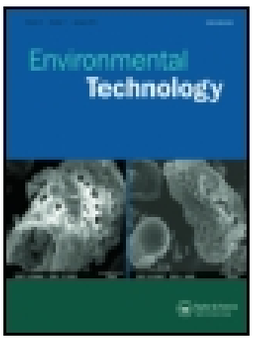


Environmental Technology

ISSN: 0959-3330 (Print) 1479-487X (Online) Journal homepage: http://www.tandfonline.com/loi/tent20

Biotransformation of Chromium (VI) in Liquid Effluents by Resistant Bacteria isolated from the Matanza-Riachuelo Basin, in Argentina

Ana Julieta González, Carolina Caimán, Natalia Gorino, María Susana Fortunato, Marcela Radice, Carlos Gómez, Carolina Mujica, Lorena Marquina, Alfredo Gallego & Sonia Edith Korol

To cite this article: Ana Julieta González, Carolina Caimán, Natalia Gorino, María Susana Fortunato, Marcela Radice, Carlos Gómez, Carolina Mujica, Lorena Marquina, Alfredo Gallego & Sonia Edith Korol (2017): Biotransformation of Chromium (VI) in Liquid Effluents by Resistant Bacteria isolated from the Matanza-Riachuelo Basin, in Argentina, Environmental Technology, DOI: 10.1080/09593330.2017.1367844

To link to this article: http://dx.doi.org/10.1080/09593330.2017.1367844

Accepted author version posted online: 04 Sep 2017.

Publisher: Taylor & Francis & Informa UK Limited, trading as Taylor & Francis Group

Journal: *Environmental Technology*

DOI: 10.1080/09593330.2017.1367844

Biotransformation of Chromium (VI) in Liquid Effluents by Resistant Bacteria isolated from the Matanza-Riachuelo Basin, in Argentina

Ana Julieta González[1,2*], Carolina Caimán[1], Natalia Gorino[1], María Susana Fortunato[1], Marcela Radice[3], Carlos Gómez[4], Carolina Mujica[4], Lorena Marquina[4], Alfredo Gallego[1], Sonia Edith Korol[1]

[1]*Cátedra de Salud Pública e Higiene Ambiental. Facultad de Farmacia y Bioquímica.Universidad de Buenos Aires. Buenos Aires. Argentina.*

[2]*CONICET. Buenos Aires. Argentina.*

[3]*Laboratorio de Resistencia Bacteriana. Cátedra de Microbiología. Facultad de Farmacia y Bioquímica.Universidad de Buenos Aires. Buenos Aires. Argentina.*

[4]*Centro de Tecnología del Uso del Agua. Instituto Nacional del Agua. Buenos Aires. Argentina.*

*Corresponding author: Ana Julieta González

Postal address: Cátedra de Salud Pública e Higiene Ambiental. Facultad de Farmacia y Bioquímica. Universidad de Buenos Aires. Junín 956, 4° Piso, (1113), Ciudad Autónoma de Buenos Aires, Argentina.

E-mail address: julietagonzalez@ffyb.uba.ar

Telephone number: 54 11 52874433

Biotransformation of Chromium (VI) in Liquid Effluents by Resistant Bacteria isolated from the Matanza-Riachuelo Basin, in Argentina

Pollution of the aquatic environment by heavy metals leads to the selection of resistant bacterial communities. These resistant microorganisms have detoxification mechanisms to survive in the polluted environment, such as biosorption and biotransformation. Optimization of these mechanisms constitutes an innovative biotechnological tool for the treatment of liquid effluents. The aims of this investigation were to evaluate the bacterial resistance to zinc, copper, chromium (VI) and lead in surface water streams from Buenos Aires, Argentina; to select a chromium-resistant strain able to remove the metal in batch process, and to evaluate the potential of this strain to remove chromium (VI) in liquid effluents. Bacterial resistance to the metals was evaluated by determining the Minimal Inhibitory Concentration (MIC). The kinetic of chromium (VI) removal by one of the resistant strains was studied in nutrient broth with 50 and 100 mg L^{-1} of the metal, as well as an effluent from an electroplating industry. High resistance to all the metals under study was observed in the bacterial communities of the Matanza-Riachuelo basin. A chromium resistant strain was isolated and identified as *Microbacterium* sp. It was able to remove 50 and 100 mg L^{-1} of Cr (VI) in 36 and 66 hours respectively, with efficiency higher than 99%. Experiments with liquid effluents showed the ability of the strain to transform 150 mg L^{-1} of the metal in 84 hours, with efficiency higher than 99%. These results show the potential of this native strain for the treatment of liquid effluents that contain chromium (VI).

Keywords: heavy metals; bacterial resistance; chromium (VI); biotransformation; industrial effluents

Introduction

Heavy metals are released to the environment, alone or associated with other pollutants, through industrial, hospital, agricultural or urban effluents, and represent a serious environmental problem both because of its toxic effects and its ability to accumulate along the food chain producing adverse effects on ecosystems and human health [1]. For this

reason, in many countries there are strict environmental regulations that require industries to implement clean production systems that include methods of treatment of low environmental impact, low cost and high efficiency for effluents rich in heavy metals [1]. In Buenos Aires, Argentina, the widespread use of chromium in industrial activities has aroused great concern since this metal, specially the hexavalent form, is highly toxic and carcinogenic [2]. The National Act on Hazardous Wastes Nº 24051 establishes guide levels for total chromium in freshwater streams of 2 μg L^{-1} for protection of aquatic life and 50 μg L^{-1} for human drink with conventional treatment. However many electroplating and tannery industries release untreated effluents to water courses in clandestine situation. This problem is especially worrying in the Matanza-Riachuelo basin since it has become one of the most polluted water sources in Argentina (www.acumar.gov.ar). For this reason, the control of these discharges as well as the improvement of treatment systems are essential to prevent further pollution.The methods conventionally employed for the removal of metals from liquid effluents consist of different physicochemical processes such as chemical precipitation, chemical reduction or oxidation, ion exchange and reverse osmosis [3]. However, despite the high removal efficiency of these processes, much has been discussed in recent years about its negative aspects such as its high cost, environmental impact and strong dependence on metal concentration, since its efficiency decreases at low concentrations [4,5]. Faced with these disadvantages and taking into account the role played by microbial processes in the environmental mobility of metals, the potential of microorganisms for the remediation of these compounds has aroused great interest [6]. In nature these organisms have developed various mechanisms of resistance to metals, such as transport through the cell membrane, biosorption to cell walls, precipitation, complex

