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Providencia rettgeri AVRB20 with multiple tolerance of Heavy metals from municipal solid waste dump site

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Abstract

The presence of toxic heavy metals (HMs) from municipal solid waste (MSW) hoists serious concern about the adverse impact to the environment. These researches focused to isolation and identification of native metal resistant bacteria and assess their efficacy against heavy metals. Metal resistant strains were isolated by pure culture technique. Identification of strains based on morphological, biochemical and molecular characteristics. The bioremediation efficacy tested by enrichment method of different metals. Twenty-five stains were selected, among these *Providencia rettgeri* AVRB20 (Genbank accession number MH327529) had the multiple metal resistance. Growth and viability of isolate was measured by enrichment of metals separately, 33.7% of viable cells observed in medium enriched with 100 mg/l of Arsenic. Likewise, 33.3 % of cells in Lead and 63.8% of cells in Nickel. Efficacy of metal degradation measured, the strain AVRB20 effectively degrade 51.4 % of Arsenic, 76.5 % of Lead and 80.9 % of Nickel. Effective metal degradation ability of *Providencia rettgeri* AVRB20 is suitable for bioremediation of HM pollution.

1. Introduction

Waste management is a challenging issue, as a result of rapid urbanization and population explosion. Globally, increasing concentration of heavy metals (HM) in municipal solid waste (MSW) is major threat to the living organism in the ecosystem. Open dumping of MSW is generating leachate by rainwater percolating through the waste layers in a landfill. It contains a large amount of contaminants among the various hazardous substance HM are harmful pollutants always associated with municipal solid waste & contaminate the surrounding environment [14]. Significant amount of HM are enter into the environs through the disposal of waste by way of open dumps and landfills. [10]. Metals like Arsenic are the rich element, present most part of our environment. It is easily attached fine particle in the air and stay for many days. Arsenic mainly used as an herbicide, insecticide, germicide, medicine

and wood preservatives also in electronics and industrial manufacturing industries [24]. When dumping of these wastes to landfill can get into to the environment through the leaching process. Continuing exposure of arsenic induces cancer, skin lacerations and cardiovascular defect, and inhalation of Arsenic affects respiratory and nervous system [22]. Many of the peoples from Argentina, Chile, China, Hungary, Mexico and USA were showing to elevated amount of As through drinking of As polluted ground water [3,17].

The widespread use of lead can contaminate environment for example, use agricultural pesticide of lead arsenate will enter in to the ground water. EPA declared that dump is a site dispose waste without environmental controls and municipal, industrial waste are disposed directly to the landfill but in sanitary landfill waste managed with environmental production standard [21, 13]. Lead is the naturally occurring toxicants, and affects gastrointestinal, cardiovascular, renal, hematological, neurological and immune function [23]. Nickel distributed in water, soil and air, and trace level of Ni essential for various metabolic activities of all organisms, however long term exposure can cause lung cancer, respiratory failure, birth defect and heart disease [5].

Microorganism present in the polluted environment can develop special mechanism to tolerate or remove toxic metals. The occurrence of organic and inorganic substances in landfills are highly attracted by microorganism especially bacteria are greatly reduce the level of HM from the environment. Bioaccumulation, bio sorption and bio reduction are the successful process for biodegradation of metals [15].

Some plants have a vital role in the heavy metal removal mechanism, according to Stofejova et al., 2021, Ar and Pb are not essential for the plant, and they accumulate on plant parts and cause toxic effects. Irrigation of metal-contaminated water or plants grown in polluted soil will increase the level of heavy metals in plants.

Hence the research goal is to examine the capability of native bacteria from landfill involved in reducing the level of HM (**As**, **Pb** and **Ni**). Naturally occurring metal resistant bacteria was isolated and characterized to reduce the level of bacteria. To ensure the level of resistance and degradation is useful for the determination of bacteria is suitable for bioremediation.

2. Methodology

2.1. **Location and sample collection**

Municipal solid waste and leachate samples were collected from landfill dumpsite Madurai, Tamil Nadu, India. The samples were kept in pre sterilized bags and bottles and stored at 4 °C for further microbiological analysis.

2.2. **Isolation and selection of HM resistant bacteria**

In the effective isolation process one gram of solid waste sample and one ml of leachate were mixed with 98 ml of saline water (8 g NaCl in 1000 ml distilled water) for 24 hours, under shaken (180 rpm). Screening and isolation of pure culture done with 0.1 ml of sample inoculated on Luria Bertani (LB) agar plate enriched by HMs (10 mg/l of Pb as $PbCl_2$) then incubated on 37^o C. After 2days of incubation, plates were witnessed the growth of any culture on plates. After preliminary screening of metal degrading bacteria, pure culture method was done to isolates desired bacteria. Control plate was prepared without HM in LB agar. Colonies were picked and purified then preserved for further analysis.

