Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 5068 - 5074
Received 15 May 2021; Accepted 20 May 2021.

Heavy Metal Tolerant *Comamonas* species Isolated from Soil Sample in Tanjaro Region of Sulaymaniyah City - Iraq

Karzan Qurbani[1*] and Haider Hamzah[2]

[1]Department of Biology, College of Science, University of Raparin, Rania, Kurdistan Region, Iraq
[2] Department of Biology, College of Science, University of Sulaimani, Sulaymaniyah, Kurdistan Region, Iraq
[*]Corresponding author
Phone +9647701579234
E-mail: karzan.ahmed@uor.edu.krd

ABSTRACT

The Tanjaro region, located southwest of Sulaymaniyah city, is polluted with industrial effluents, wastewater, and importantly, heavy metals from the nearby city. From this area, *Comamonas* sp. was isolated. *Comamonas* sp. cells are gram-negative straight or curved rod shape, non-fermentative, and produce an orange color in the presence of sodium selenite. *Comamonas* sp. was identified by biochemical tests that were confirmed by the automated VITEK2 system. *Comamonas* sp. tolerated high concentrations of mercury and cadmium. Maximum tolerance concentration (MTC) demonstrated *Comamonas* sp.'s ability to tolerate 3 mM Cd, and 6 mM Hg and optimal uptake was at 30 °C, and pH 7. Inductively, coupled plasma - optical emission spectrometry (ICP-OES) showed *Comamonas* sp. was able to reduce 15.92% of Cadmium, and 30.05% of Mercury per 24 hours in culture media. Results of the current study demonstrated that the metal resistant *Comamonas* sp. has the potential ability to remove Cd, Hg, and play a significant role in future bioremediation bioprocess.

Keywords
Gram negative bacteria, *Comamonas*, bioremediation, heavy metals.

Introduction

At Sulaymaniyah city in the Kurdistan Region of Iraq, rapid development in the industrial sector, especially in the south, many companies, municipals, and factories work with oil, gas, pharmaceuticals, buildings. These companies, municipals, and factories discharge industrial waste in the Tanjaro Area, because there are no means of reusing and recycling solid waste. This waste accumulates and profusely pollutes the Tanjaro River with heavy metals whom the surrounding villages depend on as a source of water for agriculture and pastoral farming. The polluted Tanjaro river also flows into Darbandikhan lake and consequently causes many fish to die every year due to the constant accumulation of waste in the lake [1],[2].

The removal of heavy metals from contaminated wastewater previous to releasing effluents in nature is normally achieved by physiochemical processes, but traditional technologies are high cost and ineffective [3]. Previously, the use of the activated carbon method to adsorption heavy metals from contaminated wastewater was effective, but activated carbon cost reduced its use in adsorption [4]. Now however, the use of developed special microorganisms in the process called bioremediation reduces, transformation heavy metals from contaminated soil, water. Recently, bioremediation has become an alternative to conventional remediation technologies such as excavation, containment, and pump-and-treat. There are many factors that have an effect on the bioremediation process but most important is the presence of the appropriate number of microorganisms, suitable pH and temperature, and type of contaminants redox potential of the contaminated area. Most commonly used microorganisms for bioremediation include bacteria, archaea, algae, and fungi that have the ability to degrade and transform contaminants to a nontoxic or less toxic form. The indigenous microbes that live in the contaminated site often adapt and use different available carbon sources and electron acceptors, native microbe's heavy metal removal potential can be enhanced by stimulate with addition few amount of co-substrates [5],[6]. Among the microorganism's gram-negative bacteria play significant role in field bioremediation, such as metal resistant *Alcaligenes faecalis*, *Brevundimonas* sp., and *Pseudomonas sp.* able to remove (Hg, Cd, Ni, Pb) [7]. However, *Pseudomonas* and *Desulfovibrio* used in process biodesulfurization to remove S from fuel contaminants by their enzyme or cellular extracts [8]. Recently, researchers focused on the gram-negative bacteria including high metabolic activity genera *Stenotrophomonas*, *Burkholderia* and *Pseudomonas* for remediation polycyclic aromatic hydrocarbons (PAHs) [9]. Nowadays, *Comamonas* sp. is one of the most interested genera in bioremediation pollutants [10]. Recently, researchers were found the ability of genus *Comamonas* forremove pollutant from the contaminated area with industrial effluents such as Aromatic Compound, activated sludge, herbicide(glyphosate), heavy metals, dyes, and xenobiotic [11]–[16]. Additionally, Comamonas species are able biodegrading

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 5068 - 5074
Received 15 May 2021; Accepted 20 May 2021.

polychlorinated dibenzofurans (PCDFs), Polychlorinated dibenzo-p-dioxins (PCDDs) which are persistent organic compound pollutant [17].