formation and oxidation-reduction reactions [7]. The optimization and use of these processes constitutes a valid alternative for the treatment of liquid effluents and the remediation of polluted sites. The selection of microbial populations resistant to heavy metals begins to occur in contaminated environments. These previously exposed microorganisms can then be isolated from such environments. In the literature, several bacterial strains resistant to different metals isolated from contaminated soils, sediments and surface water samples have been reported [8,9], as well as from sewage sludge and industrial effluents [10,11]. These studies are mainly focused on the genetic aspects and the biochemical mechanisms involved in resistance. On the contrary, investigations about the heavy metal resistance of bacterial communities in natural environments with different pollution degrees are still scarce. Information about this resistance is important to understand the impact of contamination on water courses.

The aims of this investigation were: a) to evaluate the bacterial resistance to zinc, copper, chromium (VI) and lead in surface water streams from Buenos Aires, Argentina; b) to select a chromium-resistant strain able to remove the metal in batch process, and c) to evaluate the potential of this strain to remove chromium (VI) in liquid effluents from an electroplating industry.

Materials and methods

Sampling points

Six samples were obtained from surface water streams in the urban area of Buenos Aires, Argentina. Sampling points were located in the high, middle and lower regions of the Matanza-Riachuelo basin. These regions are characterized by different urbanization and contamination degrees. The sampling points are shown in Figure 1 [Figure 1 near here].

The samples were collected in plastic bottles and kept under refrigeration (4 °C) until analysis. Samples to heavy metals determination were also preserved by the addition of concentrated nitric acid to pH 2.

Characterization of samples

Water samples were characterized in order to evaluate their bacteriological and chemical quality, and to determine heavy metals concentration. Total heterotrophic bacteria, fecal coliforms, *Escherichia coli* and *Enterococcus* spp. counts were performed by the agar plate method or the most probable number method, according to APHA [12]. Chemical oxygen demand (COD) was determined by using a HACH® COD test based on the closed reflux method, according to the manufacturer´s instructions. Biochemical oxygen demand (BOD) was determined by the BOD trak™ method. The BOD trak apparatus used was provided by HACH®. Heavy metals concentration was determined by graphite furnace atomic absorption spectroscopy. A Shimadzu AA -7000 GFA – 7000 spectrophotometer was used.

Heavy metals

Four heavy metals were selected for this study: zinc, copper, chromium and lead. Stock solutions were prepared by dissolving the appropriate amount of $ZnSO_4$, $CuSO_4$, K_2CrO_4 and $Pb(NO_3)_2$ respectively in distilled water to obtain a concentration of 10000 mg L^{-1} of the corresponding cation. Solutions were sterilized by filtration through a membrane filter with a 0.22 μm pore size.

Evaluation of bacterial resistance to heavy metals in surface water samples

Bacterial resistance to heavy metals was evaluated in the water samples by determining de minimal inhibitory concentration (MIC). This assay was performed in tubes with 10 mL of nutrient broth (NB) added with increasing concentrations of the metals (from 4 to 1000 mg L^{-1}). The tubes were inoculated with 0.1 mL of the water samples and incubated at 28 °Cfor 7 days. The MIC of each metal was defined as the lowest concentration of the metal that produces bacterial growth inhibition.

Isolation of Cr (VI) resistant strains

Isolation of Cr (VI) resistant strains was carried out from the tubes of the MIC assay by spreading an aliquot of the culture onto tryptone soy agar (TSA) plates supplemented with the metal. Plates were incubated for 48 hours at 28 ºC and colonies with different morphology were separated and tested for its ability to remove Cr (VI). For these individual strains the MIC of all the metals under study was also determined. This assay was performed in microplates of 96 wells by inoculating an aliquot of the bacterial suspension in 0.25 mL of nutrient broth added with increasing concentrations of the metals (from 4 to 1000 mg L^{-1}). The plates were incubated at 28 ºC for 24 hours.

Cr (VI) removal experiments

Removal experiments were performed in Erlenmeyer flasks with 100 mL of nutrient broth supplemented with 50 mg L^{-1} of Cr (VI). The flasks were inoculated with 1 mL of a bacterial suspension to obtain an initial cell concentration of 1 x 10^7 CFU mL^{-1} in the assay and incubation was carried out in a rotatory shaker, at 28 ºC and 200 rpm, during 24 hours. The concentration of chromium (VI) was determined at the beginning and the end of the removal assay.

Based on the efficiency of the isolates to remove chromium (VI) in this assays, one of them was chosen to be studied in further experiments.

Identification of the selected strain

The selected isolate was identified by molecular techniques. They consisted in the amplification of the 16S ribosomal RNA (rRNA) gene by the polymerase chain reaction (PCR) and the sequencing of the amplified fragments. For amplification the following primers (5´-3´) were used: 16SR: GYTACCTTGTTACGACTT and 16SF: AGAGTTTGATCMTGGCTCAG. Amplified fragments were purified with the QIAquick PCR Purification Kit (QIAGEN, Duesseldorf, Germany) according to manufacturer's instructions, and sequenced using an ABI Prism DNA 3700 sequencer (Applied Biosystems, California, USA). Finally, nucleotide sequences were compared with databases using the NCBIs' Basic Local Alignment Search Tool (BLAST).