2.3 Biochemical, Molecular and Phylogenetic analysis

The pure culture of metal tolerant strain was characterized by morphologically and biochemically using standard procedure from Bergey's Manual of Systematic Bacteriology 1994 [8,4]. According to Molecular identification of the strain AVRB20 was done by using 16Sr DNA analysis. DNA was extracted, and then amplification of bacterial genome carried out with polymerase chain reaction (PCR). Forward (5'-AGAGTTTGATCCTGGCTCAG-3') and reverse primers (5'-ACGGCTACCTTGTTACGACT-3) were used for PCR analysis. Steps involved in PCR amplification are initial denaturation at 94°C for 4 min; 30cycles for amplification of 60S at 94°C, 60s at 55° C, and 90s at 72° C respectively. Sequence was examined on the National Center for Biotechnology Information (NCBI). Phylogenic tree constructed by Maximum Likelihood method [20] was built to deliver a graphic representation of the patterning of relationship among individuals. MEGA version 7 software is highly useful to construct phylogenic tree [11].

2.4 Multiple metal resistance assay

Totally 25 isolates were selected for multiple metal tolerant assay. Isolates were cultured on(LB broth) enriched by As, Ni and Pb (50mg/l) and incubated 37° C for 48 hours, after incubation the multiple resistance isolate was collected and preserved and stored for further analysis.

2.5 Efficiency and growth of metal resistant strain

Among the 25 isolates one strain AVRB20 has the ability to tolerate As, Pb and Ni. To measure the efficiency of selected strain was experienced with increasing concentration of metals, and growth measured by OD at 600nm. The bacterial culture (1 ml) inoculated 100 ml of LB broth amended with increasing concentration (0, 25, 50,75and 100mg/l) of HMs (**As** as $AsCl_2$, Pb as $PbCl_2$ and Ni as $NiSo_4$) separately. All the culture was incubated at 37°C for 3 days. Growth of metal resistant bacteria were monitored the absorbance at 600 nm.

2.6 Heavy metal degradation assay

One ml of cells was inoculated in LB broth (100 ml) dissolved with 100 mg/l As, Pb and Ni respectively. Culture was incubated at 37°C for 3 days, then culture was centrifuged at 5000 rpm (15 min) then supernatant was collected and digested with concentrated HNO_3(10ml) at 80°C Allen et al., 1986) [1]. The resulting solution was analyzed for concentrations of HMs using an atomic absorption spectrophotometer (Modal-ELICO, SL173) APHA, 2005 [2]. Heavy metal degradation capacity (%) calculated by :

$$\%\text{HM utilized} = \frac{\text{HM utilized mg/L}}{\text{Concentration of HM in medium mg/L}} \text{x } 100$$

HM utilized mg/l = Initial concentration of HM in broth mg/l-Final concentration of HM in broth mg/l

3 Result and discussion

3.3 Isolation and molecular identification of HM resistant bacteria

Twenty-five isolates were obtained from municipal solid waste and leachate mixed sample. All the isolated have the ability to grow in 10 mg/l of Pb containing LB agar medium. Further the isolates were involved for multiple metal tolerance tests with 50 mg/l of **As, Pb** and **Ni** respectively. Based on the study the strain AVRB20 was more suitable for the bioremediation of heavy metal. The chosen

bacteria is a gram negative, motile and rod shaped bacteria. The biochemical analysis showed that this belongs to the family *Enterobacteriaceae* and genus *Providencia*. Molecular identification by gene (16S rRNA) amplification and sequencing showed that the isolate had 100% similarity with *Providencia rettgeri*; isolated strain was submitted in Genbank (Accession number MH327529). Phylogenetic analysis done with BLAST similarity search, and tree constructed with Neighbor joining method. (Figure 1) the present isolate designated as *Providencia rettgeri* AVRB20. Similarly a study conducted by Shardendu et al., 2017, they isolated arsenic resistant bacteria *Paracoccus denitrificance* , *Alcaligens faecalis* and *Stenotrophomonas maltophila* from waste water. In the same way a study isolated arsenic resistant *Rhizobacteria* FB4 and FB9 with maximum tolerance of arsenic 30-1000 ppm [19].