Method and Materials

Isolation of *Comamonas* sp.

Soil and sediment samples were collected from Tanjaro area (Figure 1) in a sterilized container directly from Tanjaro River located southwest of Sulaymaniyah city, the samples were stored in an ice box and were transferred to the laboratory at Biology Department, College of Science, University of Sulaimani where the samples were inoculated on Luria Bertani broth (10 g/L tryptone, 10 g/L NaCl, 5 g/L yeast extract) with different concentration of heavy metals (2 mM $CuSO_4.5H_2O$, 1 mM $K_2Cr_2O_7$, 1 mM $ZnSO_4.7H_2O$, 1 mM $CdCl_2.H_2O$, 1 mM $HgSO_4$, 1 mM N_2O_6Pb, and 1 mM Na_2SeO_3), and incubated at 30℃/120 rpm for a duration of two days. The growth was diluted and spread on Luria Bertani Agar (LBA); different colonies were selected and purified [13].

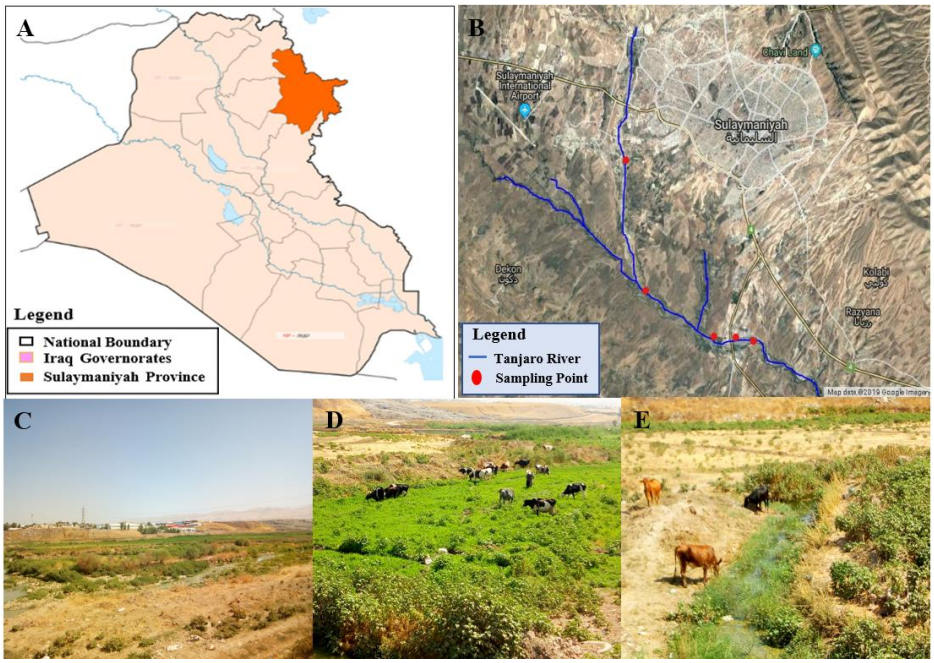

Figure 1. Tanjaro area and sampling point at Sulaymaniyah city, Kurdistan region, Iraq. A. map of Iraq; B. Satellite map of Tanjaro river direction; C, D, E. Images of sampling points.

Identification of *Comamonas* sp.

Selected bacterial isolates were inoculated on different bacteriological media such as; Nutrient agar, MacConkey agar, Eosin Methylene Blue (EMB), LBA with 1 mM Na_2SeO_3, Kligler Iron Agar (KIA), and Triple Sugar Iron Agar (TSI). Gram-negative rod shaped, oxidase positive, non-fermenter isolates were chosen and subjected to VITEK2 (BioMerieux, USA) instrument with Gram-negative VITEK2 ID card.