Kinetics and mechanism of Cr (VI) removal

Kinetic of Cr (VI) removal was studied in Erlenmeyer flasks with 100 mL of NB. The flasks containing the sterile media were supplemented with 50 or 100 mg L^{-1} of Cr (VI) and inoculated with an aliquot of a bacterial suspension in order to obtain an initial cell concentration of 1 x 10^7 CFU mL^{-1} in the experiments. Two replicates and a control of abiotic losses were used. The flasks were incubated in a rotatory shaker, at 28 ºC and 200 rpm. During incubation period 3 mL samples were aseptically removed from the flasks at appropriate intervals in order to determine bacterial growth and the remaining Cr (VI) concentration.

In order to elucidate the mechanism involved in chromium (VI) removal, samples taken at the beginning and the end of the process were also analyzed to determine total chromium in the biomass and the supernatant of the culture. For this purpose the samples were centrifuged during 10 min at 4000 rpm.The supernatant fluid was separated from the bacterial pellet, which was rinsed three times with 0.85% NaCl solution and resuspended in the same solution prior determination.

Removal of Cr (VI) in liquid effluents

A liquid effluent from an electroplating industry was used in this experiment. The effluent was characterized by determining pH, total Cr and Cr (VI) concentrations. Removal experiments were performed in Erlenmeyer flasks with 100 mL of the industrial effluent added with NB. If necessary the effluent was diluted for the experiment. The flasks were inoculated with an aliquot of a bacterial suspension in order to obtain an initial cell concentration of 1 x 10^7 CFU mL^{-1} in the experiments. Two replicates and a control of abiotic losses were used for each assay. The flasks were incubated in a rotatory shaker, at 28 ºC and 200 rpm. During incubation period 3 mL samples were aseptically removed from the flasks at appropriate intervals in order to determine bacterial growth and the remaining Cr (VI) concentration.

In this experiment, as well as in the kinetic assay, samples taken at the beginning and the end of the process were also analyzed to determine total chromium in the biomass and the supernatant of the culture.

Analytical methods

Total chromium was determined by flame atomic absorption spectrosphotometry with a Hitachi Polarized Zeeman Atomic Absorption Spectrophotometer. Chromium (VI) concentration was determined by the diphenylcarbazide method, according to APHA (2012).

Results and discussion

Characterization of samples

Water samples were obtained from the upper, middle and lower regions of the Matanza-Riachuelo basin, characterized by different urbanization and contamination degrees. The upper basin is predominantly an agricultural region, while the middle and lower regions are highly industrialized and urbanized in their vicinity, with the constant release of untreated effluents from several industries and sewage discharges [13]. For this reason samples were characterized in order to evaluate their bacteriological and chemical quality and to determine heavy metals concentrations. Results are shown in Tables 1 and 2 [Tables 1 and 2 near here]. High *Escherichia coli* and *Enterococcus* spp. counts were observed in the lower region of the basin in contrast with the upper region. This indicates high levels of fecal contamination in the urbanized region, as it could be expected. In the same way, results of heavy metals determinations show that lead and zinc concentrations increase in the lower basin. In contrast, high concentrations of chromium and copper were determined in all the sampling points, even in the upper basin. This suggests an input of these metals from another source, perhaps agro industrial activity present in the area.

Evaluation of bacterial resistance to heavy metals in surface water samples

The resistance of the bacterial communities from water samples to heavy metals was evaluated by determining de minimal inhibitory concentration (MIC). Results are shown in Table 3. MIC values reported in the literature show wide variability. In polluted environments MIC values higher than 100 mg L^{-1} are frequently found [14-20]. However much lower MIC values were also reported [9,21-23]. In this study MIC values similar to the highest reported in the literature were observed, even in the upper basin [Table 3 near here].

It has been reported that microorganisms resistant to certain heavy metal usually show simultaneous resistance to other metals [16,19,24]. This simultaneous resistance occurs by two possible mechanisms: cross-resistance (the same genetic determinant responsible for the resistance to different heavy metals) or co-resistance (different resistance determinants present on the same genetic element) [25]. These mechanisms could explain the widespread heavy metal resistance observed in all the sampling points regardless of their location and pollution degree, since the presence of high concentrations of copper and chromium in the whole basin could be responsible for the selection of microorganisms resistant to the other metals. On the other hand, it has been demonstrated that heavy metal- and antibiotic- resistance genes are linked, particularly on plasmids [25]. Therefore, the wide use of these compounds in intensive livestock rearing along the upper basin could be other reason for the heavy metal resistance observed.

Isolation of Cr (VI) resistant strains

Considering the importance of chromium as a contaminant in the Matanza-Riachuelo basin and the high concentrations of this metal found in all the sampling points (higher than the guide level established for protection of aquatic life), chromium (VI) was chosen for further

experiments. Isolation of Cr (VI) resistant strains was performed from the tubes of the MIC assay. Colonies with different morphology were also tested for their simultaneous resistance to other heavy metals and Cr (VI) removal ability. Six bacterial strains resistant to Cr (VI) were isolated, one from each sampling point. The results of the MIC assays confirmed that all of the isolates were also resistant to high concentrations of Cu, Pb and Zn (Table 4) [Table 4 near here]. Several Cr (VI) resistant strains described by other authors also showed simultaneous resistance to other heavy metals [18-20,26].

Cr (VI) removal assays

It has been reported that the resistance of a bacterial strain to heavy metals is independent from its ability to remove these metals [27]. This implies that a resistant strain is not necessarily able to remove the metal. In this investigation, Cr (VI) removal assays were performed in order to determine the ability of the isolates to remove 50 mg L^{-1} of Cr (VI) in the experimental conditions (Figure 2) [Figure 2 near here]. Results of Cr (VI) determinations at the end of the assay showed that the six isolates were able to remove the metal. However, the strain Cr 1 isolated from La Boca showed the highest removal efficiency in 24 hours. For this reason this strain was chosen for further experiments.