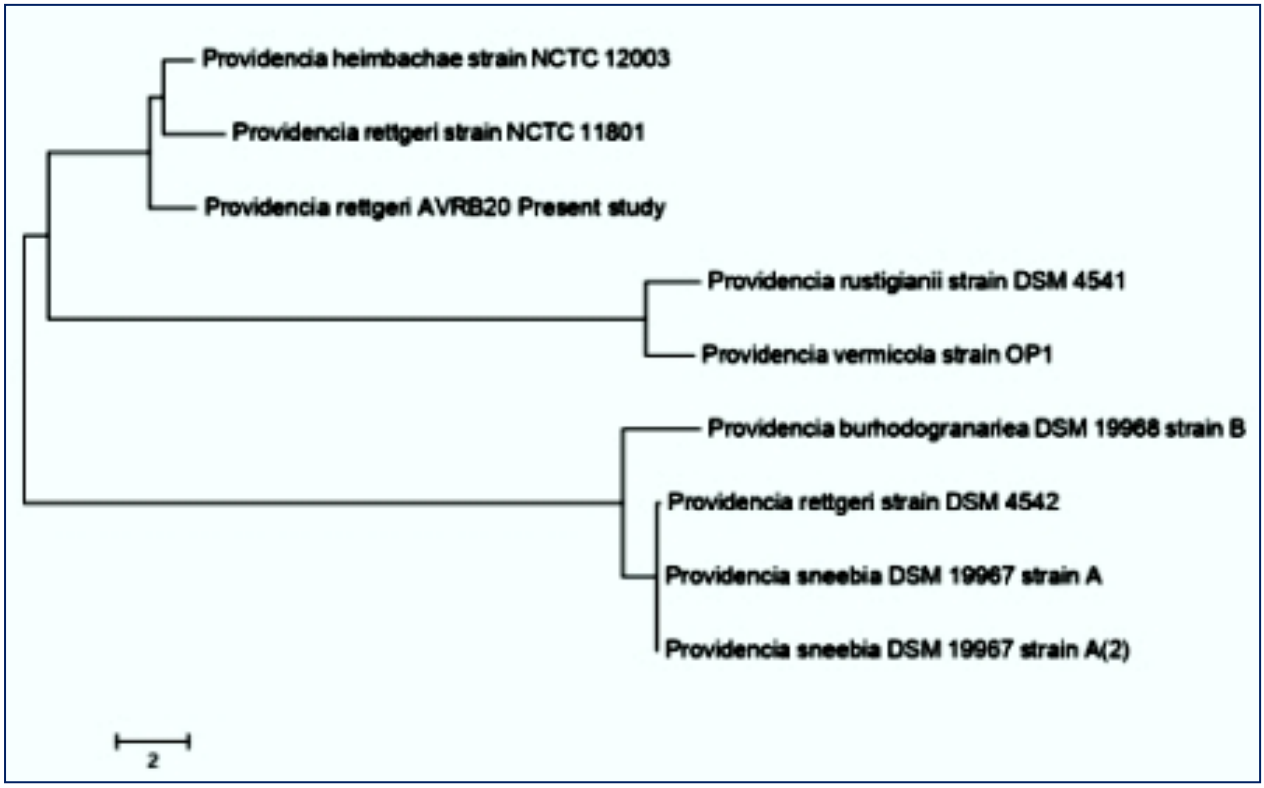

Figure 1. Phylogenetic tree of isolate *Providencia rettgeri* AVRB20

3.4 Multiple metal resistance assay

Naturally the bacteria present in contaminated area, are developed their resistance mechanism against the pollutant. For example, number of genes responsible for their resistance mechanism *Paracoccus denitrificance* gene encoding arsenical resistant protein arsenate reductase, *Alcaligens faecalis* gene encoding arsenite reductase , arsenite oxidase and arsenical pump proteins for As(V) and *Stenotrophomonas maltophila* have genes for arsenite transmenmbrane efflux pump transporter protein, arsenate reductase and arsenic resistant transmembrane for As (III)) [17]. Nickel, lead and zinc resistance observed on *Staphylococcus epidermidis*, *Proteus mirabilis* and *Escherichia coli* [12]. Bacillus is a spore producing bacteria and habitat in extreme environment, previous study isolated heavy metal resistant *Bacillus thuringiensis* and *Bacillus cereus* [6,25].