Scanning Electron Microscope (SEM)

The *Comamonas* sp. growth was pre-fixed on a grid with an aldehyde (2.5% glutaraldehyde) in 0.1 M of cacodylate buffer for 1 hour. Then rinsed with sodium cacodylate trihydrate buffer three-time (each 5 minutes); followed by post-fixation with 1% osmium in 0.1 M of cacodylate buffer, pH 7.4 for 1 hour. Then rinsed with 0.1 M cacodylate buffer for 2 minutes and 2 time with deionized water (each 2 minutes). Samples were then dehydrated by using graded ethanol series; followed by second dehydration with 1:1 (Hexamethyldisilazane: Ethanol for 15 minutes. Then, another dehydration with 100% Hexamethyldisilazane, 2 times (each 15 minutes) at room temperature. After that remove any excess liquid with filter paper for 4 hours. Finally, the sample were coated with nano-gold. Prepared samples were examined under SEM (JEOL, Japan) (Modified procedure of Fischer *et al.*, 2012).

Evaluation of heavy metal tolerance

Culture media (LB, PDB,) were prepared with different concentration of metals (Cd, Hg). The heavy metals were sterilized by autoclave. At first, they were suspended in distilled water, then autoclaved at 121°C for 20 min

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 5068 - 5074
Received 15 May 2021; Accepted 20 May 2021.

separately from culture media when cooled down to 45°C, the cultured media and metals were mixed together (to prevent any chemical reaction under high temperature). Maximum tolerable concentration detection was applied by using 96 well microtiter plate technique where each well contained different concentration of metals, then inoculated with *Comamonas* sp. (1 x10^9 cell/mL), then incubated for two days at 30°C/180 rpm. Growth rate was detected by measuring absorbance at 600nm using Microplate reader (ELx808^{TM}, Bio Tek ,USA)[19].

Heavy Metal Reduction by *Comamonas* sp.

Comamonas sp. was cultured into a conical flask containing LB Broth, incubate for 16 hours in a shaker incubator at 30°C 180 rpm, pH adjusted at 7.0. When growth reached to O. D (0.6) at 600nm for bacteria, then 2 mM of (Cd, Hg) separately was added to growth culture, incubated again 1 day at 30°C, 180 rpm. After all culture was centrifuged (CS4, VWR) 15min at 5000 rpm, supernatants were separated and volume duplicated with concentrated HNO3, then raised temperature to 100°C by Stirrer Hotplate (SB302, Keison) to discharge acid until volume is reached down initial volume, then filtered by filter paper (Whatman 42) to clean solution from any insoluble then volume returned to initial volume [20]. The heavy metal reduction was detected by inductively coupled plasma optical emission spectrometry (ICP-OES) (Optima 2100 DV, Perkin Elmer). The rate of heavy metals reduction was detected by compare results with a control.

Calculate metal reduction = heavy metal utilized (mM) ÷ heavy metals added to broth medium (mM) × 100.

Heavy metal utilized = heavy metal added to broth medium (mM) - remaining heavy metal at end of culture (mM).

Results

Identification of *Comamonas* sp.

Comamonas sp. was isolated from the Tanjaro region, where the industrial effluents and wastewater of Sulaymaniyah city including hospital discharges flow into the Tanjaro River. *Comamonas* sp. was identified using the diagnostic keys and molecular tools. The *Comamonas* sp. was isolated from soil of Tanjaro using LB broth supplemented with 1mM of different heavy metals. The *Comamonas* sp. is gram-negative bacteria, under the light microscope appeared as straight or curved rod (Fig. 2)