Identification of the selected strain

The Cr (VI) resistant strain that showed the best performance in removal assays (strain Cr 1) was first identified. The strain was a Gram positive coccus, aerobic, with small, rounded and yellow-pigmented colonies in TSA medium. The results of the analysis of the 16S rRNA sequences indicated that it belongs to the genus *Microbacterium* with an identity of 99% and 100% of sequence coverage. *Microbacterium* sp. has been reported previously as

a chromium-resistant microorganism [28,29]. This genus is widely distributed in the environment and it is not recognized as a human or animal pathogen. This is crucial for environmental purposes.

Kinetics and mechanism of Cr (VI) removal by Microbacterium sp.

The kinetic of Cr (VI) removal by *Microbacterium* sp. was first carried out in 100 mL of NB added with 50 mg L^{-1} of Cr (VI). Results are shown in Figure 3 [Figure 3 near here]. *Microbacterium* sp. was able to remove Cr (VI) from the culture medium in 36 hours, with efficiency higher than 99%. When the kinetic assay was performed with an initial Cr (VI) concentration of 100 mg L^{-1}, the complete removal of the metal was accomplished in 66 hours. It should be noted that abiotic losses of the metal remained below 2% in both experiments. The time course of Cr (VI) removal by the different microorganisms described in the literature is highly variable, as well as the culture mediums employed in those assays. Thacker et al. [30] studied Cr (VI) removal in LB broth by a strain of *Providencia* sp. The strain removed completely up to 300 mg L^{-1} of the metal within 108 hours. Zahoor and Rehman [31] reported a *Bacillus* sp. strain and a *Staphylococcus capitis* strain able to remove 100 mg L^{-1} of Cr (VI), in acetate minimal medium, within 96 hours with efficiencies of 85% and 81% respectively. Ilias et al. [11] isolated a *Staphylococcus aureus* strain and a *Pediococcus pentosaceus* strain able to remove completely 20 mg L^{-1} of Cr (VI), in LB broth, after 6 and 24 hours respectively. As it can be observed, the results of this investigation are comparable with those reported by other authors.

In order to elucidate the mechanism responsible of chromium removal, total Cr determination was also carried out at the end of the kinetic assay in both, the bacterial pellet and the supernatant of the culture. The results showed that all the chromium (VI) present at

the beginning of the experiment was transformed into Cr (III), whose concentration was calculated by the difference between total Cr and Cr (VI) concentrations (Table 5) [Table 5 near here]. This demonstrates that the mechanism involved in Cr (VI) removal was the aerobic reduction of hexavalent chromium to the trivalent form. In addition, total Cr concentrations in the two fractions at the end of the assay indicate that Cr (III) remained soluble in the supernatant of the culture, while practically no Cr (III) was detected in the bacterial pellet. Several bacterial strains able to carry out this transformation in aerobic conditions were reported in the literature [9,32-34], suggesting the ubiquity of this process in nature.

Removal of Cr (VI) in liquid effluents by Microbacterium sp.

The ability of the *Microbacterium* sp. strain to reduce Cr (VI) was also evaluated in a liquid effluent from an electroplating industry. The effluent was first characterized by determining pH, total Cr and Cr (VI) concentrations. Results showed that chromium (VI) concentration was 1501.5 mg L^{-1} while chromium (III) was not detected. The pH value was 1.5 (data not shown). Because of the acidic characteristics of the effluent and the extremely high chromium concentration, it was diluted in nutrient broth to a chromium (VI) concentration of 150 mg L^{-1} and a pH value of 7.0. The culture medium also provides the necessary nutrients to support bacterial growth.

Studies evaluating Cr (VI) removal in real industrial effluents are reported in the literature. Effluents from different origins and chromium concentrations are used in those assays. Abdulla et al. [35] described an actinomycete strain that could remove 364 mg L^{-1} of the metal from a tannery wastewater in 96 hours, with efficiency of 90%. On the other hand, the *Bacillus* sp. and *Staphylococcus capitis* described by Zahoor and Rehman [31]

316 removed up to 1.84 mg L^{-1} of Cr (VI) from an industrial effluent within 144 hours, with
317 efficiencies of 86% and 89% respectively. In an electroplating effluent with 0.028 mg L^{-1} of
318 Cr (VI), a *Pseudomonas aeruginosa* strain isolated by Pandian et al. [36] showed the ability
319 to remove 86% of the metal in 20 days. In this investigation it could be demonstrated that
320 *Microbacterium* sp. was able to reduce 150 mg L^{-1} of Cr (VI) in the effluent from an
321 electroplating industry in 84 hours, with transformation efficiency higher than 99%. The
322 performance of the strain showed to be very suitable for the treatment of industrial effluents
323 with high concentrations of Cr (VI). Results of the biotransformation assay are shown in
324 Figure 4 [Figure 4 near here].
325 Results of total Cr determination in the bacterial pellet and the supernatant of the
326 culture at the end of this assay were similar to those obtained in the kinetic assay (Table 5)
327 [Table 5 near here].
328
329 **Conclusions**
330 The bacterial resistance to heavy metals in the Matanza-Riachuelo basin, Argentina was
331 study in six sampling points with different pollution degrees. Results demonstrated high
332 resistance to all of the metals under study, in all the sampling points. A *Microbacterium* sp.
333 strain highly resistant to Cr (VI) (MIC=1000 mg L^{-1}) was then isolated from La Boca water
334 samples. The strain was also able to reduce the metal (150 mg L^{-1}) to the trivalent form in
335 the effluent of an electroplating industry, in 84 hours and with efficiency higher than 99%.
336 The results of this investigation are promising, since it could be demonstrated the great
337 potential of *Microbacterium* sp. for the treatment of industrial effluents. However several
338 aspects, such as the use of low cost substrates to support bacterial growth, the performance
339 of the strain in continuous processes and the reduction of toxicity in the treated effluent,

should be still considered in further studies focused in real applications. In addition, the information obtained about the heavy metal resistance of bacterial communities in this basin is a valuable contribution to its characterization and the study of contamination.