Municipal solid waste and leachate harbors numerous microorganisms which have evolved number of methods to survive under toxic environment. Figure 2 shows growth and viability of isolates under various trial conditions. The growth and viability of isolates were determined by turbidity in the culture

medium by measuring absorbance at 600nm spectrophotometrically. The metal resistant isolate was cultured in LB medium amended with HM. The cells were cultivated in increasing concentration of **As, Pb** and **Ni** separately 0, 25, 50, 75 and 100 mg/l. AVRB20 highly resistant against Ni with 98.3%of cells observed in medium enriched with Ni (25 mg/l concentration) 91.9, 81.6 and 63.8%of cells observed in 50,75 and 100mg/l of Ni enriched medium. Similarly, 80.8,71.9, 56.1 and 33.7 % of cells witnessed in **As** enriched medium and 74.3, 60.1, 45.3 and 33.3 5 cells in **Pb** containing medium of 25, 50, 75 and 100mg/l respectively. The overall investigation reported that AVRB20 isolate grow well in less concentration of HMs however growth slowly decreased when concentration increased.

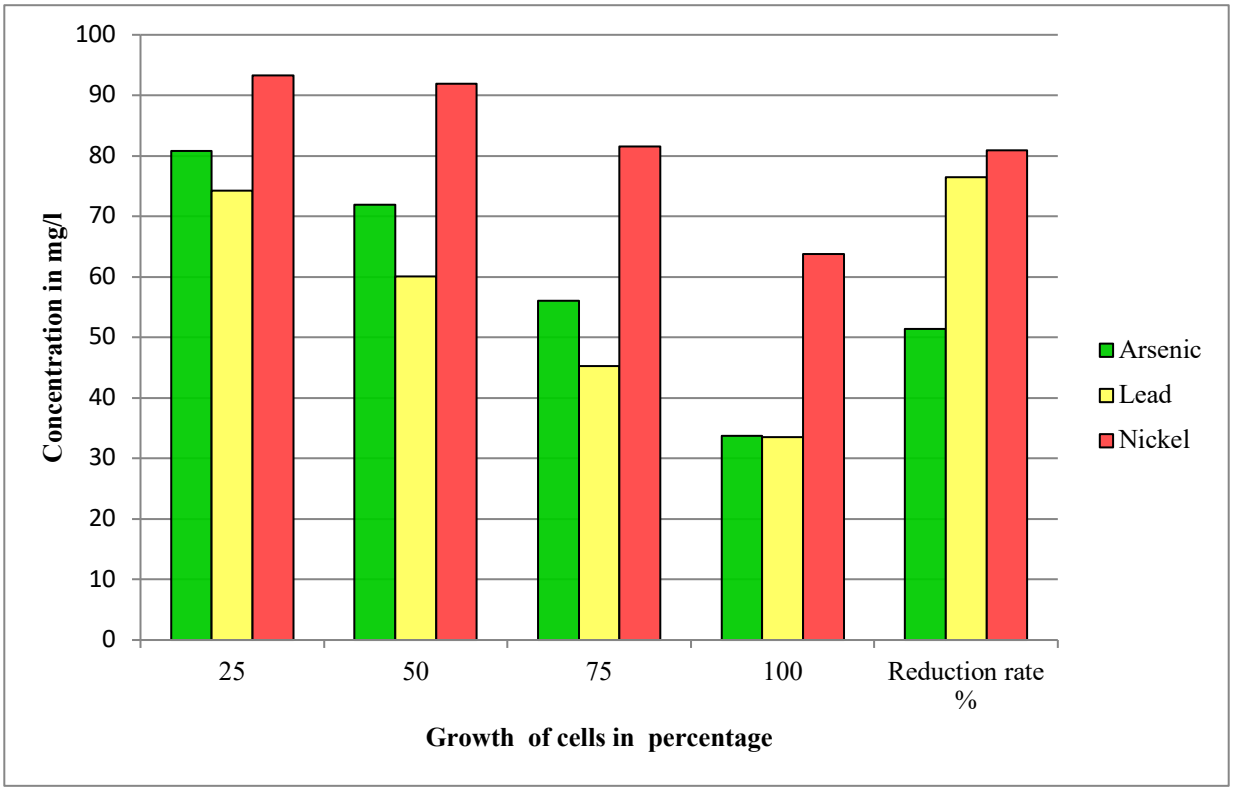

Figure 2 Shows growth and viability of isolates under various trial conditions

3.5 Growth and metal degradation efficiency of metal resistant strain

In this study the degradation of HMs (As, Pb and Ni) using *Providencia rettgeri* AVRB20 was analyzed by determining concentration of HMs in the culture growth medium. Hundred mg/l concentration of HMs were supplemented in100 ml LB broth medium separately. One ml of culture was inoculated HM enriched medium and incubated for 3 days. Then cells were centrifuged ad separated and the concentration of metals detected using atomic absorption spectroscopy. After incubation period 51.4 % of As, 76.5% of Pb and 80.9% of Ni were utilized by bacteria. Likewise the Bacteria *Chryseobacterium indoltheticum*, *Cupriavidus oxalaticus*, *Pesudomonas helmanticensis*, *Pseudomonas helmanticensis*, *Bacillus mycoides*, *Bacillus mycoides*, *Bacillus almalaya* and *Acinetobacter tjernbergiae* were isolated from soil and were able to grow in maximum of 1600 mg/ L of Pb [21]. Likewise, *Pseudomonas aerugeinosa* HF5 had the ability to grow on cobalt (590 mg/l), zinc (1310 mg/l), chromium (26mg/l), lead (570 mg/l), cadmium (843mg/l) and copper (222 mg/l) [7,26-28].

Conclusion

Municipal solid waste contains huge number of microorganisms. Generally, microbes have the capability of developing resistance mechanism when they continuously exposed in particular pollution. Metal resistance strains were isolated from municipal solid waste and leachate mixed sample. Among these *Providencia rettgeri* AVRB20 had the multiple metal (As, Pb and Ni) tolerance and high efficiency of degradation. The strong degradation and multiple tolerance of the *Providencia rettgeri* AVRB20 is suitable for the bioremediation of polluted area. It has the potential to become a commercially important strain for toxic compound bioremediation. This study recommended the combined action of microbes; microbial consortium with plant effectively reduces the bioavailability of metals on polluted sources.