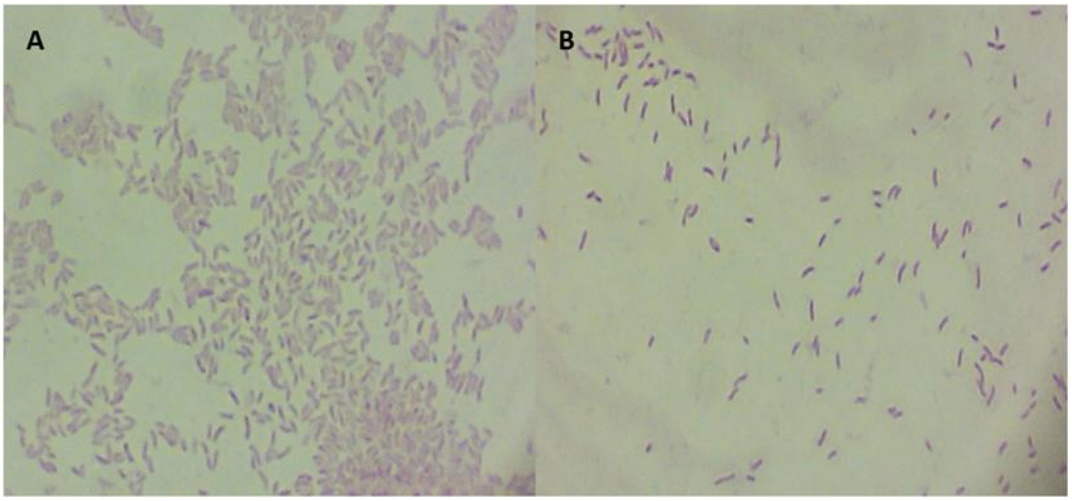

Figure 2. Gram stain of Comamonas sp. under the light microscope.

The *Comamonas* sp. biochemical properties shows non-fermenting bacteria, Oxidase positive, Catalase positive and no color production (Table 1).

Table 1. Microbiological properties of Comamonas sp.

Property	Reactions
Gram stain	Negative
Morphology	Straight, curved rod
Motility	Positive
Spore-forming	Negative

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 5068 - 5074
Received 15 May 2021; Accepted 20 May 2021.

Color production	Negative
Oxidase	Positive
Catalase	Positive
Glucose	Negative
Lactose	Negative
Sucrose	Negative

Additionally, VITEK 2 report shows that the species is *C. testosteroni* due to the card limitations (Supplementary results). Furthermore, *Comamonas* sp. under SEM appears as straight or curved rod shape (Fig. 3).

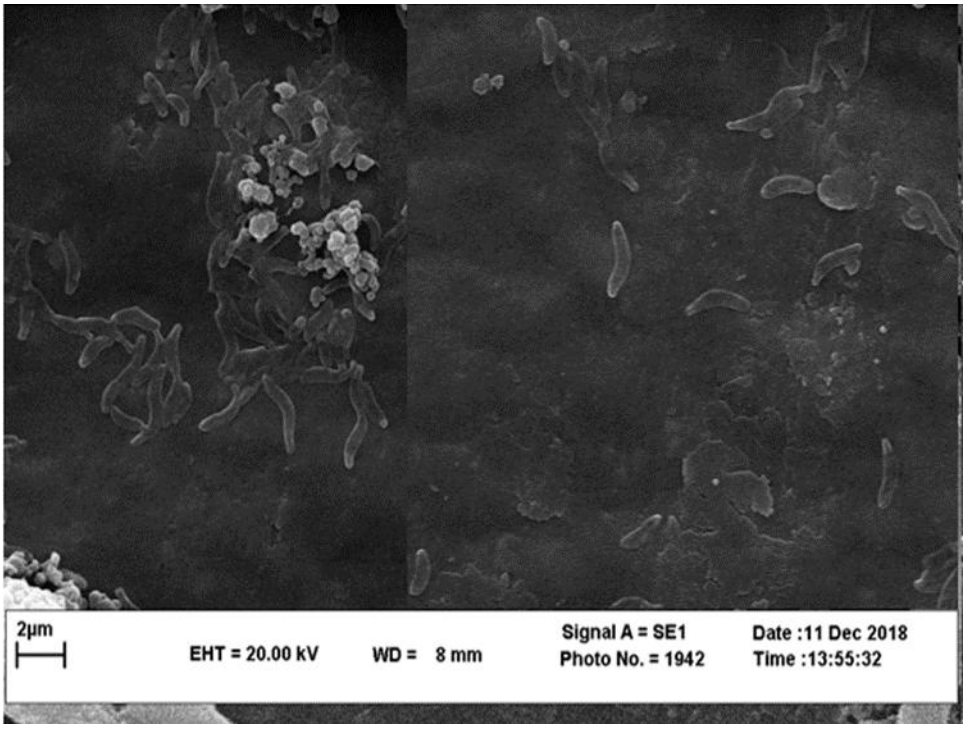

Figure 3. SEM images of Comamonas sp.