Acknowledgements. We thank the University of Buenos Aires for the grant given for this study, supported by UBACYT Program-Project 20020130100378BA 2014-2017. We also thank Dr. Gabriel Gutkind from the Laboratory of Bacterial Resistance, Faculty of Pharmacy and Biochemistry, Buenos Aires' University for his collaboration in the identification of microorganisms.

The authors have declared no conflict of interest.

References

[1] Malik A. Metal bioremediation through growing cells. Environ Int. 2004;30:261-278.

[2] Allegretti P, Furlong J, Donati E. The role of higher polythionates in the reduction of chromium (VI) by *Acidithiobacillus* and *Thiobacillus* cultures. J Biotech. 2006;122:55-61.

[3] Ahluwalia SS, Goyal D. Microbial and plant derived biomass for removal of heavy metals from wastewater. Bioresource Technol. 2007;98:2243-2257.

[4] Vijayaraghavan K, Yun, YS. Bacterial biosorbents and biosorption. Biotechnol Adv. 2008;26:266-291.

[5] Lesmana SO, Febriana N, Soetaredjo FE, Sunarso J, Ismadji S. Studies on potential applications of biomass for the separation of heavy metals from water and wastewater. Biochem Eng J. 2009;44:19–41.

[6] Umrania VV. Bioremediation of toxic heavy metals using acidothermophilic autotrophes. Bioresource Technol. 2006;97:1237-1242.

[7] Lovley DR, Coates JD. Bioremediation of metal contamination. Curr Opin Biotech. 1997;8:285-289.

[8] Roane TM, Pepper IL. Microbial Responses to Environmentally Toxic Cadmium. Microbial Ecol. 2000;38:358-364.

[9] Garavaglia L, Cerdeira SB, Vullo DL. Chromium (VI) biotransformation by β– and γ–Proteobacteria from natural polluted environments: A combined biological and chemical treatment for industrial wastes. J Hazard Mater. 2010;175:104-110.

[10] Costley SC, Wallis FM. Bioremediation of heavy metals in a synthetic wastewater using a rotating biological contactor. Water Res. 2001;35:3715-3723.

[11] Ilias M, Rafiqullah IM, Debnath BC et al. Isolation and Characterization of Chromium(VI)-Reducing Bacteria from Tannery Effluents. Indian J Microbiol. 2011;51:76-81.

[12] American Public Health Association, American Water Works Association, Water Environment Federation, *Standard Methods for the Examination of Water and Wastewater, 22nd Ed.* Washington, DC. **2012**.

[13] Cattaneo MP, López Sardi EM. Evolution of water quality in the Matanza-Riachuelo basin. Ciencia y Tecnología. 2013;13:251-278.

[14] Shakoori AR, Muneer B. Copper-Resistant Bacteria from Industrial Effluents and Their Role in Remediation of Heavy Metals in Wastewater. Folia Microbiol. 2002;47:43-50.

[15] Moraga R, Merino C, Mondaca MA. Resistance to heavy metals in bacterial isolates from the Iquique bay. Invest Mar. 2003;31:91-95.

[16] Viti C, Pace A, Giovannetti L. Characterization of Cr (VI)-Resistant Bacteria Isolated from Chromium-Contaminated Soil by Tannery Activity. Curr Microbiol. 2003;46:1-5.

[17] Pal A, Paul AK. Aerobic chromate reduction by chromium-resistant bacteria isolated from serpentine soil. Microbiol Res. 2004;159:347-354.

[18] Shukla OP, Rai UN, Singh NK, Dubey S, Baghel VS. Isolation and characterization of chromate resistant bacteria from tannery effluent. J Environ Biol. 2007;28:399-403.

411 [19] Rajbanshi A. Study on Heavy Metal resistant Bacteria in Guheswori Sewage
412 Treatment Plant. Our Nature. 2008;6:52-57.
413
414 [20] Sundar K, Vidya R, Mukherjee A, Chandrasekaran N. High Chromium Tolerant
415 Bacterial Strains from Palar River Basin: Impact of Tannery Pollution. Res J Environ Earth
416 Sci.2010;2:112-117.
417
418 [21] Camargo FAO, Okeke BC, Bento FM, Frankenberger WT. Diversity of chromium-
419 resistant bacteria isolated from soils contaminated with dichromate. Appl Soil
420 Ecol.2005;29:193-202.
421
422 [22] Vullo DL, Ceretti HM, Daniel MA, Ramírez SAM, Zalts A. Cadmium, zinc and
423 copper biosorption mediated by *Pseudomonas veronii* 2E. Bioresource
424 Technol.2008;99:5574-5581.
425
426 [23] Abskharon RN, Hassan SH, Kabir MH, Qadir SA, El-Rab SMG, Wang MH. The role
427 of antioxidants enzymes of *E. coli* ASU3, a tolerant strain to heavy metals toxicity, in
428 combating oxidative stress of copper. World J Microbiol Biotechnol. 2010;26:241-247.
429
430 [24] Raja CE, Anbazhagan K, Selvam GS. Isolation and characterization of a metal-
431 resistant *Pseudomonas aeruginosa* strain. World J Microbiol Biotechnol. 2006;22:577-585.
432
433 [25] Baker-Austin C, Wright MS, Stepanauskas R, McArthur JV. Co-selection of antibiotic
434 and metal resistance. Trends Microbiol. 2006;14:176-182.