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References

[1] S.E. Allen, H.M. Grimshaw, and A.P. Rowland, "Chemical analysis. In: Moore, P.D., Chapman, S.B. (Eds.), Methods in plant ecology. Oxford: *Blackwell scientific publication*, London, (1986) 285-344.

[2] APHA, "*Standard methods for the examination of water and wastewater"*. 21st Edition, American public health association/American water works association/water environment federation, Washington DC, (2005).

[3] P. Bhattacharya, G. Jacks, K.M. Ahmed, J. Routh, and A.A. Khan, "Arsenic in ground water of Bengal delta plain aquifers in Bangladesh" *Bulletin of environmental contamination toxicology*, 69(4) (2002) 538-545.

[4] J. Cuppucino, and N. Sherman, "Microbiology A laboratory manual". *Addison Wesley*, London, (1996) 313-337.

[5] G. Genchi, A. Carocci, G. Lauria, M. Stefania Sinicropi, and A. Catalano, " Nickel: Human health and environmental toxicology" *International Journal of Environmental Public Health*, 17(3) (2020) 679. doi:10.3390/ijerph17030679

[6] N.A. Gurave, V.V. Korde, S.S. Dhas, and M. Disale, Isolation and identification of heavymetal resistant bacteria from petroleum soil of Loni, Ahmednagar. *European Journal of Experimental Biology*,5(12)(2020) 6-11.

[7] F. Hafeez, H. Farheen, F. Mahmood, T. Shahzad, M. Shahid, M. Iqbal, S. Rasul, H. Manzoor, S. Hussin, "Isolation and characterization of a lead (Pb) tolerant Pseudomonas aeruginosa starin HF5 for decolorization of reactive red-120 and other azo dyes", *Annals of Microbiology,* 68 (2018) 93-952. https://doi.org/10.1007/s13213-018-14036.

[8] Holt, "Bergey's manual of detSerminative bacteriology". *Journal publisher Lippincott Williams and Wilkins*, (1994) Ninth edition.

[9] J. Jiang, Chaohu Pan., Aiping Xiao., Xiai Yang, and Guimin hang," Isolation, identification and environmental adaptability of heavy metal resistant bacteria from ramie rhizosphere soil around mine refinery, *3Biotechnology*, 7:5 (2017) 1-5. Doi: 10.100/s13205-017-0603-2.

[10] S. Kanmani, and R. Gandhimathi, "Assessment of heavy metal contamination in soil due to leachate migration from an open dumping site" *Applied Water Science*, 3 (2013) 193-20. Doi:10.1007/s13201-012-0072-z

[11] S. Kumar, G. Stecher, and K. Tamura, "Molecular evolutionary genetics analysis version 7.0 for bigger datasets" *Molecular biology and Evolution*, 33 (2016) 18701874. doi: 10.1093/molbev/msw054.

