putida in a concentric-tubes Airlift bioreactor, [in Spanish], Revista de la Sociedad Peruana de Microbiología, 46(1), 23-28.

Pham V.H.T., Kim J, Chang S, Chung W. Pham V.H.T., Kim J, Chang S, Chung W. Bacterial Biosorbents, an Efficient Heavy Metals Green Clean-Up Strategy: Prospects, Challenges, and Opportunities. Microorganisms. 2022; 10(3), 610, doi.org/10.3390/microorganisms10030610,

Sánchez-Ríos A., Sánchez-García M. A., Fernández-Lorenzo J.M., Martínez-Fernández M.F., 2020. Effect of the origin of Pseudomonas strains on the bioremediation of Cr (VI)-contaminated wastewater, Revista: Water Research, <10.1016/j.watres.2020.115791>

Schaller C., s.f., Structure and Reactivity in Organic, Biological and Inorganic Chemistry, Part V: Reactivity in Organic, Biological and Inorganic Chemistry 3, <employees.csbsju.edu/cschaller/Reactivity/redox/ROrednpotl.htm>

Sharma P., Singh S.P., Parakh S.K., Tong Y.W, 2022, Health hazards of hexavalent chromium (Cr (VI)) and its microbial reduction, Bioengineered,13(3), 4923-4938, <doi: 10.1080/21655979.2022.2037273.>,

Singh A.K., Tiwariu V.K., Dwivedi., 2023, Effect of temperature on the Cr (VI) reduction capacity by Pseudomonas putida, Bioresource Technolog, < 10.1016/j.biortech.2023.124389>

Volesk B. and Holan Z.R., 1995, Biosorption of heavy metals, Biotechnology Progress, 11(3), 235-50. <10.1021/bp00033a001. PMID: 7619394.

Wang P.F., Ma Y.Y., Wang C., Zhang S.H., Cheng S., 2015, Isolation and Characterization of Heavy Metal-Resistant Bacterias Capable of Removing Cr(VI, Polish Journal of Environmental Studies, 24 (1) , pp.339-345.