Evaluation of heavy metal tolerance

Maximum tolerance concentration (MTC) for (Cd, Hg) was performed on *Comamonas* sp. using microtiter plate method; where each well of microtiter plate contains a different concentration (mM) of heavy metals. The growth rate was read by spectrophotometer (Bio Tek, USA) at 600nm. *Comamonas* sp. able to tolerate 3mM of Cd (Fig. 4). However, *Comamonas* sp. able to tolerate 6 mM of Hg.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 5068 - 5074
Received 15 May 2021; Accepted 20 May 2021.

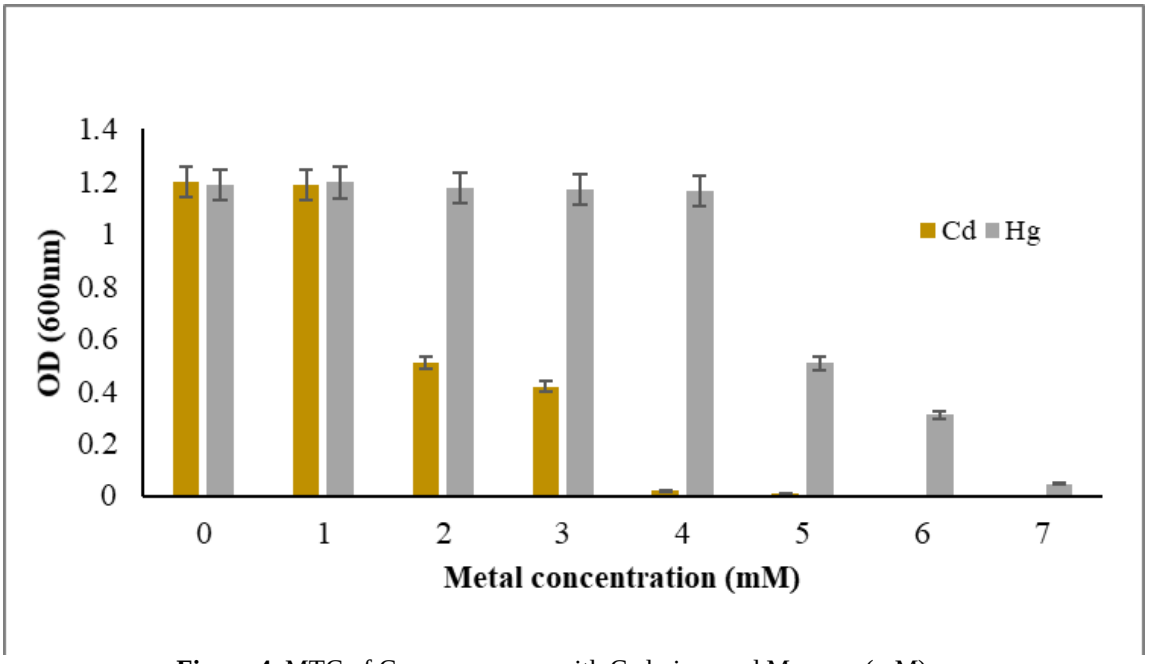

Figure 4. MTC of Comamonas sp. with Cadmium and Mercury (mM).

Additionally, results were confirmed by measuring reduced metals via *Comamonas sp.* measured through ICP-OES after 24 hours growth in media supplemented with 1 mM of Cd and Hg. *Comamonas* sp. able to reduce 1592% of the Cd, and 30.05% of Hg per 24 hours in the media (Figure 5).