435 [26] Tamil Selvi A, Anjugam E, Archana Devi R, Madhan B, Kannappan S,
436 Chandrasekaran B. Isolation and Characterization of Bacteria from Tannery Effluent
437 Treatment Plant and Their Tolerance to Heavy Metals and Antibiotics. Asian J Exp Biol
438 Sci. 2012;3:34-41.
439
440 [27] Ge S, Zhou M, Dong X, Lu Y, Ge S. Distinct and effective biotransformation of
441 hexavalent chromium by a novel isolate under aerobic growth followed by facultative
442 anaerobic incubation. Appl Microbiol Biot. 2013;97:2131-2137.
443
444 [28] Pattanapipitpaisal P, Brown N, Macaskie L. Chromate reduction and 16S rRNA
445 identification of bacteria isolated from a Cr (VI) contaminated site. Appl Microbiol Biot.
446 2001;57:257-261.
447
448 [29] Liu Z, Wu Y, Lei C, Liu P, Gao M. Chromate reduction by a chromate-resistant
449 bacterium, *Microbacterium* sp. World J Microb Biot. 2012;28:1585-1592.
450
451 [30] Thacker U, Parikh R, Shouche Y, Madamwar D. Hexavalent chromium reduction by
452 *Providencia* sp. Process Biochem. 2006;41:1332-1337.
453
454 [31] Zahoor A, Rehman A. Isolation of Cr (VI) reducing bacteria from industrial effluents
455 and their potential use in bioremediation of chromium containing wastewater. J Environ
456 Sci. 2009:21:814-820.
457

458 [32] Zakaria ZA, Zakaria Z, Surif S, Ahmad WA. Hexavalent chromium reduction by
459 *Acinetobacter haemolyticus* isolated from heavy-metal contaminated wastewater. J Hazard
460 Mater. 2007;146:30–38.
461
462 [33] Pal A, Datta S, Paul AK. Hexavalent Chromium Reduction by Immobilized Cells of
463 *Bacillus sphaericus* AND 303. Braz Arch Biol Techn. 2013;56:505-512.
464
465 [34] Wani PA, Omozele AB. Cr (VI) Removal by Indigenous *Klebsiella* Species PB6
466 Isolated from Contaminated Soil under the Influence of Various Factors. Curr Res
467 Bacteriol. 2015;8:62-69.
468
469 [35] Abdulla HM, Kamal EM, Mohamed AH, El-Bassuony AD. Chromium removal from
470 tannery wastewater using chemical and biological techniques aiming zero discharge of
471 pollution, *Proceeding of Fifth Scientific Environmental Conference, Zagazig University.*
472 Egypt.**2010**.
473
474 [36] Pandian K, Thatheyus AJ, Ramya D.Bioremoval of Chromium, Nickel And Zinc in
475 Electroplating Effluent by *Pseudomonas aeruginosa*. Open J Water Pollut Treat.
476 2014;1:75-82.
477
478

479 Table 1. Chemical and bacteriological characterization of water samples.

480

Sampling point	*BOD ($mg\ L^{-1}$)*	*COD ($mg\ L^{-1}$)*	*Total heterotrophic bacteria ($CFU\ mL^{-1}$)*	*Fecal Coliforms*	*Escherichia coli*	*Enterococcus spp.*
La Boca	28	82	3.5×10^{5}	$7.5 \times 10^{3+}$	$2.5 \times 10^{3+}$	$2.5 \times 10^{2+}$
Alsina bridge	26	79	1.7×10^{5}	$1.0 \times 10^{3+}$	$4.2 \times 10^{2+}$	$4.0 \times 10^{1+}$
La Noria bridge	5	90	5.6×10^{5}	3.3×10^{3}	1.3×10^{3}	3.7×10^{2}
Ricchieri freeway	9	89	5.0×10^{4}	$1.2 \times 10^{3+}$	$8.0 \times 10^{1+}$	$2.0 \times 10^{1+}$
Morales creek	6	69	1.6×10^{5}	$3.1 \times 10^{3+}$	$3.0 \times 10^{2+}$	$5.0 \times 10^{1+}$
Rodríguez creek	<5	29	1.6×10^{4}	$8.0 \times 10^{1+}$	3	$2.0 \times 10^{1+}$

481

482 (*) Most probable number in 100 mL; ($^{+}$)CFU mL^{-1}

483

Table 2. Heavy metals concentrations in water samples.

Sampling point	*Total Cu ($\mu g\ L^{-1}$)*	*Total Cr ($\mu g\ L^{-1}$)*	*Total Pb ($\mu g\ L^{-1}$)*	*Total Zn ($\mu g\ L^{-1}$)*
La Boca	20	8	<10*	49
Alsina bridge	11	11	13	<30*
La Noria bridge	7	11	<10*	nd
Ricchieri freeway	5	4	nd	nd
Morales creek	12	7	nd	nd
Rodríguez creek	<5*	8	nd	nd

(nd) Not detected. Metal concentration is lower than the detection limit of the method; (*) Metal concentration is lower than the quantification limit.

Table 3. Bacterial resistance to heavy metals in water samples

Sampling point	*MIC (mg L^{-1})*			
	Cu	*Cr*	*Pb*	*Zn*
La Boca	1000	>1000	>500	>1000
Alsina bridge	1000	>1000	>500	>1000
La Noria bridge	1000	>1000	>500	>1000
Ricchieri freeway	1000	>1000	>500	>1000
Morales creek	500	>1000	>500	>1000
Rodríguez creek	1000	>1000	>500	>1000

Table 4. Resistance of each isolated strain to all the metals under study

Sampling point	*Strain*	*MIC (mg L^{-1})*			
		Cu	*Cr*	*Pb*	*Zn*
La Boca	Cr1	1000	1000	>500	500
Alsina bridge	Cr2	1000	1000	>500	500
La Noria bridge	Cr3	1000	1000	>500	500
Ricchieri freeway	Cr4	500	>1000	>500	500
Morales creek	Cr5	1000	>1000	>500	500
Rodríguez creek	Cr6	500	>1000	>500	500