[12] E.C. Nwagwu,V. Moltong Yilwa, N.Eucharia Egbe, and G.B. Onwumre., "Isolation and characterization of heavy metal tolerant bacteria from Panteka stream, Kaduna, Nigeria and their potential for bioremediation" *African Journal of Biotechnology*, 16(1) (2017) 32-40. Doi:10.5897/AJB2016.15676.

[13] T.L. Pedersen, "Drinking water contamination by dumps and landfills" (1997) http://extoxnet.orst.edu/faqa/safedrink/

[14] A.Y. Rukah, and A.O. Kofahi, "The assessment of the effect of landfill leachate on ground waterQuality, A case studies, El-Akader landfill site, North Jordan" *Journal of Arid Environment*, 49 (2000) 615.

[15] Z. Ran, W. Bi, C. Qing Tao, L. Xiao Xia, L. Min, H. Dong, G. Dong Bei, W. Juan, and F. Chun, "Bioremediation of hexavalent chromium pollution by *Sporosarcinasaromensis* M52 Isolated from offshore sediments in Xiamen, China" *Biomedical and Environmental Science* 29(2) (2016) 127-136. Doi: 10.3967/bes2016.014

[16] Shardendu, Kumari Tripti., D. Narain Singh, and D. Sayantan, "Evaluation of asenic removal potential of arsenic resistant bacteria with the role of physiological and genomic factors".*Indian Journal of experimental Biology*, 55 (2017) 251-261.

[17] P.L. Smedley, and D.G. Kinniburgh, "A review of the source, behavior and distribution of arsenic in natural waters". *Applied Geochemistry* 17(5) (2002) 517-568.

[18] L. Stofejova, J. Fazekas, and D. Fazekasova, "Analysis of Heavy metal content in soil and plants in the dumping ground of magnesite mining facory Jelsava-Lubenik (Slovakia)", *Sustainability*, 13 (2021) 4508. https://doi.org/10.3390/su13084508.

[19] A.M. Taher, Q.F. Quadir, M.Z. Hossen, T. Nazneen, A. Rahman," Screening and isolation of arsenic tolerant *Rhizobacteria* from arsenic contaminated area of Bangladesh", *Progressive Agriculture,* 30(1) (2019) 17-25.

[20] K. Tamura, and M. Nei, "Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees" *Molecular biology and Evolution* 10 (1993) 512-526.

[21] T. Tongesayi, J. Kugara, and S. Tongesayi, "Waste dumpsites and public health: a case for lead exposure in Zimbabwe and potential global implications" *Environmental Geochemistry and Health*, (2017)1-10. doi 10.1007/s10653-017-9917-6

[22] WHO, news room, (2018).https://www.who.int/news-room/fact-sheets/detail/arsenic

[23] WHO, "Global health observatory: Regulations and controls on lead paint" (2019) http://www.who.int/gho/phe/chemical_safty/lead_paint_regulations/en

[24] J. Yung Chang, S. Do Yu, and Y. Seoub Hong, "Environmental source of Arsenic exposure" *Journal of Preventive Medicine and Public Health,* 47 (2014) 253-257. http://dx.doi.org/10.3961/jpmph.14.036

[25] U. Utami, L. Harianie, N. R. Dunyana and Romaidi, "Lead resistant bacteria isolated from oil wastewater sample for bioremediation of lead", *Water science technology,* 81. 10 (2020) 2244-2249.

[26] F. Ghadamgahi, S. Tarighi, P. Taheri, G.V. Saripella, A. Anzalone, P.B. Kalyandurg, V. Catara, R. Ortiz, R.R. Vetukuri, Plant GrowthPromoting Activity of Pseudomonas aeruginosa FG106 and Its Ability to Act as a Biocontrol Agent against Potato, Tomato and Taro Pathogens. *Biology,* 11 (2022) 140. https://doi.org/10.3390/biology11010140

[27] A. Borges, M. Simões, T. R. Todorović, N. R. Filipović, & A. T. García-Sosa, Cobalt Complex with Thiazole-Based Ligand as New Pseudomonas aeruginosa Quorum Quencher, Biofilm Inhibitor and Virulence Attenuator. *Molecules (Basel, Switzerland),* 23(6) (2018) 1385. https://doi.org/10.3390/molecules23061385

[28] S. P. Diggle, & M. Whiteley, Microbe Profile: Pseudomonas aeruginosa: opportunistic pathogen and lab rat. *Microbiology (Reading, England),* 166(1) (2020) 30–33. https://doi.org/10.1099/mic.0.000860

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