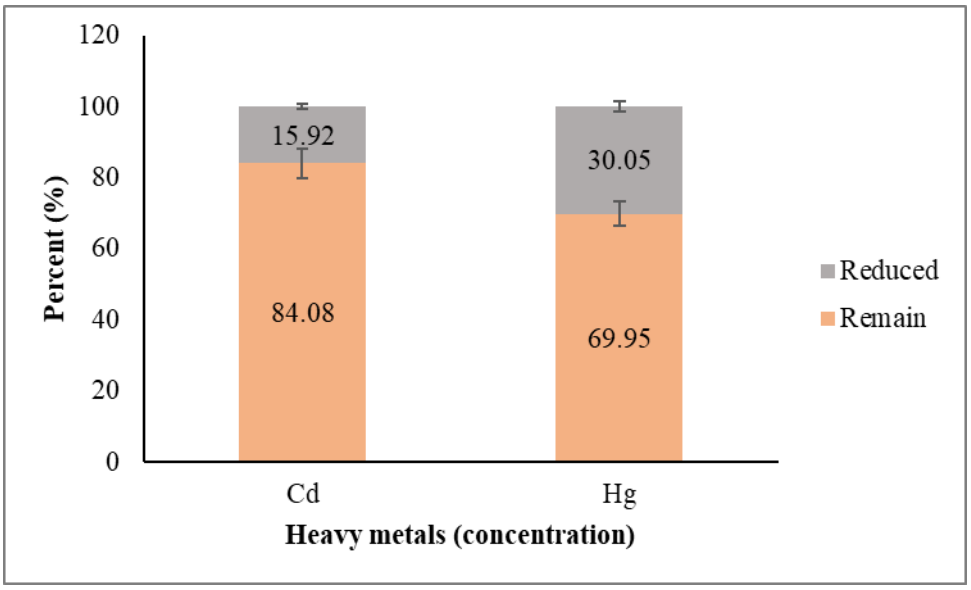

Figure 5. Measurement reduced Cd and Hg by Comamonas sp. using ICP-OES.

Discussion

Nowadays, scientists focused on identification and application of wild metal resistant strains that have potential in removing heavy metal toxicity and other contaminants from contaminated area. Non- fermentative *Comamonas* sp. have great potential to tolerate and reduce contaminants. This study is first research focused on identification of metal resistant *Comamonas* sp. in all Iraq and was used in field bioremediation. The genus *Comamonas* was first reported by [21] belongs to class Betaproteobacteria, family *Comamonadaceae*,and have more

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 5068 - 5074
Received 15 May 2021; Accepted 20 May 2021.

than seventeen species. The *Comamonas* species are gram-negative straight or slightly curved rod shaped, non-spore forming, and non-fermentative bacteria [22]. The *Comamonas* sp. able to reduce selenite to selenium and produce orange color in medium supplemented to sodium selenite [10]. The *Comamonas* species have been isolated form diverse environments, such as soil, fresh water, sediment, activated sludge, , wetland, wheat straw compost, and termite gut [23]–[25].

The Tanjaro region located southwest of Sulaymaniyah city is heavily contaminated with heavy metals, where untreated wastewater is directly discharged into the river, including the wastewater of the city and industrial areas. The polluted water is directly used for irrigation and watering farm animals which ultimately has negative impacts on the human health [2],[26],[27]. Today, cleaning metal pollutants from contaminated industrial sites by using metal resistant bacteria is most preferred. This study is the first research that focuses on the biosystem of the Tanjaro area, using native metal resistant microbes for the remediation of heavy metals. The *Comamonas* sp. isolated from Tanjaro region tolerates a high concentration of metals (Cd, Hg) of (3, 6) mM respectively. Moreover, *Comamonas sp.* able to potentially tolerates and removes heavy metals [13],[28]. However, it's used as biosystem in bioremediation of organic pollutants [11],[13],[16],[28]–[34].

Conclusion

In this study, *Comamonas* sp. was isolated and identified from Tanjaro region, Sulaymaniyah city, Kurdistan – Iraq. *Comamonas* sp. was able to grow and uptake heavy metals at 30 ℃, and pH 7, using different culture media. The cells were gram-negative, non-spore forming, and non-fermenting bacteria. SEM showed the typical curved and rod shapes of *Comamonas*. The *Comamonas* sp. tolerated 3 mM and 6 mM of Cd and Hg, respectively. ICP-OES results confirmed the ability of *Comamonas* sp. to reduce the two metals significantly.

References

[1] Tahir TA. (2017). Increasing solid waste generation in Sulaimania city as a new challenge to the environment of the city. Eurasian J Sci Eng. 3(2):68–81.

[2] Othman N, Kane T, Mohammed K, Alkaradaghi K, Salih F, Abdulla T, Hamafaraj K, Ali T. (2017). Environmental Health Assessment in Sulaymaniyah City and Vicinity. Kurdistan Institution for Strategic Studies and Scientific Research, William Joiner institute, University of Massachusetts Boston; 2017.