516 Table 5. Determination of Cr (VI) and total Cr in the different fractions of the bacterial
517 culture, at the beginning and the end of the assays with supplemented chromium (NB + Cr
518 VI) and electroplating effluent (NB + Effluent)
519

Time	*Culture fraction*	*Chromium (VI) concentration (mg L^{-1})*			
		NB + Cr (VI)		*NB + Effluent*	
		Cr (VI)	*Total Cr*	*Cr (VI)*	*Total Cr*
Inital	-	52.0	52.0	150.1	150.1
Final	Supernatant	nd	51.3	nd	148.0
	Biomass	*	0.7	*	2.1

520

521 (nd) Not detected. Metal concentration is lower than the detection limit of the method

522 *Not determined

523

524 Captions for figures

525

526 Figure 1.

527 Sampling points. (1) La Boca, (2) Alsina bridge, (3) La Noria bridge, (4) Ricchieri freeway,

528 (5) Morales creek, (6) Rodríguez creek.

529

530 Figure 2.

531 Removal of Cr (VI) by the resistant strains in nutrient broth with 50 mg L^{-1} of the metal. %

532 Remaining Cr (VI) at the beginning ■; and at the end ■ of the assay (24 hours). Origin of

533 the strains: (Cr 1) La Boca; (Cr 2) Alsina bridge; (Cr 3) La Noria bridge; (Cr 4) Ricchieri

534 freeway; (Cr 5) Morales creek; (Cr 6) Rodríguez creek.

535

536 Figure 3.

537 Kinetics of Cr (VI) removal by *Microbacterium* sp. in nutrient broth added with different

538 initial concentrations of the metal. (A) 50 mg L^{-1}; (B) 100 mg L^{-1}. ● Bacterial growth; ○

539 remaining Cr (VI) concentration; ■ remaining Cr (VI) concentration in controls of abiotic

540 losses.

541

542 Figure 4.

543 Kinetic of Cr (VI) removal by *Microbacterium* sp. in the effluent of an electroplating

544 industry. ● Bacterial growth; ○ remaining Cr (VI) concentration; ■ remaining Cr (VI)

545 concentration in the control of abiotic losses.

546 Table 1. Chemical and bacteriological characterization of water samples.

547

Sampling point	*BOD ($mg\ L^{-1}$)*	*COD ($mg\ L^{-1}$)*	*Total heterotrophic bacteria ($CFU\ mL^{-1}$)*	*Fecal Coliforms*	*Escherichia coli*	*Enterococcus spp.*
La Boca	28	82	3.5×10^{5}	$7.5 \times 10^{3+}$	$2.5 \times 10^{3+}$	$2.5 \times 10^{2+}$
Alsina bridge	26	79	1.7×10^{5}	$1.0 \times 10^{3+}$	$4.2 \times 10^{2+}$	$4.0 \times 10^{1+}$
La Noria bridge	5	90	5.6×10^{5}	3.3×10^{3}	1.3×10^{3}	3.7×10^{2}
Ricchieri freeway	9	89	5.0×10^{4}	$1.2 \times 10^{3+}$	$8.0 \times 10^{1+}$	$2.0 \times 10^{1+}$
Morales creek	6	69	1.6×10^{5}	$3.1 \times 10^{3+}$	$3.0 \times 10^{2+}$	$5.0 \times 10^{1+}$
Rodríguez creek	<5	29	1.6×10^{4}	$8.0 \times 10^{1+}$	3	$2.0 \times 10^{1+}$

548

549 (*) Most probable number in 100 mL; ($^{+}$) CFU mL^{-1}

550

551 Table 2. Heavy metals concentrations in water samples.
552

553

Sampling point	*Total Cu* ($\mu g\ L^{-1}$)	*Total Cr* ($\mu g\ L^{-1}$)	*Total Pb* ($\mu g\ L^{-1}$)	*Total Zn* ($\mu g\ L^{-1}$)
La Boca	20	8	<10*	49
Alsina bridge	11	11	13	<30*
La Noria bridge	7	11	<10*	nd
Ricchieri freeway	5	4	nd	nd
Morales creek	12	7	nd	nd
Rodríguez creek	<5*	8	nd	nd

554
555
556
557
558
559

560 (nd) Not detected. Metal concentration is lower than the detection limit of the method; (*)
561 Metal concentration is lower than the quantification limit.

562

563 Table 3. Bacterial resistance to heavy metals in water samples

564

565

Sampling point	*CIM (mg L^{-1})*			
	Cu	*Cr*	*Pb*	*Zn*
La Boca	1000	>1000	>500	>1000
Alsina bridge	1000	>1000	>500	>1000
La Noria bridge	1000	>1000	>500	>1000
Ricchieri freeway	1000	>1000	>500	>1000
Morales creek	500	>1000	>500	>1000
Rodríguez creek	1000	>1000	>500	>1000

566 Table 4. Resistance of each isolated strain to all the metals under study (cross resistance)

567

568

569

Sampling point	*Strain*	*CIM (mg L^{-1})*			
		Cu	*Cr*	*Pb*	*Zn*
La Boca	Cr1	1000	1000	>500	500
Alsina bridge	Cr2	1000	1000	>500	500
La Noria bridge	Cr3	1000	1000	>500	500
Ricchieri freeway	Cr4	500	>1000	>500	500
Morales creek	Cr5	1000	>1000	>500	500
Rodríguez creek	Cr6	500	>1000	>500	500

570 Table 5. Determination of Cr (VI) and total Cr in the different fractions of the bacterial
571 culture, at the beginning and the end of the assays with supplemented chromium (NB + Cr
572 VI) and electroplating effluent (NB + Effluent)

Time	*Culture fraction*	*Chromium (VI) concentration (mg L^{-1})*			
		NB + Cr (VI)		*NB + Effluent*	
		Cr (VI)	*Total Cr*	*Cr (VI)*	*Total Cr*
Inital	-	52.0	52.0	150.1	150.1
Final	Supernatant	nd	51.3	nd	148.0
	Biomass	*	0.7	*	2.1