[3] Ahluwalia SS, Goyal D. (2007). Microbial and plant derived biomass for removal of heavy metals from wastewater. Bioresour Technol. 98(12):2243–57.

[4] Bishnoi NR, Garima. (2005). Fungus - An alternative for bioremediation of heavy metal containing wastewater : A review. J Sci Ind Res. 64:93–100.

[5] Ortíz I, Velasco A, Le Borgne S, Revah S. (2013). Biodegradation of DDT by stimulation of indigenous microbial populations in soil with cosubstrates. Biodegradation. 24(2):215–25.

[6] Liu W, Luo Y, Teng Y, Li Z, Ma LQ. (2010). Bioremediation of oily sludge-contaminated soil by stimulating indigenous microbes. Environ Geochem Health. 32(1):23–9.

[7] Giovanella P, Cabral L, Costa AP, de Oliveira Camargo FA, Gianello C, Bento FM. (2017). Metal resistance mechanisms in Gram-negative bacteria and their potential to remove Hg in the presence of other metals. Ecotoxicol Environ Saf. 140(February):162–9.

[8] Martínez I, El-Said Mohamed M, Santos VE, García JL, García-Ochoa F, Díaz E. (2017). Metabolic and process engineering for biodesulfurization in Gram-negative bacteria. J Biotechnol [Internet]. 262:47–55. Available from: https://doi.org/10.1016/j.jbiotec.2017.09.004

[9] Kuppusamy S, Thavamani P, Megharaj M, Venkateswarlu K, Lee YB, Naidu R. (2016). Pyrosequencing analysis of bacterial diversity in soils contaminated long-term with PAHs and heavy metals: Implications to bioremediation. J Hazard Mater. 317:169–79.

[10] Zheng S, Su J, Wang L, Yao R, Wang D, Deng Y, Wang R, Wang G, Rensing C. (2014). Selenite reduction by the obligate aerobic bacterium Comamonas testosteroni S44 isolated from a metal-contaminated soil. BMC Microbiol. 14(204):1–13.

[11] Azwani F, Suzuki K, Honjyo M, Tashiro Y, Hiroyuki F. (2017). Draft genome sequence of Comamonas testosteroni R2, consisting of aromatic compound degradation genes for phenol hydroxylase. Genome Announc. 5(36):e00875-17.

[12] Gumaelius L, Magnusson G, Pettersson B, Dalhammar G. (2001). Comamonas denitrificans sp. nov., an efficient denitrifying bacterium isolated from activated sludge. Int J Syst Evol Microbiol. 52(3):999–1006.

[13] Ghane M, Tabandeh F, Bandehpour M, Ghane M. (2013). Isolation and characterization of a heavy metal resistant Comamonas sp from industrial effluents. Iran J Sci Technol. A2:173–9.
[14] Xiong J, Li D, Li H, He M, Miller SJ, Yu L, Rensing C, Wang G. (2011). Genome analysis and characterization of zinc efflux systems of a highly zinc-resistant bacterium, Comamonas testosteroni S44. Res Microbiol. 162:671–9.
[15] Jadhav UU, Dawkar V V., Ghodake GS, Govindwar SP. (2008). Biodegradation of Direct Red 5B, a textile dye by newly isolated Comamonas sp. UVS. J Hazard Mater. 158(2–3):507–16.
[16] Weiss M, Kesberg AI, Labutti KM, Pitluck S, Bruce D, Hauser L, Copeland A, Woyke T, Lowry S, Lucas S, Land M, Goodwin L, Kjelleberg S, Cook AM, Buhmann M, Thomas T, Schleheck D. (2013). Permanent draft genome sequence of Comamonas testosteroni KF-1. Stand Genomic Sci. 8(2):239–54.
[17] Wang Y, Yamazoe A, Suzuki S, Liu C Te, Aono T, Oyaizu H. (2004). Isolation and characterization of dibenzofuran-degrading Comamonas sp. strains isolated from white clover roots. Curr Microbiol. 49(4):288–94.
[18] Fischer ER, Hansen BT, Nair V, Hoyt FH, Dorward DW. (2012). Scanning Electron Microscopy. In: Curr Protoc Microbiol. USA: Current Protocol Microbiology; 2012. p. 2B.2.1–2B.2.47.
[19] Qurbani K, Hamzah H. (2020). Intimate communication between Comamonas aquatica and Fusarium solani in remediation of heavy metal-polluted environments. Arch Microbiol [Internet]. 202(6):1397–406. Available from: https://doi.org/10.1007/s00203-020-01853-8
[20] Marzan LW, Hossain M, Mina SA, Akter Y, Chowdhury AMMA. (2017). Isolation and biochemical characterization of heavy-metal resistant bacteria from tannery effluent in Chittagong city, Bangladesh: Bioremediation viewpoint. Egypt J Aquat Res. 43(1):65–74.
[21] De Vos P, Kersters K, Falsen E, Pot B, Gillis M, Segers P, De Ley J. (1985). Comamonas Davis and Park 1962 gen. nov., nom. rev. emend., and Comamonas terrigena Hugh 1962 sp. nov., nom. rev. Int J Syst Evol Microbiol. 35(4):443–53.
[22] Zhu D, Xie C, Huang Y, Sun J, Zhang W. (2014). Description of Comamonas serinivorans sp. nov., isolated from wheat straw compost. Int J Syst Evol Microbiol. 64(12):4141–6.
[23] Chou JH, Sheu SY, Lin KY, Chen WM, Arun AB, Young CC. (2007). Comamonas odontotermitis sp. nov., isolated from the gut of the termite Odontotermes formosanus. Int J Syst Evol Microbiol. 57(4):887–91.
[24] Narayan KD, Pandey SK, Das SK. (2010). Characterization of Comamonas thiooxidans sp. nov., and comparison of thiosulfate oxidation with Comamonas testosteroni and Comamonas composti. Curr Microbiol. 61(4):248–53.
[25] Zhang J, Wang Y, Zhou S, Wu C, He J, Li F. (2013). Comamonas guangdongensis sp. nov., isolated from subterranean forest sediment, and emended description of the genus Comamonas. Int J Syst Evol Microbiol. 63(3):809–14.
[26] Aziz N, Salih SM, Hama-Salh NY. (2012). Pollution of Tanjero river by some heavy metals generated from sewage wastewater and industrial wastewater in Sulaimani district. J Kirkuk Univ –Scientific Stud. 7(1):67–83.
[27] Rasheed RO, HamaKarim TA. (2017). Impact assessment of wastewater and planning for a treatment plant within Sulaimani City, Iraq. Arab J Geosci. 10(23):507.
[28] Black R, Sartaj M, Mohammadian A, Qiblawey HAM. (2014). Biosorption of Pb and Cu using fixed and suspended bacteria. J Environ Chem Eng. 2(3):1663–71.
[29] Pandit RJ, Patel B, Kunjadia PD, Nagee A. (2013). Isolation, characterization and molecular identification of heavy metal resistant bacteria from industrial effluents, Amala-khadi - Ankleshwar, Gujarat. Int J Environ Sci. 3(5):1689–99.
[30] Staniland S, Coppock M, Tuffin M, Zyl L Van, Roychoudhury AN, Cowan D. (2010). Cobalt Uptake and Resistance to Trace Metals inComamonas testosteroni Isolated From a Heavy- Metal Contaminated Site in the Zambian Copperbelt. Geomicrobiol J. 27(8):656–68.
[31] Rudakiya DM, Pawar KS. (2013). Evaluation of remediation in heavy metal tolerance and removal by Comamonas acidovorans MTCC 3364. IOSR J Environ Sci Toxicol Food Technol. 5(5):26–32.
[32] Siunova T V., Siunov A V., Kochetkov V V., Boronin AM. (2009). The cnr-like operon in strain Comamonas sp. encoding resistance to cobalt and nickel. Russ J Genet. 45(3):292–7.
[33] Rudakiya DM, Pawar K. (2017). Bactericidal potential of silver nanoparticles synthesized using cell-free extract of Comamonas acidovorans: in vitro and in silico approaches. 3 Biotech. 7(2):92.
[34] Zhang Y, Ma YF, Qi SW, Meng B, Chaudhry MT, Liu SQ, Liu SJ. (2007). Responses to arsenate stress by Comamonas sp. strain CNB-1 at genetic and proteomic levels. Microbiology. 153(11):3713–21.