573

574 (nd) Not detected. Metal concentration is lower than the detection limit of the method

575 *Not determined

576

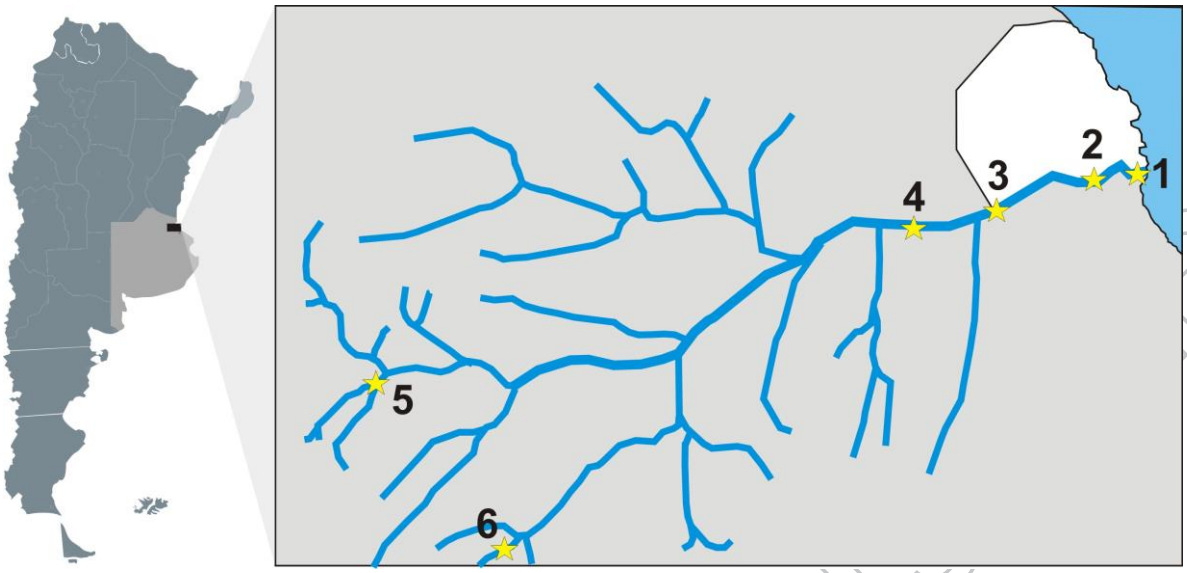
577

578

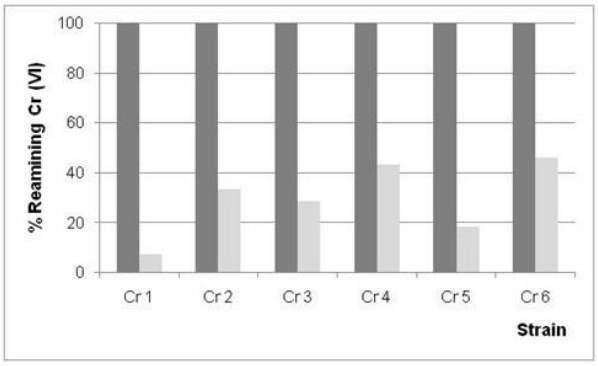
579

580

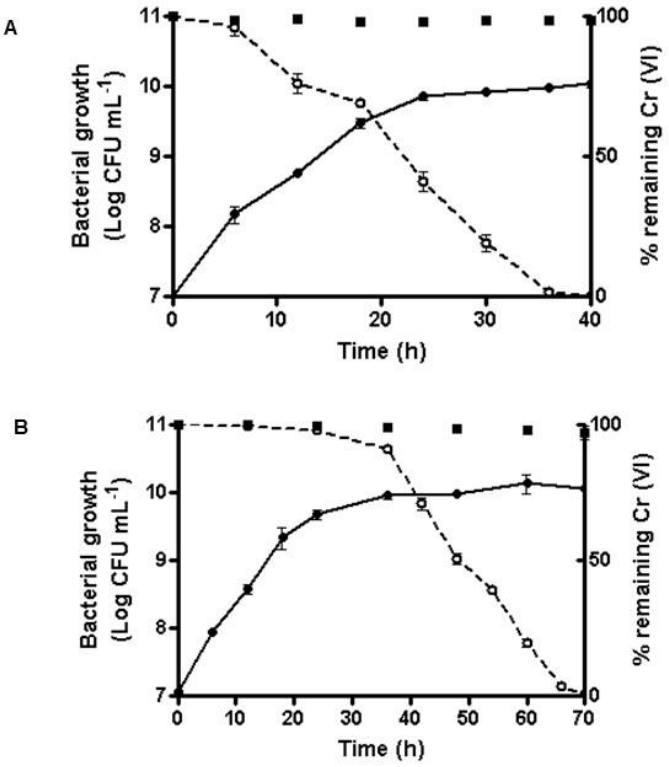
581



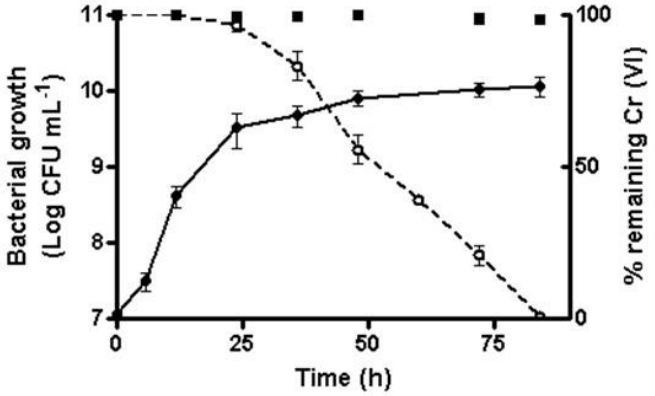
582



583



584



585